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Model Complexity has a Significant Effect on the Numerical Value and Interpretation of Metabolic Sensitivity Coefficients

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Logarithmic sensitivity coefficients were promulgated for the analysis of metabolic regulation about 20 years ago. Interest in their use has risen significantly since their introduction. However, no comprehensive evaluation of the utility of these metabolic sensitivity coefficients is available for realistic metabolic models. In this study, logarithmic sensitivity coefficients calculated from three progressively simpler metabolic models of red blood cell metabolism were compared. Two simpler models were obtained from a comprehensive red cell model by first removing volume regulation, and second by removing two pathways. The comparisons of sensitivity coefficients obtained for these three models showed that model complexity has significant effects on the numerical values and interpretation of the metabolic sensitivity coefficients. Additionally, it was found that the physiochemical volume regulatory mechanism, namely electroneutrality and osmotic balances, play an important role in the red cell metabolic flux control. In general, there is no proportionate relationship between sensitivity coefficients calculated from the three different red cell metabolic models or other simple red cell models reported in the early literature. Some sensitivity coefficients determined by different models even have opposite signs. Thus, analysis of incomplete metabolic models can be seriously misleading and produce inappropriate indicators of the characteristics of a full model for the same metabolic network.

Introduction

Mathematical models of metabolism are of much contemporary interest and practical importance. In particular, recent efforts to direct metabolic fluxes for commercial production of primary or secondary metabolites have emphasized the need for a systematic analysis of metabolic networks (Bailey, 1991; Stephenopoulos & Vallino, 1991). Efforts to model metabolism mathematically date back at least 20 years. In the late 1960s and early 1970s various metabolic flux sensitivities and measures were defined (Savageau, 1969; Kacser & Burns, 1973; Heinrich & Rapoport, 1974) and they have gained widespread acceptance. These sensitivity coefficients have been discussed extensively in the current literature (for example, Srere *et al.*, 1989; Cornish-Bowden & Cardenas, 1990). All the abbreviations used in the paper are defined in Table 1.

Although these metabolic sensitivity coefficients have been studied theoretically and for an abstract model system, no comprehensive evaluation of their utility and shortcomings for realistic metabolic models has been reported. This void is in part a result of the lack of comprehensive realistic kinetic metabolic models that would

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serve as a basis for such an evaluation. However, recently a mathematical model for red blood cell metabolism has been formulated that accounts for all major metabolic events in this cell; including glycolysis, pentose pathway, nucleotide synthesis, transmembrane transport of key ions, magnesium complexation, pH dependence, hemoglobin binding, electroneutrality, and osmotic balance (Joshi & Palsson, 1989, 1990; Lee & Palsson, 1990). The first steps toward a similar model for yeast have also been undertaken (Galazzo & Bailey, 1990).

The red blood cell model thus provides a suitable forum to address elementary questions associated with the use and interpretation of the metabolic sensitivity coefficients. One primary issue in the formulation of insightful and useful dynamic metabolic models is the assessment of which biochemical and physiochemical processes need to be included in a model in order to make it realistic. It is thus important to address the question of how the metabolic sensitivity coefficient is affected by model complexity in order to determine if such coefficients evaluated from partial models serve a useful purpose. This issue is particularly easy to address with the red blood cell model, given its comprehensive accounting of all metabolic processes that underlie the red cell function. We can simply systematically remove metabolic and other processes from the full model to form sub-models and examine how the sensitivity coefficients change with the exclusion of known pathways or transmembrane processes. Here, we will undertake such a study and proceed in two stages; first we will eliminate the physiochemical processes that couple red blood cell metabolism to the extracellular environment (namely, the balancing of osmotic pressure and electroneutrality), and second, we will remove metabolic pathways. In the latter case, we will simplify the models to glycolysis only so that we can compare the full model to the first red cell models that were published in the 1970s that accounted for glycolysis only (for example, Heinrich *et al.*, 1977).

Given the highly specialized nature of the red blood cell, it is likely that the particular results obtained have little bearing on the analysis of metabolism in complex mammalian cells or in commercially important organisms. However, we can effectively address the issue of the effects of model complexity on the numerical value and interpretation of the metabolic sensitivity coefficients using the red cell model.

Mathematical Model and Numerical Methods

FULL MATHEMATICAL MODEL

The mathematical model of red cell metabolism used in this study is described in Joshi & Palsson (1989, 1990) and Lee & Palsson (1990). This model accounts for glycolysis, pentose pathway, nucleotide synthesis, transmembrane transport of key ions, magnesium complexation with adenine nucleotides, pH dependence of kinetic parameters, binding of oxygen, carbon dioxide, and metabolites to hemoglobin, internal and external electroneutrality, and osmotic balance (Fig. 1). The model accounts for 33 metabolitic balances that contain 41 enzymatic reactions (Table 1), two physiochemical constraints (electroneutrality and osmotic balance), and several chemical equilibria. The mathematical description of the red blood cell metabolic

