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# Metabolic Capabilities of *Escherichia coli*: I. Synthesis of Biosynthetic Precursors and Cofactors

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Metabolism of living cells converts substrates into metabolic energy, redox potential and metabolic end products that are essential to maintain cellular function. The flux distribution among the various biochemical pathways is determined by the kinetic properties of enzymes which are subject to strict regulatory control. Simulation of metabolic behavior therefore requires the complete knowledge of biochemical pathways, enzyme kinetics as well as their regulation. Unfortunately, complete kinetic and regulatory information is not available for microbial cells, thus preventing accurate dynamic simulation of their metabolic behavior. However, it is possible to define wider limits on metabolic behavior based solely on flux balances of biochemical pathways. We present here comprehensive information about the catabolic pathways of the bacterium *Escherichia coli*. Using this biochemical database, we formulate a stoichiometric model of the bacterial network of fueling reactions. After logical structural reduction, the network consists of 53 metabolic fluxes and 30 metabolites. The solution space of this under-determined system of equations presents the bounds of metabolic flux distribution that the bacterial cell can achieve. We use specific objective functions and linear optimization to investigate the capability of *E. coli* catabolism to maximally produce the 12 biosynthetic precursors and three key cofactors within this solution space. For the three cofactors, the maximum yields are calculated to be 18·67 ATP, 11·6 NADH and 11 NADPH *per* glucose molecule, respectively. The yields of NADH and NADPH are less than 12 owing to the energy costs of importing glucose. These constraints are made explicit by the interpretation of shadow prices. The optimal yields of the 12 biosynthetic precursors are computed. Four of the 12 precursors (3-phosphoglycerate, phosphoenolpyruvate, pyruvate and oxaloacetate) can be made by *E. coli* with complete carbon conversion. Conversely, none of the sugar monophosphates can be made with 100% carbon conversion and analysis of the shadow prices reveals that this conversion is constrained by the energy cost of importing glucose. Three of the 12 precursors (acetyl-coA, α-ketoglutarate, and succinyl-coA) cannot be made with full carbon conversion owing to stoichiometric constraints; there is no route to these compounds without carrying out a decarboxylation reaction. Metabolite flux balances and linear optimization have thus been used to determine the catabolic capabilities of *E. coli*.

## 1. Introduction

The chemistry, stoichiometry and regulation of metabolism is perhaps the most thoroughly studied and best known aspect of the microscopic functioning of living

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cells. Although detailed knowledge exists about the biochemistry of individual metabolic events, less is known about the systemic nature of metabolic function and how it impacts the overall function of a cell. Several attempts to systematically model metabolic dynamics have been carried out (for example, see reviews in Garfinkel *et al.*, 1970; Heinrich *et al.*, 1977; Reich & Sel'kov, 1981), but they have been hampered by the lack of kinetic and regulatory information on the function of all enzymes in a particular cell. The only cell for which a comprehensive kinetic metabolic model currently exists is the human red blood cell (Joshi & Palsson, 1989*a*, *b*, 1990*a*, *b*; Lee & Palsson, 1990). The red blood cell model enables several kinetic studies, but these investigations and the conclusions derived from them, although useful, are necessarily limited, given the highly differentiated state and specialized function of the red blood cell. On the other hand, much effort has been devoted towards the development of a theoretical framework for the analysis of metabolic regulation, mostly through the use of logarithmic sensitivity coefficients (Savageau, 1969; Kacser & Burns, 1973; Heinrich & Rapoport, 1974*b*). Extensive literature exists on this topic and useful overviews are available (Cornish-Bowden & Cardenas, 1990; Srere *et al.*, 1990). The applicability and usefulness of these theories remain to be examined within the context of realistic metabolic models, although some recent progress has been made (Palsson & Lee, 1992).

Progress on the systemic analysis of kinetic behavior of metabolic networks is thus constrained by the availability of comprehensive kinetic and regulatory information on metabolic enzymes in a single cell type. Recently, it has been shown that a flux-balance based analysis is surprisingly useful in analyzing the steady-state behavior of metabolic networks in the absence of detailed kinetic knowledge (Savinell & Palsson, 1992*a*, *b*, *c*, *d*). This approach relies only on the stoichiometry of the system under investigation and the metabolic demands that are placed on it. Within this framework one can answer questions that are related to the capability of networks to perform certain functions, while constrained by stoichiometry alone. Since intermediary metabolism—the chemical machine that drives the living process—is fairly similar in all cell types, this approach is useful to define the wider limits of the metabolic capabilities that a cell possesses. These stoichiometrically set limits are then further narrowed by the kinetic and regulatory function of metabolic enzymes. These two limits placed on metabolic function are illustrated in Fig. 1.

Here, we set out to examine and define the stoichiometrically determined metabolic capabilities of the bacterium *Escherichia coli*; first with respect to the production of biosynthetic precursors, and second with respect to making a balanced mix of cellular constituents. The *E. coli* cell is well known in terms of its chemical composition and therefore the metabolic demands that are imposed by cell growth and function can be defined. These demands have recently been thoroughly documented (Ingraham *et al.*, 1983). Unfortunately, no single source exists that compiles and documents the active metabolic pathways, with their associated stoichiometry, in *E. coli*. Since flux-based analysis is based upon a reliable knowledge of metabolic stoichiometry, in the form of the stoichiometric matrix, our first step to reach the stated goals is to compile and document our present knowledge of *E. coli* catabolic reactions. We then use the catabolic network to study the ability of *E. coli* to make

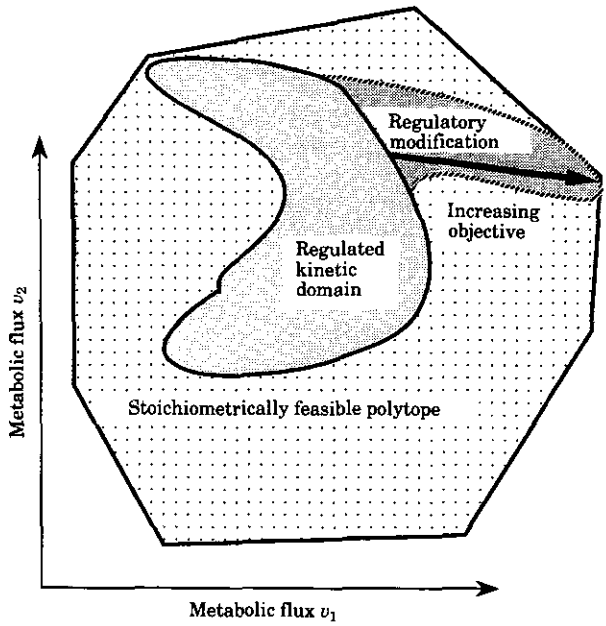


FIG. 1. The stoichiometrically feasible domain of steady-state reaction fluxes within the cell describe a wider limit of metabolic behavior. A two-dimensional region is shown to schematically illustrate the stoichiometrically feasible values of two hypothetical metabolic fluxes. The regulated domain of fluxes chosen by the cell forms a subset of the stoichiometrically feasible region.

the three key metabolic cofactors (NADH, NADPH and ATP) as well as the 12 basic biosynthetic precursor molecules during aerobic growth on glucose. We seek to determine optimal pathway utilization, and maximal yields of each metabolite and to assess the systemic constraints that *E. coli* is faced with in their production. In the subsequent part we assess *E. coli*'s ability to meet balanced growth demands.

## 2. Stoichiometric Analysis

The history of stoichiometrically based metabolic analyses is relatively short. Linear programming can be used to study the stoichiometric systemic constraints on metabolic networks (Watson, 1986). Where the number of metabolic fluxes exceeds the number of flux balances, this approach has been applied to adipocite metabolism (Fell & Small, 1986). Acetate secretion from *E. coli* under ATP maximization conditions (Majewski & Domach, 1990) and the metabolic behavior of hybridoma cells (Savinell & Palsson, 1992*a*, *b*) have been studied.