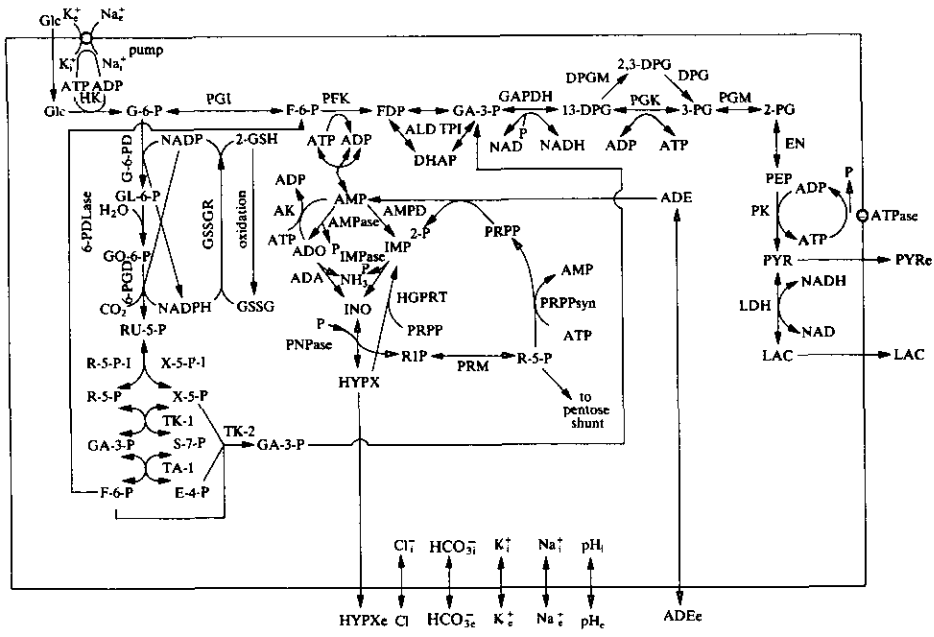

FIG. 1. The red blood cell metabolic model studied here included glycolysis, pentose pathway, nucleotide synthesis, cation transports, magnesium complexation, pH dependence, hemoglobin binding, electroneutrality, and osmotic balance.

model thus consists of a system of 33 ordinary differential equations, that have 33 metabolite concentrations as the dependent variables, and a set of coupled algebraic equations that describe the chemical and physiochemical constraints. The metabolic model can predict experimental data of the physiological steady state of red cell metabolism (Joshi & Palsson, 1990) and dynamic response of the red cell to an extracellular pH perturbation (Lee & Palsson, 1990). It also simulates: the red cell metabolic response to transient changes in the extracellular environment, such as glucose, adenine, phosphate, lactate concentrations, pH, and osmolarity; the hemoglobin binding curves at different ligand concentrations, such as 2,3-DPG, oxygen, carbon dioxide, chloride, and hydrogen ion. Details of the computer simulator are described in Lee & Palsson, 1992).

Separate subroutines are used to evaluate individual kinetic rate laws, physiochemical processes, and chemical equilibrium constraints in the red blood cell metabolic model. To ignore particular enzymes or processes, the corresponding subroutines can simply be removed from the simulator and the variables so eliminated are given fixed numerical values that are treated as constants.

SIMPLIFIED MODELS

Two different sub-models derived from the full model just described are formulated in order to study the effects of model complexity on the sensitivity coefficients

TABLE I

Red cell intermediates and enzymes accounted for in the full model

Metabolic intermediates	Abbreviation	Enzymes	Abbreviation
Glucose-6-phosphate	G-6-P	Hexokinase	HK
Fructose-6-phosphate	F-6-P	Phosphoglucoisomerase	PGI
Fructose 1,6-diphosphate	FDP	Phosphofructokinase	PFK
Dihydroxyacetone phosphate	DHAP	Aldolase	ALD
Glyceraldehyde-3-phosphate	GA-3-P	Triose phosphate isomerase	TPI
3-Phosphoglycerate	3-PG	Glyceraldehyde phosphate dehydrogenase	GAPDH
2-Phosphoglycerate	2-PG	Phosphoglycerate kinase	PGK
Phosphoenolpyruvate	PEP	Diphosphoglycerate mutase	DPGM
Pyruvate	PYR	Diphosphoglycerate phosphatase	DPGase
Lactate	LAC	Phosphoglyceromutase	PGM
1,3-Diphosphoglycerate	1,3-DPG	Enolase	EN
2,3-Diphosphoglycerate	2,3-DPG	Pyruvate kinase	PK
Nicotinamide adenine dinucleotide	NAD	Lactate dehydrogenase	LacDH
Adenosine tri-phosphate	ATP	Pyruvate export	PYRex
Adenosine di-phosphate	ADP	Lactate export	LACex
Adenosine mono-phosphate	AMP	Adenosine monophosphate phosphohydrolase	AMPase
Adenosine	ADO	Adenosine deaminase	ADA
Adenine	ADE	Adenosine kinase	AK
6-Phosphoglucono-δ-lactone	GL-6-P	Adenylate kinase	ApK
6-Phosphogluconate	GO-6-P	Adenosine monophosphate deaminase	AMPDA
Nicotinamide adenine phosphate	NADP	Adenosine triphosphate phosphohydrolase	ATPase
Glutathione	GSH	Adenine phosphoribosyl transferase	AdPRT
Ribulose-5-phosphate	RU-5-P	Glucose-6-phosphate dehydrogenase	G-6-PDH
Ribose-5-phosphate	R-5-P	6-Phosphogluconolactonase	PGLase
Xylulose-5-phosphate	X-5-P	6-Phosphogluconate dehydrogenase	PG-6-DH
Sedoheptulose-5-phosphate	S-7-P	Glutathione reductase	GSSGR
Erythrose 4-phosphate	E4P	Glutathione oxidase	GSHR
5-Phosphoribosyl-1-pyrophosphate	PRPP	Ribose-5-phosphate isomerase	R-5-P-I
Inosine mono-phosphate	IMP	Xylulose-5-phosphate epimerase	Xu-5-P-I
Inosine	INO	Transketolase	TK1
Hypoxanthine	HK	Transketolase	TK2
Ribose-1-phosphate	R-1-P	Transaldolase	TA1
Potassium	K	Inosine monophosphatase	IMPase
Sodium	Na	Purine nucleoside phosphorylase	PNPase
		Phosphoribomutase	PRM
		Phosphoribosyl pyrophosphate synthetase	PRPPsyn
		Hypoxanthine-guanine phosphoryl transferase	HGPRT
		Hypoxanhine export	HXex
		Leak of potassium out of the red cell	Leak K
		Leak of sodium into the red cell	Leak Na
		Sodium/potassium pump	Pump

TABLE 2

Three models of red cell metabolism are considered in this study. Model 1 is the full model. The physicochemical processes are removed in model 2, and model 3 consists of only glycolysis

Process	Model 1	Model 2	Model 3
Glycolysis	Yes	Yes	Yes
Pentose pathway	Yes	Yes	No
Nucleotide synthesis	Yes	Yes	No
Physicochemical processes	Yes	No	No

(Table 2). Model 1 is the full model described above and it serves as "control" or reference to compare the sub-models to. We examine the effects of the physiochemical constraints by removing them from the full model and from model 2. In model 3, all metabolic pathways are eliminated except glycolysis. Model 3 serves to evaluate interpretations of metabolic sensitivity coefficients in early red cell models (Heinrich *et al.*, 1977).

EVALUATION OF THE SENSITIVITY COEFFICIENTS

The sensitivity coefficients of primary interest are defined as

$$C_{E_k}^{J_i} = \frac{\partial J_i / J_i}{\partial E_k / E_k} = \frac{\partial \ln J_i}{\partial \ln E_k}. \tag{1}$$

This quantity is known as the control coefficient. The sensitivity coefficient $C_{E_k}^{J_i}$ is a measure of how the flux through reaction i, J_i, changes as a result of the change in enzyme concentration E_k. Thus, the coefficient indicates how sensitive a particular flux is to the change in the amount of a particular enzyme. Therefore, the coefficient $C_{E_k}^{J_i}$ is potentially useful for the purpose of identifying which enzymes are good choices for genetic manipulation in order to achieve the overproduction of a particular metabolite. Although these sensitivity coefficients are insightful and were originally based on intuitive arguments, they are somewhat awkward in a strict mathematical sense (Reder, 1988).

The values of $C_{E_k}^{J_i}$ can be estimated numerically by using a limiting procedure

$$\frac{\partial J_i / J_i}{\partial E_k / E_k} = \lim_{\Delta E_k \to 0} \frac{\Delta J_i / J_i}{\Delta E_k / E_k} \tag{2}$$

where $\Delta E_k = E_{k1} - E_{k2}$ is the difference of two concentrations of enzyme E_k, and $\Delta J_i / J_i$ is the difference between the two steady-state fluxes corresponding to the two enzyme concentrations. To calculate $C_{E_k}^{J_i}$ according to eqn (2), the value of $C_{E_k}^{J_i}$ is determined with decreasing values of $\Delta E_k / E_k$. Our experience with the red cell model shows that the sensitivity coefficient approaches a constant value relatively quickly

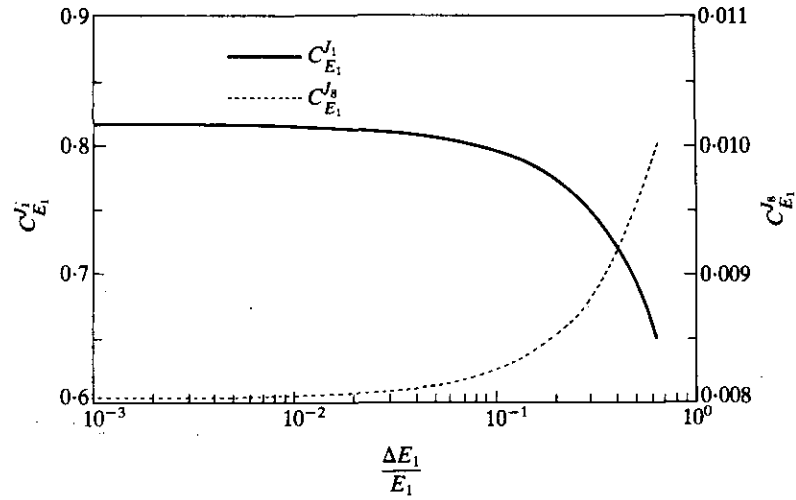

FIG. 2. Calculation of the sensitivity coefficient $C^{J_i}_{E_k}$ in model 3. It converges as $\Delta E_k/E_k$ approaches 0.

(see examples in Fig. 2). Some steady-state metabolic fluxes J_i's in the red cell model have magnitudes as small as 10^{-6}. To obtain reasonable accuracy for the evaluation of the difference between two small fluxes, ΔJ_i's, the error criterion for the fluxes, J_i's, must be set to at least three or four orders of magnitude lower than their own values. In our calculation, convergence of the sensitivity coefficients to 0·1% error is achieved by setting the absolute error criterion for J_i's to 10^{-10}. To gain a better accuracy of the sensitivity coefficients, the error criterion for the Newton's routine must be lower in order to prevent numerical instability.

Since 41 enzymatic reactions are involved in the red cell metabolic network, there are 41×41 sensitivity coefficients that characterize the full model. A useful verification of the accuracy of the calculation is to check if the summation theorem (Kacser & Burns, 1973; Heinrich & Rapoport, 1974):

$$\sum_{k=1}^{41} C^{J_i}_{E_k} = 1 \tag{3}$$

holds. All such summations for individual J_i's are within 1% derivation from unity, with one exception only (Table 3). HGPRT in the full model gives a slightly larger deviation from unity, because HGPRT is a very low flux reaction.