Our goal here is to apply this powerful and insightful, yet simple and biochemically reliable, approach to analyze the metabolic capabilities of *E. coli*. The reactions that lead to the synthesis of a bacterial cell can be logically divided into the categories of fueling, biosynthetic, polymerization and assembly reactions (Ingraham *et al.*, 1983; Neidhardt, 1987). The input to the fueling reactions consists of a carbon

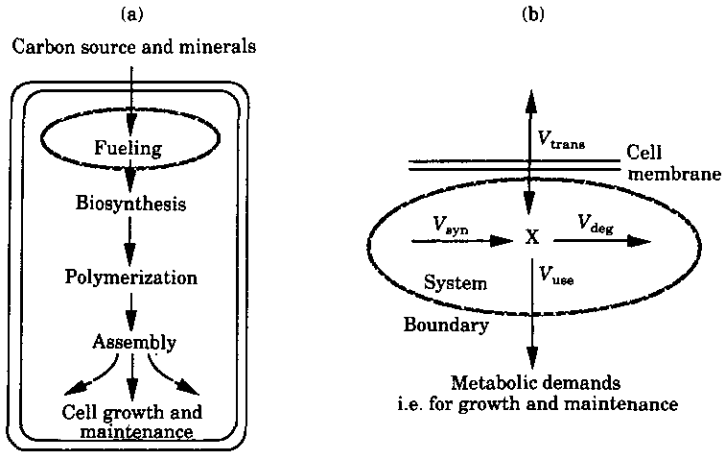


FIG. 2. (a) The fueling reactions form the system of reactions chosen for metabolic analysis. The products of the metabolic network are processed further and used for cell growth and maintenance. (b) The fluxes affecting the concentration of a metabolite, $X$, in the cell.

source and minerals. The output from the fueling reactions consists of the metabolites required for growth, maintenance and possibly secretion. The two sections of intermediary metabolism, fueling and biosynthesis, are connected by 12 such metabolites that are called the biosynthetic precursors. We can therefore logically decompose the problem under consideration into two parts. In this paper we investigate the capability of *E. coli* metabolism to produce individual biosynthetic precursors. In the following paper we study the ability of the metabolic network to meet a balanced set of demands that simulate the growth requirements on the precursor pools.

## FLUX BALANCE-BASED ANALYSIS

The general methods of flux balance-based analysis outlined in the literature (Fell & Small, 1986; Watson, 1986; Majewski & Domach, 1990; Savinell & Palsson, 1992*a*, *b*, *c*, *d*) are used. The method uses flux balances to define limits on metabolic capabilities. The governing steady-state equation is written as:

$$\mathbf{S} \cdot \boldsymbol{v} = \boldsymbol{b}, \tag{1}$$

where $\mathbf{S}$ is the stoichiometric matrix of the metabolic network, $\boldsymbol{v}$ is the vector of reaction fluxes, and $\boldsymbol{b}$ is the net output from cellular metabolism. The stoichiometric matrix used in the present analysis consists of the catabolic reactions of *E. coli*. Figure 2 depicts the fluxes affecting a metabolite. Equation (1) is typically underdetermined since the number of fluxes normally exceeds the number of metabolites. Thus, a plurality of solutions exists and a particular solution may be found using linear optimization by stating an objective and seeking its maximal value within the stoichiometrically defined domain.

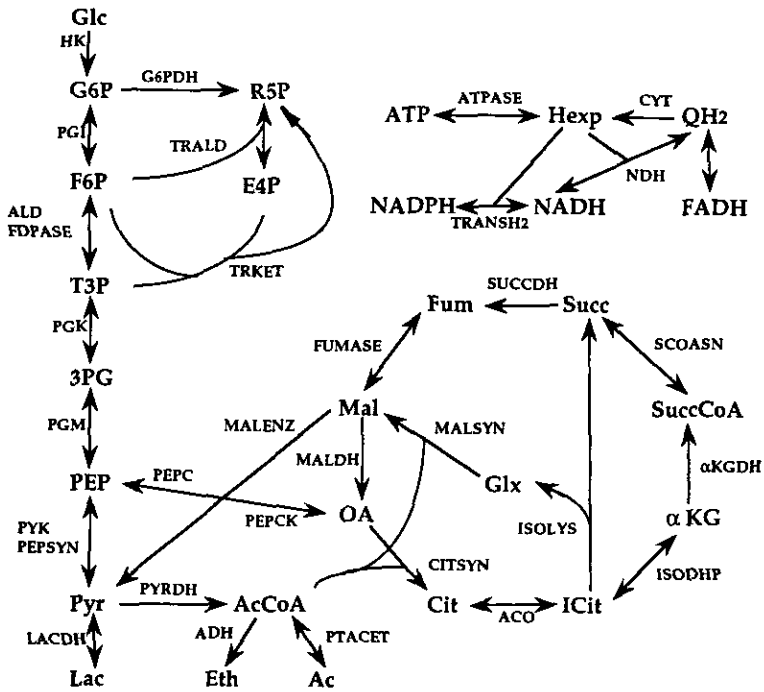


FIG. 3. The fueling reaction network of *E. coli* metabolism. The arrows represent the reaction fluxes with the enzyme abbreviation next to them.

## OBJECTIVE

An investigation of metabolic optimality can be carried out within the stoichiometrically defined domain by specifying physiologically meaningful objective functions.

For the present analysis we are interested in maximizing the production of a particular metabolite. The objective function could be represented as maximizing a drain flux, defined for the metabolite in the stoichiometric matrix as a flux causing the removal of the metabolite.

$$\text{Minimize } Z = c_i \cdot v_{i,\,drain}. \tag{2}$$

The weight $c_i$ for the drain flux for that metabolite would have an arbitrary negative value, while all other weights would have a zero value.

## SHADOW PRICES

The mathematical dual of the linear optimization problem (Murty, 1983) is also evaluated to determine the dual solution. Interpretation of the dual variables ($\gamma_i$) as

the shadow prices, eqn (3), provides a useful intrinsic measure of the value of a metabolic intermediate towards optimizing the objective.

$$\gamma_i = \frac{\partial Z}{\partial b_i}. \tag{3}$$

## 3. Network Formulation

The biochemical pathway information described in the Appendix can be presented in the mathematical form of eqn (1). Several reductions of the flux balance equations are possible without altering the mathematical nature of the problem.

### STOICHIOMETRIC REDUCTION

The stoichiometric representation of metabolism can be made compact by using simple rules, thus reducing the dimensions of S. The application of these rules does not alter the basic structure of the catabolic network. These rules are:

(i) Intermediates of reaction pathways that have only one route for generation and one route for consumption can be ignored and one flux may denote the flux producing the intermediate and the flux consuming it. For example, the set of reactions producing R5P from G6P can be represented by a single flux converting G6P to R5P.

(ii) Metabolites that are freely interconvertible, without the involvement of a third compound, can be represented by a single metabolite. An example may be found in the enzymatically convertible compounds, DHAP and G3P. They are both represented in our network as T3P.

(iii) Compounds of no interest here need not be included in the reaction network. They are then implicitly assumed to have an infinite source or sink. Thus, we do not need to keep track of $O_2$ in the reaction network. Cofactor molecules such as coenzyme A and NAD which are carriers for specific molecular species have their flux balances intrinsically balanced, and can therefore be ignored in the network. Inclusion of such metabolites only leads to the generation of a dependent row.

(iv) Although the reactions have not been described here, the high energy phosphate bond of the various nucleotides can be traded among the nucleotides. Therefore the utilization of the high energy bond associated with a non-adenosine nucleotide, as well as the second phosphate bond of adenosine phosphate, have been considered equivalent to the third phosphate bond of ATP.

Further, only physiologically occurring reactions are included. Thus, although the reaction catalyzed by pyruvate kinase is reversible *in vitro*, only the physiological forward reaction producing pyruvate is included in the network.

### THE *E. COLI* NETWORK OF FUELING REACTIONS

Applying these simple rules to the fueling reactions described above, we have derived the basic network that describes aerobic metabolism in *E. coli*, Fig. 3.