Results

We will now describe the effects of model simplification on the numerical values of the sensitivity coefficients. It is important to point out that the enzyme kinetic rate laws used for a particular enzyme are identical in every model.

MODEL 1 VS. MODEL 2

Osmotic balancing and electroneutrality significantly influence the values of the sensitivity coefficients, and consequently the interpretation based on these coefficients. The difference between the two models is that model 2 lacks the physiochemical constraints of electroneutrality and osmotic balance. The physiological function of these constraints is to balance ionic charges and osmotic pressure across the cell membrane and thus to regulate the cell volume and the Donnan ratio. Comparisons between the sensitivity coefficients $C_{E_k}^{J_i}$ calculated from model 1 and model 2 are shown in Fig. 3. In each diagram, one enzyme concentration is varied, and the resulting change in the 41 metabolic fluxes is calculated and plotted in the form of $C_{E_k}^{J_i}$.

The following interpretations of red cell metabolic dynamics are based on the control coefficients calculated from model 2. The same conclusions are not derived from model 1, implying that misleading information may be obtained from simpler models:

(i) fluxes through pentose pathway enzymes are not affected by the concentration of any enzyme in glycolysis, oxidative and non-oxidative pentose pathway, or nucleotide synthesis; the pentose pathway fluxes are only affected by the GSHR enzyme concentration in model 2; on the other hand, pentose pathway fluxes are controlled by all other enzymes in model 1;

(ii) the oxidative pentose pathway enzyme concentrations themselves show no influence on any fluxes in the metabolic network; on the other hand, non-oxidative pentose enzyme concentrations affect, to some degree, fluxes through glycolysis and nucleotide synthesis enzymes, but do not affect the fluxes through the pentose pathway enzymes themselves;

(iii) interestingly, the sodium and potassium leaks, and Na/K pump have little effect on any other fluxes except themselves in model 2, because they regulate red cell metabolism mainly by controlling the cell volume; but in model 1; These cation transmembrane processes show control over all enzyme activities.

(iv) there is no proportionate relationship between the sensitivity coefficients determined by model 1 and model 2; the absolute numerical values for some of the control coefficients $C_{E_k}^{J_i}$ are higher in model 1 (for example: E_K= ATPase, leak K and Na/K pump), some are higher in model 2 (for example: E_K=ALD and GSHR), and some even change sign (for example: E_K= DPGase, ATPase).

Although the flux sensitivity coefficients in model 1 are not in proportion to those in model 2, their difference has a direct correlation with volume sensitivity (Table 4). If the volume sensitivity $C_{E_k}^{V}$ is positive with respect to an enzyme E_k, then cell volume increases with increased enzyme concentration. Owing to the volume increase when raising E_k, all metabolite and enzyme concentrations as well as all metabolic fluxes drop, which results in lower absolute flux sensitivity coefficients in model 1 and model 2. For a similar reason, if the volume sensitivity $C_{E_k}^{V}$ is negative, the absolute values of flux sensitivity coefficients with respect to E_k will be higher in model 1.