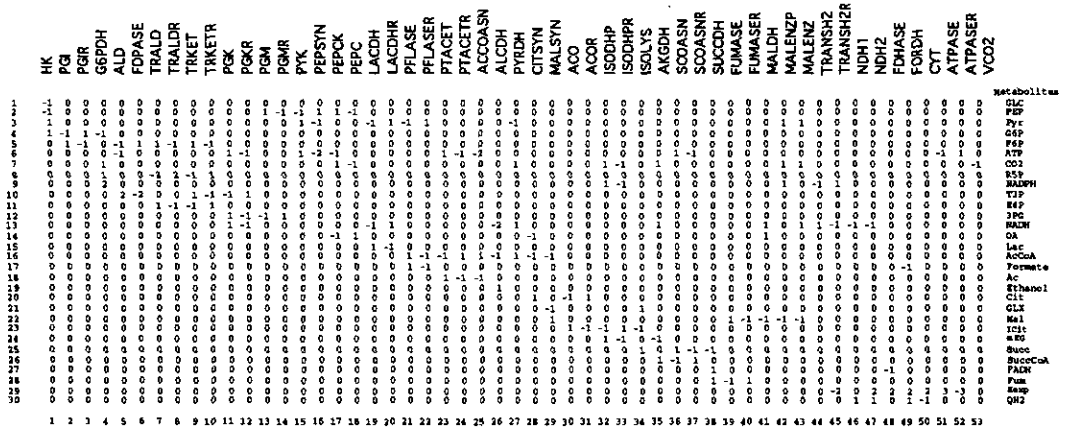


FIG. 4. The stoichiometric matrix for the fueling reactions of *E. coli*. The rows correspond to the flux balance of the metabolite indicated. The columns are the specific fluxes indicated by the enzyme abbreviation.

Reaction fluxes are denoted by letter codes which represent the enzyme involved. For cases where more than one enzyme is required for the reaction pathway shown, any one of the enzymes has been used to represent the entire pathway.

The network of catabolic reactions shown in Fig. 3 may be represented in the mathematical form of eqn (1). The stoichiometric matrix, **S**, in eqn (1) is shown in Fig. 4. The rows denote the flux balances, while the columns denote the fluxes. Included are the 30 flux balances that contain 53 fluxes.

We have restricted ourselves in the formulation of the network in Fig. 3 to the aerobic state of the cell. Aerobiosis implies that oxygen is present as the terminal electron acceptor. Therefore, only those reactions are included that are possible under aerobic conditions. For instance, reductive reactions requiring NADH as a cofactor are not allowed in this scheme. However, transhydrogenation as a means of transferring electrons between NAD and NADP is included.

## NETWORK FLEXIBILITY

A few observations about the flexibility of this stoichiometric model are in order. A particular solution for steady-state pathway fluxes can result in the generation of surplus energy or reductive power. In the network formulated above, surplus energy can be eliminated by several metabolic loops. A well-known example of a futile cycle may be found in the conversion of F6P into T3P and the reconversion back to F6P which has the net effect of hydrolyzing one high-energy phosphate bond.

Similarly, surplus energy associated with the proton gradient across the membrane can be transformed into a high-energy phosphate bond in ATP by the enzyme ATPase, and then dumped into a futile cycle. Surplus reductive power in the aerobic network can be transformed into the transmembrane proton gradient using oxygen as the electron acceptor. The transmembrane proton gradient is converted into high-

energy phosphate bonds and dissipated through the futile cycles. Of course, anerobic conditions would not allow the transformation of surplus reductive power into energy and some other sink for electrons is required.

The network can deal with a surplus of a particular metabolite by two mechanisms. Dissipation can occur through a complete oxidation to $CO_2$ and water, or by a secretion pathway that would have to be incorporated in the network. Thus, the basic network that we have synthesized and shown in Fig. 3 accounts for known fueling reactions in *E. coli*. It has the metabolic flexibility that the bacterial cell possesses.

## 4. Optimal Production of Cofactors

The cofactors ATP, NADH and NADPH play a central role in bacterial metabolism. We now use linear optimization to determine the maximal production of these cofactors. The type of objective function used has been defined in eqn (7). The solutions for maximum ATP and NADPH production are shown in Figs 5 and 6, respectively. These solutions represent the maximal capability of the *E. coli* catabolic network to produce metabolic energy and biosynthetic redox. These solutions thus represent important and fundamental biological constraints. The key characteristics of these solutions are summarized in Table 1.

We find that a maximum of 18·7 moles of ATP can be generated *per* mole of glucose oxidized to $CO_2$ and water, see Table 1. The optimal solution does not use

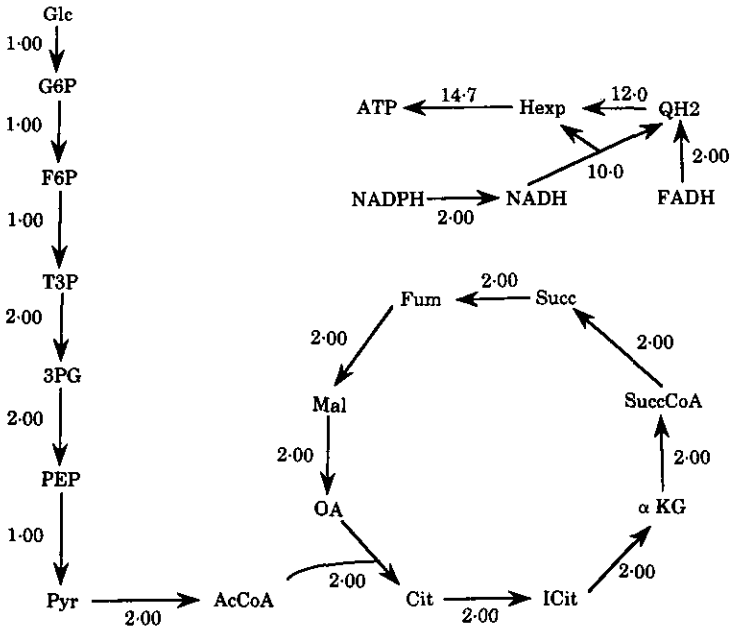


FIG. 5. Flux distribution map for maximal ATP yield. Only non-zero fluxes are depicted. The maximal yield is 18·7 ATP/molecule glucose.

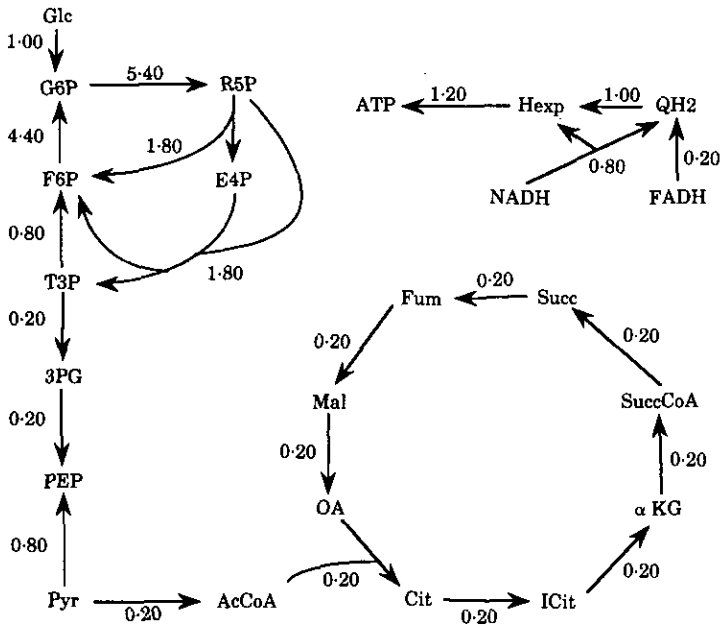


FIG. 6. Flux distribution map for maximal NADPH production. The maximal yield of NADPH is 11 molecules/molecule glucose.

the pentose phosphate pathway. The use of the TCA cycle for optimal production of ATP is consistent with the general belief that the pentose phosphate pathway functions primarily to generate pentoses and NADPH and is not cycled to produce metabolic energy in the form of ATP. The glyoxalate shunt is believed to have the function of producing TCA cycle intermediates. Consistent with this belief, the glyoxalate shunt is not used for the optimal production of ATP. Inactivating the TCA cycle and forcing the flux through the glyoxalate bypass lowers the maximum ATP yield on glucose to 16·7 mol $mol^{-1}$.