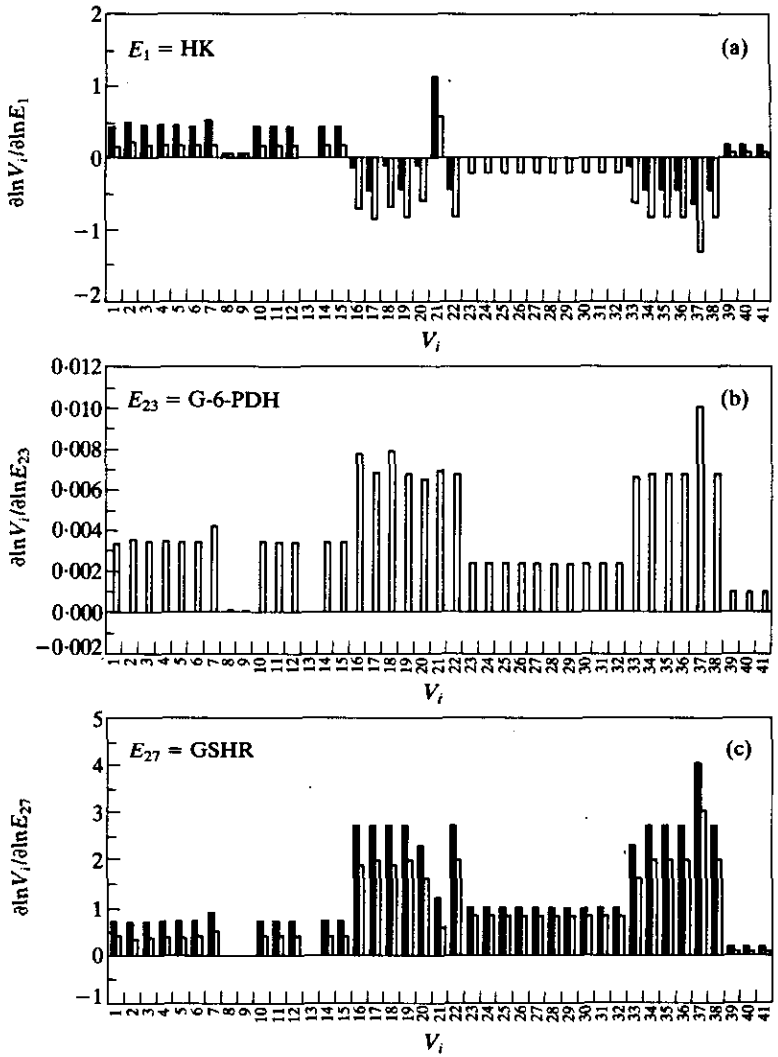

FIG. 3. Comparison of sensitivity coefficients $C_{E_k}^{J_i}$ of model 1 and model 2 for selected E_k. In each plot, an enzyme concentration is varied and the corresponding changes in 41 fluxes are calculated. In the horizontal co-ordinate, 1 = HK, 2 = PGI, 3 = PFK, 4 = ALD, 5 = TPI, 6 = GAPDH, 7 = PGK, 8 = DPGM, 9 = DPGase, 10 = PGM, 11 = EN, 12 = PK, 13 = PYRex, 14 = LACDH, 15 = LACex, 16 = AMPase, 17 = ADA, 18 = AK, 19 = APK, 20 = AMPDA, 21 = ATPase, 22 = AD-PRT, 23 = G-6-PDH, 24 = PGLase, 25 = PG-6-DH, 26 = GSSGR, 27 = GSHR, 28 = R-5-P-I, 29 = Xu-5-P-I, 30 = TK1, 31 = TK2, 32 = TA1, 33 = IMPase, 34 = PNPase, 35 = PRM, 36 = PRPPsyn, 37 = HG-PRT, 38 = HXex, 39 = leak K, 40 = leak Na, 41 = NK pump. □, model 1; ■, model 2.

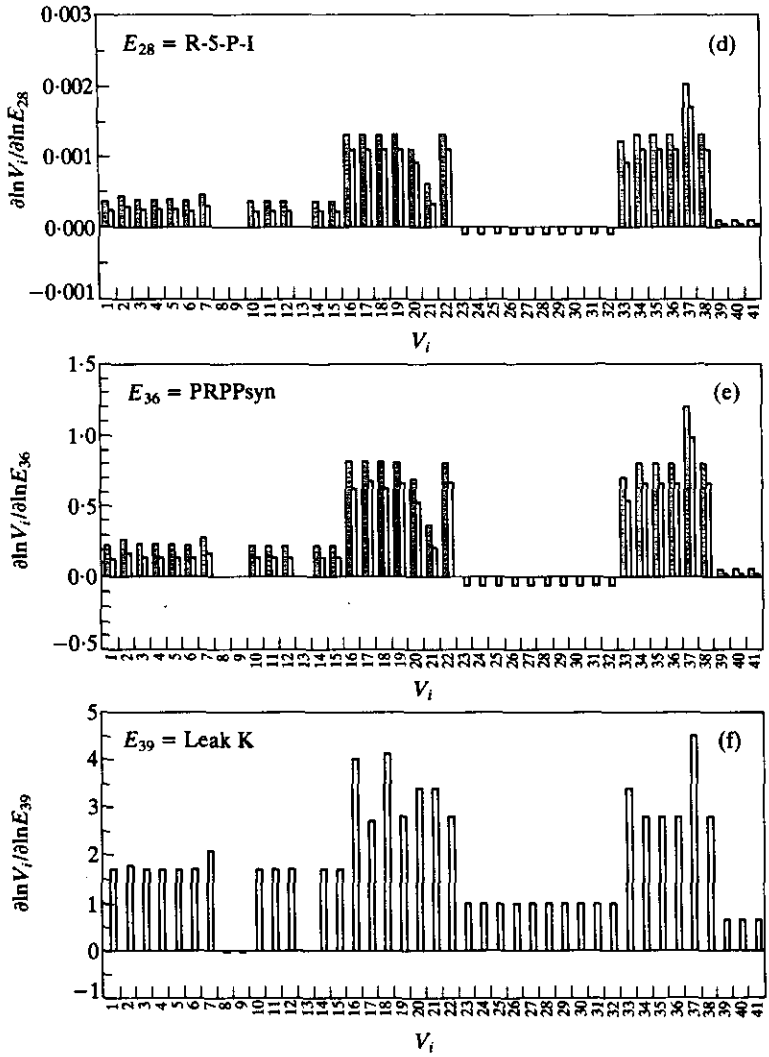

FIG. 3—continued.

Without physiochemical volume regulation (model 2), enzymes leak K, leak Na, and Na/K pump, which are responsible for cation transport, have little influence on other parts of the metabolic network (Fig. 3). However, with the inclusion of volume regulatory mechanism (model 1), these cation transport processes become significant in red cell metabolic regulation. For example: the negative volume sensitivity $C^V_{E_k}$ with respect to the carrier of potassium (leak K) results in positive flux sensitivity $C^{J_i}_{E_k}$ for all fluxes with respect to the carrier. When the cell volume shrinks owing to an increase in the potassium carrier concentration, all metabolite concentrations

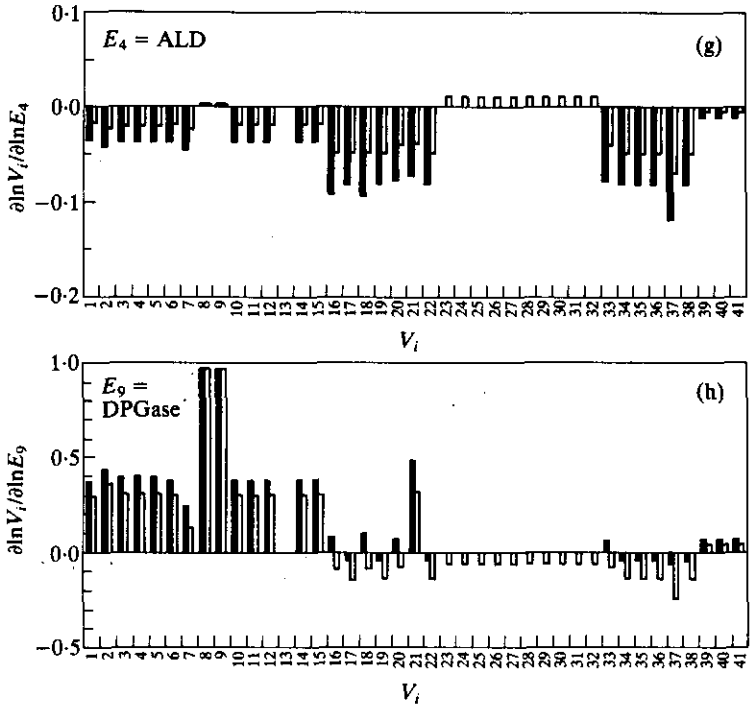

FIG. 3—continued.

increase. As a result, activities of all enzymes accelerate and all metabolic fluxes rise. Similarly, the sensitivity for all fluxes with respect to sodium leak (leak Na) and Na/K pump are negative as $C^V_{E_k}$ with respect to these enzymes has positive values.

MODEL 2 VS. MODEL 3

Metabolic model complexity significantly influences the sensitivity coefficients, which is illustrated by the comparisons of sensitivity coefficients between model 2 and model 3 in Fig. 4. Model 3 is a sub-model of model 2, both of which consist only of enzymatic reactions, and neither accounts for the physiochemical constraints. Model 3 simply describes glycolysis, which is one of the three pathways included in model 2.

A comparison between the two models shows that the absolute sensitivity coefficients $C^{J_i}_{E_k}$ are higher in model 3 when E_k is HK, DPGM, GAPDH, or LACex; are higher in model 2 when E_k is ALD; and change sign when E_k is PFK. In other cases, such as E_k = DPGase and PK, the sensitivity coefficients calculated from the two models are relatively close to each other in magnitude. In yet another two cases, i.e. GAPDH and LACex, the sensitivity coefficients in model 2 are negligible compared to those in model 3. Both models show that most glycolytic enzymes have

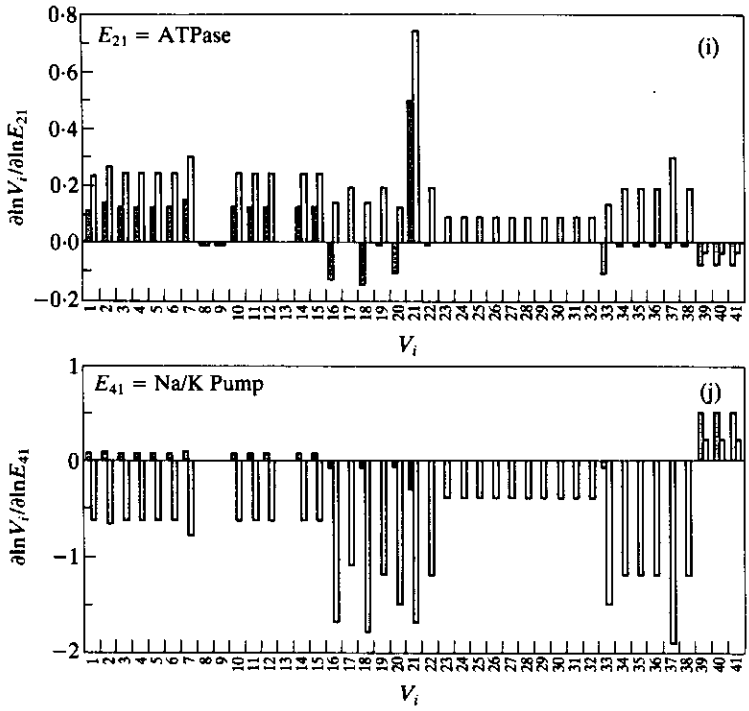

FIG. 3—continued.

smaller influence on fluxes through 2, 3-DPG bypass, DPGM and DPGase than on themselves.

A major difference between model 2 and model 3 lies in the number of branch points in the glycolytic pathway. As expected the flux sensitivity coefficients $C_{E_k}^{J_i}$ are the same for enzymes that are linearly connected in the pathway without the interruption of branch points (Figs 3 and 4). For example, the fluxes through PGM, EN, and PK in model 1, model 2, and model 3; and the fluxes through G-6-PD, PGLase, and 6-PGD in model 1 and model 2. Most of the glycolytic enzymes in model 3 are linearly connected, with the only branch points occurring at 13-DPG and 3-PG; therefore, most flux sensitivity coefficients with respect to an enzyme in model 3 have the same value except for the fluxes around the branch points. However, the glycolytic pathway is separated by additional branch points in model 2 at G-6-P, F-6-P, GA-3-P, ADP, and ATP. As a result, the whole pathway is divided into many segments in the more complex model. The flux sensitivity coefficients are identical for all fluxes within the same segment with respect to the change in an enzyme concentration. Since more branches divert from or condense to glycolytic fluxes in the more complicated metabolic model (model 2), the realistic behavior of metabolic regulation cannot be accurately predicted by the simpler model (model 3), which considers most enzymes in the glycolysis sequentially connected without the interruption of branch points.