TABLE 1

*Maximum energy and reductive potential generation on glucose using* E. coli *fueling reactions. The PPS flux is given as the percentage of carbon flow through the oxidative branch of the pentose phosphate pathway with a maximum of six times the glucose input or* 600%

| Metabolite | Yield | PPS | ATP shadow price | Constraint |
|---|---|---|---|---|
| ATP | 18·667 | 0% | −1 | — |
| NADH | 11·573 | 471% | −0·214 | Energy |
| NADPH | 11·000 | 540% | −0·5 | |

The complete oxidation of glucose requires a sink for 24 electrons. Therefore, a potential reduction of 12 redox carriers can be obtained. However, in the presence of systemic constraints, the base network can produce a maximum of only 11·6 NADH. The difference can be explained based on the ATP shadow price in the optimal NADH solution:

$$\frac{\partial \text{NADH}}{\partial \text{ATP}} \simeq \frac{\Delta \text{NADH}}{\Delta \text{ATP}} = 0{\cdot}214. \tag{4}$$

Thus, the additional amount of ATP needed to make the full amount of 12 NADH from glucose is

$$\Delta \text{ATP} = \frac{\Delta \text{NADH}}{0{\cdot}214} = \frac{12 - 11{\cdot}573}{0{\cdot}214} = 2. \tag{5}$$

These two ATPs correspond to the cost of resynthesizing the PEP using PEP synthease from the pyruvate formed during glucose phosphorylation by the phosphotransferase system. Thus, when the whole network is considered, one finds that it is not possible to generate 12 reduced NADH molecules from glucose due to the cost of glucose import.

Similarly, the theoretical maximum reducing power that can be generated in the form of NADPH is 12 NADPH *per* molecule of glucose. The amount that can actually be generated considering the whole network is lower, or 11 NADPH *per* glucose molecule. As for NADH, the cost of glucose uptake prevents the basic fueling network from generating 12 molecules of NADPH. The additional ATPs required to yield the 12 NADPH molecules can be calculated from the shadow price for ATP:

$$\frac{\partial \text{NADPH}}{\partial \text{ATP}} \simeq \frac{\Delta \text{NADPH}}{\Delta \text{ATP}} \Rightarrow \Delta \text{ATP} = \frac{12 - 11}{0{\cdot}5} = 2. \tag{6}$$

Again, the recovery of PEP used for glucose import requires two ATPs. The difference in the ATP shadow price for the above two examples is due to the different optimal pathway utilizations for the production of NADPH and NADH.

From the above discussion it is apparent that energy is the constraining factor for maximal generation of reductive power. The action of the enzyme transhydrogenase is to transfer reductive power between NAD and NADP. From the above optimal solutions we note that the maximal synthesis of NADPH does not utilize transhydrogenation. Biosynthesis requires reductive power in the form of NADPH, while NADH is oxidized to produce energy under aerobic conditions (Ingraham *et al.*, 1983). The absence of a requirement of transhydrogenase for maximal NADPH generation in the catabolic network is experimentally indicated by the lack of a phenotype for transhydrogenase mutants (Zahl *et al.*, 1978).

From a study of optimal flux distributions we note that the oxidizing pathway of PPS is used only for redox generation. The optimal generation of the precursors E4P and R5P, discussed next, occurs through the non-oxidative branch. Experimentally determined pathway utilizations (Wood, 1985) also agree with these observations of optimal flux distributions.

## 5. Optimal Production of Biosynthetic Precursors

All carbon sources are degraded into a minimum set of 12 biosynthetic precursors (Ingraham *et al.*, 1983). The biosynthetic reactions of the cell utilize these precursors to produce the monomers that go into making the macromolecular constituents of the cell. We now determine the capability of the basic *E. coli* catabolic network to produce each of these precursors individually using glucose as the sole carbon source. The results from optimizing the production of each precursor molecule on glucose are summarized in Table 2. Full optimal solutions are shown in Figs 7 and 8 for the maximal production of E4P and PEP from glucose as examples of what the optimal solutions look like. The carbon conversion listed in Table 2 is the percentage of the carbon in glucose that ends up in the precursor molecule being produced.

The three glycolytic intermediates, 3PG, PEP and Pyr, can be produced with 100% carbon conversion. Their maximum yield from glucose has no energy-related constraints (the shadow price for ATP is zero) and, in fact, a surplus of energy is generated which is dissipated through a futile cycle.

The surplus ATP production is readily illustrated by an example. Consider the generation of PEP from glucose, Fig. 8. To make two PEP from glucose, two ATP are required for glucose uptake, one ATP is required for the PFK reaction and two ATP are produced by PGK. Thus, direct stoichiometric coupling of ATP to PEP production results in the consumption of one ATP. However, two NADH are also produced which subsequently yield 2·66 ATP upon oxidation through the electron transport system. Thus, an overall surplus of 1·66 ATP *per* glucose is produced. Note that this amount is dissipated via a PEP→OA→Mal→Pyr→PEP cycle whose net effect is the dissipation of two ATPs, leading to a futile cycle flux of $1{\cdot}666/2 = 0{\cdot}833$. The network possesses several other equivalent futile cycles which could be used to

TABLE 2

*Maximum stoichiometric yields of biosynthetic precursors on glucose for an aerobic non-growing cell*

| Metabolite | Yield | Carbon conversion | ATP shadow price | Constraint |
|---|---|---|---|---|
| 3PG | 2 | 100% | 0 | None |
| PEP | 2 | 100% | 0 | |
| Pyr | 2 | 100% | 0 | |
| OA | 2 | 133·3% | 0 | |
| G6P | 0·908 | 90·8% | −0·046 | Energy |
| F6P | 0·908 | 90·8% | −0·046 | |
| R5P | 1·08 | 90% | −0·055 | |
| E4P | 1·33 | 88·7% | −0·068 | |
| T3P | 1·73 | 86·5% | −0·088 | |
| AcCoA | 2 | 66·7% | 0 | Stoichiometry |
| αKG | 1 | 83·3% | 0 | |
| SuccCoA | 1 | 66·7% | 0 | |

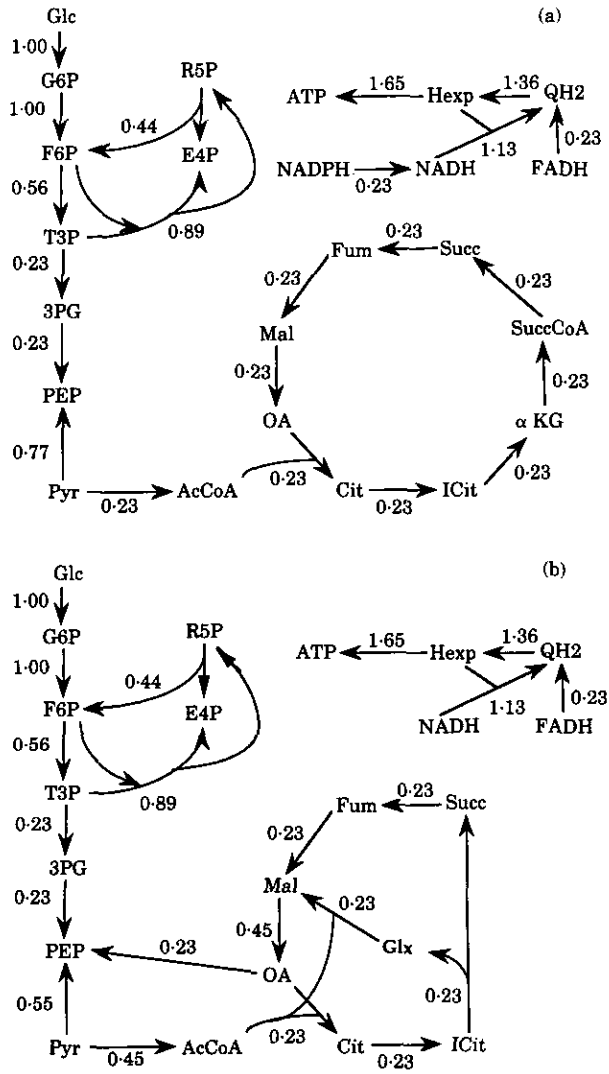


FIG. 7. Flux distribution maps for maximal E4P production. The maximal yield of E4P is 1·33 molecules/molecule glucose at an 88·7% carbon conversion. Utilization of the TCA pathway or glyoxalate shunt are shown to lead to the same maximal production of E4P.

generate the same result. We will discuss this issue further below and in the accompanying article (Varma & Palsson, 1993).

Optimal production of oxaloacetate results in a carbon recovery in excess of 100%. A carbon conversion of 133% is possible because of the $CO_2$ fixing reaction catalyzed by PEPC. There are no energy limitations and the TCA cycle is not used. In the base fueling network we do allow unlimited access to $CO_2$. Restricting the

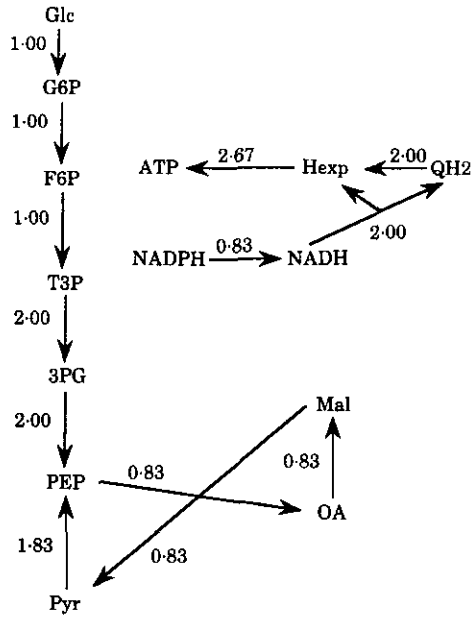


FIG. 8. Flux distribution map for maximal PEP production. The maximal yield of PEP is two molecules/molecule glucose at a 100% carbon conversion. A futile cycle converting PEP→OA→Mal→Pyr→PEP is also shown to be operative.

$CO_2$ availability reduces the carbon yield to 100%. ATP overproduction is dissipated through a futile cycle as just discussed for PEP.

## CONSTRAINTS ON THE PRODUCTION OF BIOSYNTHETIC PRECURSORS

In attempting to maximize the production of a metabolite in the network, the cell may be confronted with systemic constraints that prevent a 100% carbon conversion from substrate to metabolite. For the 12 biosynthetic precursors the cell encounters two constraints; energy or network stoichiometry. These constraints are listed in Table 2 for the biosynthetic precursors. Note that redox constraints do not appear in the production of precursors. Reductive power is primarily required for the biosynthesis of monomers (Ingraham *et al.*, 1983).

The constraint for a particular optimization can be determined by studying the solution of the dual optimization problem. Energy constraints are evidenced by nonzero shadow prices of ATP. Stoichiometric limitations are indicated by less than 100% carbon conversion and the absence of an energy constraint.

### *Energy*

The monophosphate sugars, G6P, F6P, R5P, E4P and T3P, cannot be produced at a 100% carbon conversion, Table 2. Energy is a constraint for all five cases and some carbon must be oxidized fully to provide the required energy. As illustrated

above for optimal cofactor production from glucose, the ATP shadow price brings this fact out clearly. For G6P we have that $\partial G6P/\partial ATP = 0{\cdot}046$, therefore, 100% carbon conversion requires

$$\Delta ATP = \frac{\Delta G6P}{0{\cdot}046} = \frac{1-0{\cdot}908}{0{\cdot}046} = 2 \tag{7}$$

additional molecules of ATP which correspond to the cost of importing and phosphorylating glucose. A similar calculation for T3P yields:

$$\Delta ATP = \frac{\Delta T3P}{0{\cdot}088} = \frac{2-1{\cdot}73}{0{\cdot}088} = 3. \tag{8}$$

Thus, not only are two ATPs required for PEP recovery, but also a third ATP molecule is needed in the PFK reaction. E4P and R5P are produced by the non-oxidative branch of the pentose phosphate pathway in an attempt to reduce loss of carbon through decarboxylation in the oxidative steps. Again, the reduced carbon conversion can be readily explained based on the ATP shadow price. For E4P, the additional ATP required for 100% carbon conversion is:

$$\Delta ATP = \frac{\Delta E4P}{0{\cdot}068} = \frac{1{\cdot}5-1{\cdot}33}{0{\cdot}068} = 2{\cdot}5. \tag{9}$$

This ATP requirement is consistent with the stoichiometry of E4P synthesis from glucose through the non-oxidative branch.

$$2\ \text{Glucose} \rightarrow F6P + 2T3P \rightarrow 3E4P.$$

As discussed above, F6P requires two ATPs (similar to G6P) and two T3Ps require three ATPs. Thus, five ATPs are required to synthesize three E4Ps from two glucose molecules which is equivalent to 2·5 ATP *per* glucose molecule.

The optimal production of the monophosphate sugars is therefore clearly constrained by energy. A sample flux distribution is displayed in Fig. 7(a) for E4P production. The solution shows the conversion of Pyr to PEP by the enzyme PEP-synthase. The energy required for the reaction is optimally produced by the TCA cycle coupled with the ETS. As shown earlier, the TCA cycle is the optimal pathway for the generation of ATP. However, there is an alternative pathway to convert Pyr to PEP, which is energetically equivalent. If we restrict the complete TCA cycle, the same yields for sugar monophosphates are obtained by the use of the glyoxalate shunt. A sample solution displaying the use of the glyoxalate shunt for E4P production is shown in Fig. 7(b). The flexibility of the fueling network is such that it has more than one equivalent way to make the ATPs necessary for making sugar phosphates.

This situation is one of multiple optimal solutions and the linear optimization program arbitrarily selects any one of the optimal solutions. The glyoxalate shunt provides an alternate route for the flux from Pyr to PEP using the enzyme PEP carboxykinase. Thus, the diversion of flux from the reaction catalyzed by PEP synthase results in a saving of energy which makes the net use of the glyoxalate

shunt, energetically comparable to the TCA cycle for these special cases. However, simply for the production of energy, the TCA cycle maintains an edge over the glyoxalate shunt as discussed earlier.

*Stoichiometry*

Acetyl CoA can only be produced with a 66·7% carbon conversion and the optimal solution is not constrained by energy. The catabolic network possesses only one route for generation of acetyl CoA and it is by the decarboxylation of pyruvate. Therefore, maximally a two-third carbon recovery is possible, simply due to stoichiometric limitations.

The maximal production of the TCA intermediates α-ketoglutarate and succinyl CoA is not limited by energy requirements. Yet the yield is less than 100%. Again, the stoichiometric structure of the catabolic network forces the loss of carbons in essential reactions needed for the production of α-ketoglutarate and Succ-CoA. The production of α-ketoglutarate is associated with decarboxylation in the reactions catalyzed by pyruvate dehydrogenase and isocitrate dehydrogenase. The loss of carbons is mitigated to some extent by the use of the $CO_2$-fixing anaplerotic reaction catalyzed by PEP-carboxylase. The production of succinyl CoA is associated with a further loss of carbon by decarboxylation in the reaction catalyzed by α-ketoglutarate dehydrogenase.

## 6. Discussion

Stoichiometric analysis of metabolic networks is expected to yield rich dividends in terms of systematizing knowledge of metabolic systems, presenting us with the opportunity to explore the complex biochemical process that underlies the function of living cells. In this paper we have formulated the stoichiometric matrix for the catabolic reaction pathways of *E. coli* that enables the use of linear programing to explore the boundaries of achievable metabolic performance.