TABLE 3

The summation theorem as a test for the convergence in the evaluations of the flux sensitivity coefficients

	$\sum_{k=1}^{n} C_{E_k}^{J_i}$		
J_i	Model 1	Model 2	Model 3
HK	1·001	1·000	0·999
PGI	1·001	1·001	0·999
PFK	1·001	1·000	0·999
ALD	1·001	1·000	0·999
TPI	1·001	1·000	0·999
GAPDH	1·001	1·000	0·999
PGK	1·002	1·001	0·999
DPGM	0·999	0·999	0·999
DPGase	0·999	0·999	0·999
PGM	1·001	1·000	0·999
EN	1·001	1·000	0·999
PK	1·001	1·000	0·999
LacDH	1·001	1·000	0·999
Lacex	1·009	1·000	0·999
AMPase	1·007	1·006	
ADA	1·009	1·006	
AK	1·007	1·005	
APK	1·006	1·006	
AMPDA	1·002	1·004	
ATPase	1·006	1·001	
ADPRT	1·001	1·006	
G-6-PDH	1·001	0·999	
PGLase	1·001	0·999	
PG-6-DH	1·001	0·999	
GSSGR	1·001	0·999	
GSHR	1·001	0·999	
R5P-I	1·001	0·999	
Xu5P-I	1·001	0·999	
TK1	1·001	0·999	
TK2	1·001	0·999	
TA1	1·001	0·999	
IMPase	1·001	1·004	
PNPase	1·006	1·006	
PRM	1·007	1·006	
PRPPsyn	1·007	1·006	
HGPRT	1·014	1·009	
HXex	1·006	1·006	
Leak K	0·999	0·999	
Leak Na	0·999	0·999	
NK Pump	0·999	0·999	

Conclusions

Three models of red blood cell metabolism with different degrees of complexity have been used to calculate the flux sensitivity coefficients. We found that the metabolic model complexity has significant effects on the evaluation and interpretation

TABLE 4

The difference in flux sensitivity coefficients of model 1 and model 2 has a direct correlation with volume sensitivity

E_k	$C^{J_i}_{E_k,\text{model 1}} - C^{J_i}_{E_k,\text{model 2}}$ for J_i = any flux	$C^{V}_{E_k,\text{model 1}}$
HK	−	+
PGI	−	+
PFK	−	+
ALD	+	−
TPI	+	−
GAPDH	+	−
PGK	−	+
DPGM	+	−
DPGase	−	+
PGM	−	+
EN	−	+
PK	−	+
LacDH	+	−
Lacex	+	−
AMPase	+	−
ADA	+	−
AK	−	+
APK	−	+
AMPDA	+	−
ATPase	+	−
ADPRT	−	+
G-6-PDH	−	+
PGLase	−	+
PG-6-DH	+	−
GSSGR	−	+
GSHR	−	+
R-5-P-I	−	+
Xu-5-P-I	+	−
TK1	+	−
TK2	+	−
TA1	+	−
IMPase	+	−
PNPase	+	−
PRM	+	−
PRPPsyn	−	+
HGPRT	+	−
HXex	−	+
Leak K	+	−
Leak Na	−	+
NK pump	−	+

of flux sensitivity coefficients. Flux sensitivity coefficients based on one model are not always consistently proportional to those based on another model. In some cases they have opposite signs in two different models, for example: $C^{J_i}_{E_k}$ with respect to E_k = DPGase and ATPase in model 1 and model 2. Therefore, the simplified models cannot provide an accurate prediction of the realistic behaviors of metabolism.

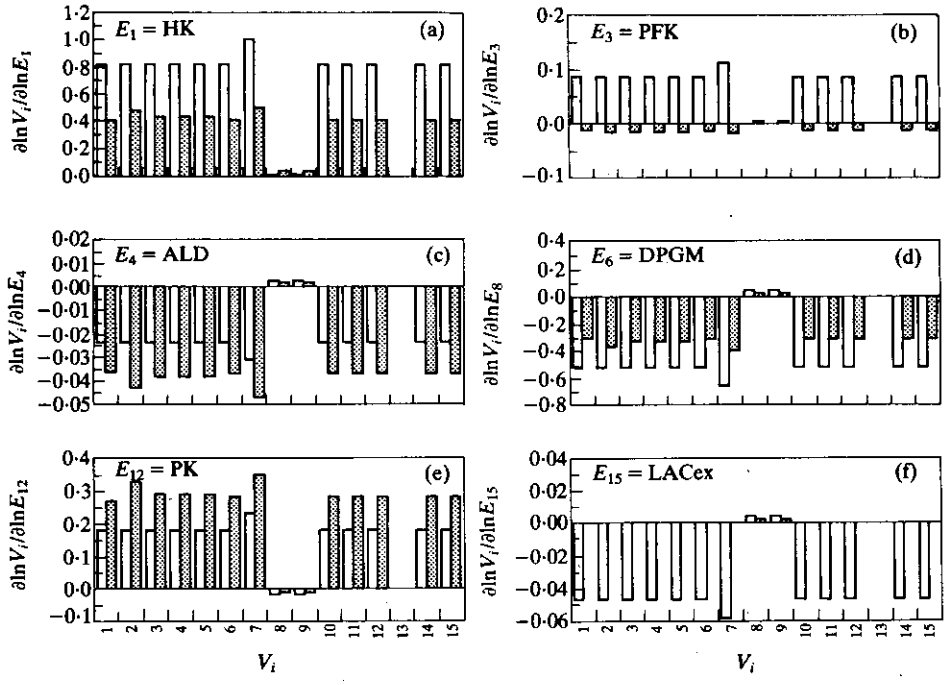

FIG. 4. Comparison of sensitivity coefficients $C^J_{E_i}$ of model 2 and model 3 for selected E_k. In each plot, an enzyme concentration is varied and the corresponding changes in 15 glycolytic fluxes are calculated. In the horizontal co-ordinate, 1 = HK, 2 = PGI, 3 = PFK, 4 = ALD, 5 = TPI, 6 = GAPDH, 7 = PGK, 8 = DPGM, 9 = DPGase, 10 = PGM, 11 = EN, 12 = PK, 13 = PYRex, 14 = LACDH, 15 = LACex. □, model 3; ■, model 2.