Linear optimization thus allows the identification of the optimal reaction pathway utilization to fulfill specific metabolic needs of the cell, such as the production of energy, reductive potential, or biosynthetic requirements. The relative value of various metabolites and substrates in achieving an objective can be determined using the shadow prices. The objective may be cell growth or production of a particular metabolic intermediate. The optimum flux distribution for a desired goal (such as the commercial production of a metabolite) can be determined. We can thereby identify the important reactions which are subject to genetic engineering to achieve the stated goal.

A flux-based approach for the analysis of metabolic networks has several advantages. First, the information required, the stoichiometry of the cellular reactions is fairly well known for most organisms. Although rates of reactions are not considered, limited thermodynamic information is included in the form of physiological reversibility or irreversibility of the reactions. Linkage to energy in the form of the high-energy phosphate bond of ATP provides some additional thermodynamic informa-

tion. In addition, the problem formulation allows the incorporation of experimental knowledge of maximal activities of specific metabolic reactions.

There are, however, some limitations of this approach to metabolic modeling. We do not consider the regulation of enzymes catalyzing the cellular reactions. The solution obtained may therefore not be acceptable to the regulatory mechanisms of the cells. Of course, genetic engineering can be used to obtain organisms with modified metabolic regulation. The second limitation is the absence of the explicit accounting of metabolic concentrations. We are therefore unable to predict the concentrations of the metabolites within the cell. Lastly, we have not incorporated any thermodynamic information in the form of rate expressions. Time as a variable is therefore absent and we are unable to make any predictions as to the time constants of the cellular processes.

The network formulated in Fig. 3 with the stoichiometry listed in Fig. 4 displays the central catabolic pathways of *E. coli*. The possible input substrates to the network are glucose, lactate, acetate and any intermediate in the network. Any other substrate would require an extension of the network to include pathways for its degradation into one of the intermediates of the network shown. Also, the discovery of new pathways (Draths & Frost, 1991) and stoichiometries or a modification of the existing pathways may require further modifications to the network formulated here.

The set of catabolic pathways within the cell serve to degrade all substrates into a common set of biosynthetic precursors and cofactors. We have determined the capability of the bacterial catabolic network to produce these precursors and cofactors aerobically from glucose. All cellular synthesis utilizes the carbon skeletons provided by this pool of precursors, while the cofactors provide energy and redox power to the cell. The maximal achievable yields of biosynthetic precursors and cofactors, therefore, represents fundamental determinants of metabolic performance.

Formulation of the flux balance-based model of *E. coli* catabolism has enabled the determination of the capabilities of *E. coli* to make three key cofactors and the 12 biosynthetic precursors. The results from these computations thus represent fundamental systemic constraints on *E. coli* metabolism and therefore important quantities in bacterial physiology. Confirmation of optimal pathway utilization by experimental literature demonstrates the correctness of the metabolic network formulated and demonstrates the applicability of the flux balance-based method for metabolic analysis.

More complex metabolic functions, such as support for growth, can be represented as balanced sets of demands on the cofactor and biosynthetic precursor pools, which is the subject of the following paper.

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# APPENDIX A

## A1. Fueling Reactions of *E. coli*

The fueling reactions of *E. coli* have been divided into sets of standard metabolic pathways. These include the glycolytic pathway, the pentose phosphate pathway and the tricarboxylic acid (TCA) cycle with the glyoxalate shunt. Some of the intermediates of these pathways are drained off for biosynthesis. In order to replenish the metabolite pools, there exist a set of anaplerotic reactions which connect glycolytic and TCA intermediates. Finally, energy in the form of ATP, reducing power in the form of NADH and NADPH, as well as the proton gradient across the cytoplasmic membrane, are interlinked through specific reactions of the electron transfer system.

We now describe important features of the fueling reactions occurring in *E. coli*. Abbreviations used for metabolites are listed in Table A1 and abbreviations used for enzymes are listed in Tables A2 and A3. The reactions themselves are listed in Tables A4–A6. Some of the more interesting aspects of the reaction pathways in *E. coli* that are not compiled in standard literature sources are now discussed.

### A1.1 MAJOR PATHWAYS

*Glycolysis*

The Embden–Meyerhof–Parnas pathway active in *E. coli* is responsible for the degradation of sugars into pyruvate. In the process it also produces precursor metabolites, 3PG, PEP and Pyr that are used for the biosynthetic reactions. Net metabolic energy is also generated by these reactions.

TABLE A1
*Abbreviations used for the fueling metabolites*

| Full name | Abbrev. | Full name | Abbrev. |
|---|---|---|---|
| Acetate | Ac | Isocitrate | ICit |
| Acetyl coenzyme A | AcCoA | Malate | Mal |
| Acetyl phosphate | AcetP | Nicotinamide adenine dinucleotide | NADH |
| Adenosine triphosphate | ATP | | |
| α-Ketoglutarate | αKG | Nicotinamide adenine dinucleotide phosphate | NADPH |
| Citrate | Cit | | |
| Dihydroxy acetone phosphate | DHAP | Oxaloacetate | OA |
| Erythrose 4-phosphate | E4P | Phosphoenolpyruvate | PEP |
| Flavin adenine dinucleotide | FADH | 3-Phosphoglycerate | 3PG |
| Fructose 6-phosphate | F6P | Protons exported | Hexp |
| Fructose diphosphate | FDP | Pyruvate | Pyr |
| Fumarate | Fum | Ribose 5-phosphate | R5P |
| Gluconate 6-phosphate | Gu6P | Ribulose 5-phosphate | Ru5P |
| Gluconolactone 6-phosphate | GL6P | Succinate | Succ |
| Glucose | Glc | Succinyl CoA | SuccCoA |
| Glucose 6-phosphate | G6P | Triose 3-phosphate | T3P |
| Glyceraldehyde 3-phosphate | G3P | Quinone hydrogenated | QH2 |
| Glyoxalate | Glx | Xylulose 5-phosphate | X5P |
| Guanosine triphosphate | GTP | | |

TABLE A2
*Abbreviations used for the fueling enzymes*

| Full name | Abbrev. |
|---|---|
| Acetate kinase | AcK |
| Acetyl CoA synthetase | AcCoAsyn |
| Aconitase | Aco |
| Alcohol dehydrogenase | AlcDH |
| Adenosine triphosphatase | ATPase |
| Citrate synthase | CitSyn |
| Cytochrome oxidase complex | Cyt |
| Enolase | Eno |
| Fructose 1,6-diphosphate aldolase | Ald |
| Fructose 1,6-diphosphatase | FDPase |
| Formate dehydrogenase | ForDH |
| Fumarase | Fumase |
| Fumarate reductase | FumRed |
| Glucose 6-phosphatase | G6Pase |
| Glucose 6-phosphate dehydrogenase | G6PDH |
| Glycerate 3-phosphate dehydrogenase | G3PDH |
| Hexokinase | HK |
| Isocitrate dehydrogenase (NADH) | IsoDH |
| Isocitrate dehydrogenase (NADPH) | IsoDHP |
| Isocitrate lyase | IsoLys |
| α-Ketoglutarate dehydrogenase | AKGDH |

*Pentose phosphate shunt*

The primary function of the pentose phosphate shunt (PPS) is to provide the biosynthetic precursors, R5P and E4P, which are required for the synthesis of macromolecules essential to growth. These precursors can either be produced via the decarboxylating, oxidative pathway or through the non-oxidative pathways. The non-oxidative rearrangement of the glycolytic sugar monophosphates to the PPS sugar monophosphates represents the simplest possible mechanism (Melendez-Hevia & Isidoro, 1985).

A cyclical operation of the pentose phosphate pathway could lead to the complete degradation of sugar. Some cyclic use of the shunt has been postulated for a wild-type strain (Katz & Rognstad, 1967). However, phosphofructokinase mutants are almost completely unable to grow on glucose (Daldal *et al.*, 1982), leading one to conclude that the PPS does not function cyclically. Another important function of the PPS is the production of reducing power in the form of NADPH with a concomitant decarboxylation. However, the PPS is not the only source of NADPH (Csonka & Fraenkel, 1977) and the reactions catalyzed by transhydrogenase, isocitrate dehydrogenase and malic enzyme can also supply *E. coli* with NADPH.

*Tricarboxylic acid (TCA) cycle*

The TCA cycle is a well-known mechanism for the generation of energy under aerobic conditions. There are several reviews of the cycle (Krebs & Johnson, 1937; Kornberg, 1959; Lowenstein, 1967) and all the enzymes and reactions are well

TABLE A3
*Abbreviations used for the fueling enzymes*

| Full name | Abbrev. |
|---|---|
| Lactate dehydrogenase | LacDH |
| Malate dehydrogenase | MalDH |
| Malate synthase | Malsyn |
| Malic enzyme (NADH) | MalEnz |
| Malic enzyme (NADPH) | MalEnzP |
| NADH dehydrogenase ($2H^+$ exported) | NDH1 |
| NADH dehydrogenase | NDH2 |
| Phosphoenol pyruvate carboxylase | PEPC |
| Phosphoenol pyruvate carboxykinase | PEPCK |
| Phosphoenol pyruvate synthase | PEPsyn |
| Phosphofructokinase | PFK |
| Phosphoglucokinase | PGK |
| 6-Phosphogluconate dehydrogenase | GL6PDH |
| 6-Phosphogluconolactonase | PGLase |
| Phosphoglucose isomerase | PGI |
| Phosphogluco mutase | PGluM |
| Phosphoglycerate mutase | PGM |
| Phosphotransacetylase | PTAcet |
| Phosphotransferase system | PTS |
| Pyruvate dehydrogenase | PyrDH |
| Pyrivate formate lyase | PFLase |
| Pyruvate kinase | PyK |
| Ribose phosphate epimerase | RPE |
| Ribose 5-phosphate isomerase | RPI |
| Succinate dehydrogenase | SuccDH |
| Succinyl CoA synthase | SCoAsn |
| Transaldolase | TrAld |
| Transhydrogenase | TransH2 |
| Transketolase | TrKet |
| Triose phosphate isomerase | TPI |

known. Under anerobic conditions the TCA cycle is found to be repressed in *E. coli* (Gray *et al.*, 1966), and functions only to the extent required to produce precursors.

*Glyoxalate shunt*

The tricarboxylic acid cycle operating cyclically can completely oxidize acetate to carbon dioxide without the consumption or production of the intermediates. Intermediates of the TCA cycle such as oxaloacetate and α-ketoglutarate are consumed in the production of macromolecules. Replenishment of the intermediate $C_4$-acids is the function of the glyoxalate shunt (Kornberg & Krebs, 1957). The glyoxalate shunt is activated during growth on acetate.

*Anaplerotic reactions*

Bacterial growth requires pathways for the replenishment of TCA cycle intermediates drained off for biosynthesis and there exist pathways that accomplish the generation of TCA cycle intermediates during growth on glucose. Similarly, there are

TABLE A4

*Glycolytic, anaplerotic reactions and the electron transport system*

| Enzyme | Reaction catalyzed | Source |
|---|---|---|
| PTS | Glc + PEP → G6P + Pyr | Kundig *et al.* (1964) |
| PGI | G6P ⇌ F6P | Fraenkel & Levisohn (1967) |
| PFK | F6P + ATP → FDP + ADP | Blangy *et al.* (1968) |
| ALD | FDP ⇌ G3P + DHAP | Doelle *et al.* (1974) |
| FDPase | FDP → F6P + Pi | Fraenkel & Horecker (1965) |
| TPI | DHAP ⇌ G3P | Cooper & Anderson (1970) |
| G3PDH | T3P + NAD ⇌ 1,3DPG + NADH | Hillman & Fraenkel, (1975); Irani & Maitra (1977) |
| PGK | 1,3DPG + ADP ⇌ 3PG + ATP | |
| PGM | 3PG ⇌ 2PG | Hillman & Fraenkel (1975); Irani & Maitra (1977) |
| Eno | 2PG ⇌ PEP | Hillman & Fraenkel (1975); Irani & Maitra (1977) |
| PyK | PEP + ADP → Pyr + ATP | Pertierra & Cooper (1977) |
| PyrDH | Pyr + NAD → AcCoA + NADH + $CO_2$ | |
| AcCoAsn | Ac + ATP + CoA → AcCcA + AMP + PPi | Brown *et al.* (1977) |
| AcK | Ac + ATP ⇌ AcetP + ADP | Rose *et al.* (1954) |
| PTAcet | AcetP + CoA ⇌ AcCoA + Pi | Shimizu *et al.* (1969); Suzuki (1969) |
| PEPC | PEP + $CO_2$ → OA + Pi | |
| PEPCK | OA + ATP → PEP + $CO_2$ + ADP | Kornberg (1965, 1970) |
| MalEnzP | Mal + NADP → Pyr + NADPH + $CO_2$ | Kornberg (1965, 1970) |
| MalEnz | Mal + NAD → Pyr + NADH + $CO_2$ | Kornberg (1965, 1970) |
| PEPsyn | Pyr + ATP ⇌ PEP + AMP + Pi | Cooper & Kornberg (1965) |
| ATPase | ATP ⇌ ADP + 3Hexp + Pi | Kashket (1982, 1983); Maloney (1987) |
| Cyt | $QH_2 + \frac{1}{2}O_2$ → Q + $H_2O$ + 2Hexp | Lawford & Haddock (1973); Matsushita *et al.* (1984) |
| TransH2 | NADPH + NAD → NADP + NADH | Bragg *et al.* (1972); Cox & Gibson (1974); Skulachev (1970); Voordouw *et al.* (1983); Zahl *et al.* (1978) |
| TransH2R | NADH + NADP + 2Hexp → NADPH + NAD | Bragg *et al.* (1972); Cox & Gibson (1974); Skulachev (1970); Voordouw *et al.* (1983); Zahl (1978) |
| NDH1 | NADH + Q → NAD + QH2 + 2Hexp | |
| NDH2 | NADH + Q → NAD + QH2 | Poole & Haddock (1974) |

TABLE A5

*Pentose phosphate pathway reactions*

| Enzyme | Reaction catalyzed | Source |
|---|---|---|
| G6PDH | G6P + NADP → GL6P + NADPH | Fraenkel & Vinopal (1973) |
| PGLase | GL6P → Gu6P | Fraenkel & Vinopal (1973) |
| GL6PDH | Gu6P + NADP → Ru5P + NADPH + $CO_2$ | Fraenkel & Vinopal (1973) |
| RPE | X5P ⇌ Ru5P | |
| RPI | Ru5P ⇌ R5P | |
| TrAld | 2R5P ⇌ F6P + E4P | |
| TrKet | R5P + E4P ⇌ F6P + T3P | Josephson & Fraenkel (1969) |

TABLE A6
*Tricarboxylic acid cycle reactions*

| Enzyme | Reaction catalyzed | Source |
|---|---|---|
| CitSyn | $OA + AcCoA \rightarrow Cit$ | Sanwal (1970) |
| Aco | $Cit \rightleftharpoons ICit$ | |
| IsoDHP | $ICit + NADP \rightleftharpoons \alpha KG + NADPH + CO_2$ | LaPorte & Koshland (1982); Nimmo (1984) |
| AKGDH | $\alpha KG + NAD \rightarrow SuccCoA + NADH + CO_2$ | Amarsingham & Davis (1965) |
| ScoAsn | $SuccCoA + GDP + Pi \rightleftharpoons Succ + GTP$ | |
| SuccDH | $Succ + FAD \rightleftharpoons Fum + FADH$ | Hirsch *et al.* (1963) |
| Fumase | $Fum \rightleftharpoons Mal$ | |
| MalDH | $Mal + NAD \rightleftharpoons OA + NADH$ | |
| IsoLys | $ICit \rightarrow Succ + Glx$ | |
| Malsyn | $Glx + AcCoA \rightarrow Mal$ | Dixon *et al.* (1960) |

pathways for the conversion of TCA intermediates into glycolytic intermediates which are active during growth on TCA intermediates. These are the anaplerotic reactions and they are summarized in Table A4. During growth on acetate, the glyoxalate shunt generates the necessary TCA intermediates.