The differences in the results from model 1 and model 2 are a result of the physiochemical processes, which regulate the cell volume and affect metabolite concentrations and enzyme activities. When the cell volume expands following the increase in an enzyme concentration, metabolite concentrations drop and metabolic fluxes decrease. Accordingly, model 1, considering volume regulation, predicts lower absolute flux sensitivity coefficients than model 2. The enzymes involved in cation transport, such as leak K, leak Na, and Na/K pump, show significant influence on metabolic fluxes in model 1, but show negligible effect on the metabolic fluxes in model 2. This discrepancy is because cation transport processes affect the metabolism through the volume regulatory mechanism. The conclusion that one arrives at based on this comparison is that the consideration of transmembrane processes is important in theoretical studies of metabolic regulation.

Model 2 and model 3 also give different values for flux sensitivity coefficients because of the differences in the number of branch points. In model 3, all sensitivity coefficients of glycolytic fluxes with respect to the change in an enzyme concentration are identical except for the fluxes through PGK, DPGM, and DPGase, since all enzymes are linearly connected with the only branch points occurring at 13-DPG

and 3-PG. On the other hand, the sensitivity coefficients with respect to an enzyme vary in different segments of the glycolytic pathway in model 2 as the pathway is divided by many branch points in the model. Model complexity thus significantly influences the numerical values of the sensitivity coefficients and the interpretations derived from them, even if the rate laws used in full and sub-models are identical.

With these conclusions in hand, it is instructive to look back over the history of metabolic control coefficients and red blood cell metabolic models. The sensitivity coefficients for red blood cell glycolytic fluxes have been estimated from a number of simple red cell metabolic models (Rapoport *et al.*, 1974, 1976; Heinrich *et al.*, 1977; Schuster *et al.*, 1988; Heinrich, 1989). A comparison of the results obtained from these models are listed in Table 5. The simpler models ([1] and [2] in Table 5) predict the importance of hexokinase in controlling the glycolytic flux. However, with the help of more complete models ([3], [4], [5], and model 1), it becomes clear that the influences of cation transport, ATPase, and 2,3-DPG bypass on the glycolytic flux are more significant than hexokinase as measured by the control coefficient (Table 6). This comparison further demonstrates that incomplete metabolic models can

TABLE 5

Sensitivity coefficients calculated from different red cell metabolic models

Model No. of enzymes considered	[1] 5	[2] 5	[3] 5	[4] 9	[5] 20	Model 3 15	Model 2 41	Model 1 41 + Vol. reg.
HK + PFK		1·37	0·24	1·13				
HK	0·69				0·076	0·82	0·40	0·14
PGI						0·00	0·00	0·00
PFK	0·31				0·023	0·08	−0·01	−0·017
ALD						−0·02	−0·04	−0·018
TPI						0·00	0·00	0·00
GADH						−0·02	0·00	0·00
PGK				−0·31		0·00	0·01	0·005
DPGM	0·0	−0·04	0·02	0·31	−0·074	−0·52	−0·30	−0·15
DPGase	0·0	−0·04	0·21	−0·30	0·221	0·52	0·37	0·29
PGM						0·00	0·00	0·00
EN						0·00	0·00	0·00
PK	0·0	0·04	−0·02	0·0	0·015	0·18	0·27	0·14
LDH					0·005	0·00	0·00	0·00
ATPase			0·55	0·16	0·700		0·11	0·23
Na/K pump				−0·05			0·07	−0·63
Leak K				−4·19			0·00	1·7
Leak Na				4·30			0·06	−0·79
G-6-PDH					0·0		0·00	−0·004
PG-6-DH					0·0		0·00	0·003
GSSGR					0·0		0·00	0·00
GSHR					0·005		0·73	0·41
PRPPsyn					0·028		0·22	0·13

The sensitivity coefficients shown are variations of HK flux with respect to the enzyme indicated $\partial \ln J_{HK}/\partial \ln E_k$.

Source: [1] = Rapoport *et al.* (1974); [2] = Rapoport *et al.* (1976); [3] = Heinrich *et al.* (1977); [4] = Heinrich (1989); [5] = Schuster *et al.* (1988).

TABLE 6

Hexokinase flux controlling enzymes predicted from red cell metabolic models of different complexity. The enzymes are listed in the order of decreasing importance for each model

Model	[1]	[2]	[3]
No. of enzymes considered	5	5	5
HK flux controlling enzymes	HK > PFK	HK + PFK > ATPase	ATPase > HK + PFK > DPGase

Model	[4]	[5]	Model 1
No. of enzymes considered	9	20	41
HK flux controlling enzymes	Leak Na > Leak K > HK + PFK > DPGM > PGK > DPGase > ATPase	ATPase > DPGase	Leak K > Leak Na > Pump > GSHR > TA1 > DPGase > ATPase > DPGM > HK > PK > PRPPsyn > AK > ADA

Source: [1] = Rapoport *et al.* (1974); [2] = Rapoport *et al.* (1976); [3] = Heinrich *et al.* (1977); [4] = Heinrich (1989); [5] = Schuster *et al.* (1988).

result in misleading interpretations. Finally, based on the results presented here, it seems unlikely that metabolic control coefficients will be useful in guiding metabolic engineering in the absence of comprehensive metabolic models.

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