TCA intermediate generation from the glycolytic metabolites is accomplished by the irreversible carbon dioxide-fixing conversion of PEP to OA catalyzed by the enzyme phosphoenolpyruvate carboxylase (PEPC). A different enzyme phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the reverse reaction with the concomitant consumption of a high-energy phosphate bond.

Another TCA cycle intermediate, malate, can be converted to pyruvate through the action of malic enzyme. This reaction reduces one molecule of NADP to NADPH. A second malic enzyme exists that can use NAD as a cofactor.

Although the reactions catalyzed by the enzymes PEPCK and malic enzyme are reversible, physiologically they are found to operate only unidirectionally. Evidence for the unidirectional operation, PEPCK producing PEP and malic enzyme producing pyruvate, comes from the observation that mutants of enterobacteriaceae deficient in PEPC are able to grow on $C_4$-compounds but not on $C_3$-compounds (Kornberg, 1965, 1970). Furthermore, mutants deficient in PEPCK are able to grow on $C_3$-compounds but not on $C_4$-compounds.

It should be noted that some of the reactions result in the fixation of carbon dioxide in the bacterial cell. In fact, carbon dioxide has been found to be essential to the growth of the cell (Valley & Rettger, 1927; Repaske & Clayton, 1978). Normally, cells pick up the necessary carbon dioxide from the carbonate buffer in the media.

The growth of *E. coli* on pyruvate or lactate requires the production of PEP from pyruvate. An enzyme responsible for the conversion of Pyr to PEP, PEP synthase (PEPSYN) has been isolated and the reaction stoichiometry determined (Cooper & Kornberg, 1965). The reaction consumes two high-energy phosphate bonds.

Growth on $C_4$-dicarboxylic acids such as malate requires that the cell be able to produce PEP for gluconeogenesis as well as to produce pentoses and certain amino acids. There are two pathways existing to fulfill these PEP demands (Hansen & Juni, 1974, 1975). One pathway involves the conversion of malate to pyruvate by malic

enzyme followed by the synthesis of PEP from pyruvate by PEPSYN. The other pathway is the conversion of OA to PEP by the action of PEPCK. Although malic enzyme can use either NAD and NADP as a cofactor, it is observed that in the growth on $C_4$-dicarboxylic acids only the malic enzyme with NAD as a cofactor can be used. The reason is probably because any surplus NADPH produced cannot easily give up electrons via the electron transfer system (Hansen & Juni, 1974). It appears that NADPH is produced only to the extent required for biosynthesis.

### A2. ELECTRON TRANSFER SYSTEM (ETS) AND DEHYDROGENASES

The ETS in *E. coli* produces the bulk of the cell's ATP under aerobic conditions. Here, we summarize what is known about the stoichiometry of the key enzymes in the ETS.

#### *Adenosine triphosphatase (ATPase)*

F0F1-type ATPases can be found in the membranes of bacteria as well as chloroplasts and mitochondria. The structure and mechanism of ATPase has been well described in some recent review articles (Mitchell, 1985; Senior, 1990; Penefsky & Cross, 1991). ATPase causes the reversible translocation of protons across a membrane against a proton gradient at the cost of dephosphorylating an ATP to ADP. The net reaction is:

$$ATP \rightleftharpoons ADP + P_i + nH^+ \text{ exported.}$$

A number of studies have been reported in literature on the stoichiometry of this reaction to determine the parameter $n$; *E. coli* during anerobic growth (Kashket, 1983), *E. coli* during aerobic growth (Kashket, 1982), submitochondrial particles (Berry & Hinkle, 1983), spinach chloroplast thylakoids (McCarty & Portis, 1976; Portis & McCarty, 1976). Most authors have found the value of $n$ to be close to $3H^+/ATP$. Therefore, the integral value of $3H^+/ATP$ will be used here as the experimental methods used do not allow the accurate fractional determination of the stoichiometry (Maloney, 1987).

#### *Cytochrome oxidase system*

Of the four to five major classes of cytochrome oxidases, cytochrome-o is the most widespread (Poole, 1983). It is a tightly membrane-bound complex and has been purified from a cytochrome-d deficient mutant of *E. coli* (Matsushita *et al.*, 1984). Cytochrome oxidase complexes catalyze the transfer of electrons to electron acceptors (usually $O_2$), while at the same time translocating protons across the cytoplasmic membrane against a proton gradient. The overall reaction may be represented as:

$$QH_2 + 1/2O_2 \rightarrow Q + H_2O + 2H^+ \text{ exported.}$$

Studies of succinate oxidation (Lawford & Haddock, 1973) and the use of reconstituted vesicles (Matsushita *et al.*, 1984) have determined some ranges for the stoichiometry of proton translocation. Based on these ranges we have used an

integral stoichiometry of $2H^+/O$ for the overall reaction of the cytochrome oxidase complex.

*Transhydrogenase*

Transhydrogenase found in the inner membrane of mitochondria, the cytoplasmic membrane of some bacteria and the cytoplasm of some heterotrophic bacteria catalyzes the reversible transfer of a hydride ion between NADP and NAD. The cytoplasmic transhydrogenase is non-energy linked, while the membrane-bound enzyme is energy linked and causes the simultaneous flow of protons across the membrane:

$$\text{NADPH} + \text{NAD} \rightarrow \text{NADP} + \text{NADH}$$

$$\text{NADP} + \text{NADH} + 2\text{H}^+ \text{ exported} \rightarrow \text{NADPH} + \text{NAD}.$$

Some mechanisms for the energy linkage have been reviewed (Skulachev, 1970). Based on studies of the degree of reduction of the NADH and NADPH pools, it has been suggested that the non-energy linked transhydrogenase catalyzes the transfer of the hydride from NADPH to NAD, while the energy-linked enzyme transfers the hydride from NADH to NADP (Cox & Gibson, 1974; Voordouw *et al.*, 1983). The repression of *E. coli* transhydrogenase activity by mixture of amino acids also suggests that the role of the enzyme in *E. coli* is to produce NADPH for biosynthesis (Bragg *et al.*, 1972). Transhydrogenase from *E. coli* has been cloned, partially characterized and reconstituted (Clarke & Bragg, 1985*a*, *b*). The enzyme was thought to have an energy linked as well as non-energy linked activity. It has been observed that a single mutation leads to a loss of transhydrogenase activity in *E. coli* (Zahl, 1978). Therefore, it is likely that there is only one transhydrogenase enzyme in *E. coli* and that the enzyme activity is energy linked. However, there are some reversible dehydrogenases in the bacterium which are not very specific about the cofactor (NAD or NADP). Activity of these enzymes can result in a net non-energy linked transhydrogenation from NADPH to NAD.

*NADH dehydrogenase*

NADH dehydrogenase is located in the cytoplasmic membrane of *E. coli*. It performs the important function of transferring reducing equivalents generated by the central catabolic reactions to the membrane-bound energy-generating system. NADH dehydrogenase from *E. coli* has been synthesized *in vitro* and the nucleotide sequence and, hence, the amino acid sequence, have been determined (Poulis *et al.*, 1981). The enzyme has been found to occur with or without a proton translocating capability.

The evidence for the loss of proton translocating capability comes from measurements of the $H^+/O$ stoichiometries. Under sulfate limitation, the bacterium has been found to synthesize NDH2, the non-proton translocating enzyme (Poole & Haddock, 1974). Therefore, it has been suggested that *E. coli* contains only one NADH dehydrogenase and that the loss of the proton translocation capability represents the loss of associated iron-sulfur containing compounds.