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Feedforward Inhibition in Biosynthetic Pathways: Inhibition of the Aminoacyl-tRNA Synthetase by Intermediates of the Pathway

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The stability of an amino acid biosynthetic pathway controlled by end-product inhibition is significantly improved if, in addition, the corresponding aminoacyl-tRNA synthetase is inhibited by an intermediate in the pathway. The more proximal the feedforward modifier is to the initial substrate, the more stable is the system. The temporal responsiveness of a system having both feedback and feedforward inhibition also is improved by having the feedforward modifier located at the beginning of the pathway. According to all other criteria that have been used previously to determine the functional effectiveness of biosynthetic pathways, the behavior of such a system essentially is unaffected by the position of the feedforward modifier in the pathway.

1. Introduction

The principle of feedforward control was discovered and applied in a mechanical context by the early millwrights. Mead's regulator (1787) was designed with a continuously adjustable gear ratio inversely proportional to the rotational speed of the input shaft. Thus, the speed of the output shaft would remain relatively constant in spite of fluctuations in the speed of the input shaft.

This principle was rediscovered by Black (1924), who later became famous for his applications of negative feedback in the design of electrical circuits. Feedback control immediately became an important feature of design in electrical circuits, whereas feedforward control remained a curiosity. However, a fuller awareness of the advantages of feedforward control has been achieved recently. In contrast to feedback control, in which a sample of the output is returned to the input and a comparison of non-synchronous signals is made, feedforward control involves processing a sample of the input in parallel with the primary flow of information and a comparison with

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the synchronous output. It is now known that this parallel processing of primary and corrective signals results in faster temporal response and greater stability without a concomitant reduction in overall gain of the system. This knowledge, together with recent technological advances, has renewed interest in feedforward control of electrical systems (see Jurgen, 1972).

Feedforward control by activation was discovered in amphibolic pathways (Leloir *et al.*, 1959; Leloir & Goldenberg, 1960) and the simplest models were analyzed by Higgins (1967), but this mechanism has never been reported in biosynthetic pathways. The inhibition of an aminoacyl-tRNA synthetase by the penultimate product of an amino acid biosynthetic pathway appears to be the first example of feedforward control discovered in biosynthetic pathways (Ames & Hartman, 1961), but this mechanism appears to offer no significant advantage over control by end-product inhibition alone (Savageau, 1979). Perhaps the length of the "feedforward pathway" in these cases is insufficient for the realization of the advantages noted with electrical systems.

To test this hypothesis we have analyzed the behavior of amino acid biosynthetic pathways in which the position of the feedforward modifier is more proximal to the input or initial substrate. The results show that the stability of the system and its temporal responsiveness are significantly improved as the position of the feedforward modifier approaches that of the initial substrate. The implications of this result are discussed in light of available experimental evidence.

2. Method of Analysis

The general model of a biosynthetic pathway represented in Fig. 1, including both feedback and feedforward interactions, will be analyzed. The descriptive equations for this model can be written in the non-linear formalism previously described (Savageau, 1976, 1979).

$$
\begin{aligned}
\dot{X}_1 &= \alpha_1 X_0^{g_{10}} X_n^{g_{1n}} - \beta_1 X_1^{h_{11}} \\
\dot{X}_2 &= \beta_1 X_1^{h_{11}} - \beta_2 X_2^{h_{22}} \\
&\vdots \\
\dot{X}_p &= \alpha_p X_p^{g_{pp'}} X_{p-1}^{g_{p,p-1}} - \beta_p X_p^{h_{pp}} \\
&\vdots \\
\dot{X}_n &= \alpha_n X_n^{g_{nn'}} X_{n-1}^{g_{n,n-1}} - \beta_n X_k^{h_{nk}} X_n^{h_{nn}} X_{n+1}^{h_{n,n+1}} \\
\dot{X}_{n+1} &= \beta_n X_k^{h_{nk}} X_n^{h_{nn}} X_{n+1}^{h_{n,n+1}} - \beta_{n+1} X_{n+1}^{h_{n+1,n+1}}
\end{aligned}
\qquad (1)
$$

The corresponding steady-state equations can be written as

$$\begin{bmatrix} (b_1 - g_{10}y_0) \\ b_2 \\ b_3 \\ \vdots \\ (b_p - g_{pp'}y_{p'}) \\ \vdots \\ b_k \\ \vdots \\ (b_n - g_{nn'}y_{n'}) \\ b_{n+1} \end{bmatrix} = \begin{bmatrix} -h_{11} & 0 & 0 & 0 & \cdots & g_{1n} & 0 \\ h_{11} & -h_{22} & 0 & 0 & \cdots & 0 & 0 \\ 0 & h_{22} & -h_{33} & 0 & \cdots & 0 & 0 \\ \vdots & & \ddots & & & \vdots & \vdots \\ 0 & \cdots & g_{p,p-1} & -h_{pp} & \cdots & 0 & 0 \\ \vdots & & & \ddots & & \vdots & \vdots \\ 0 & \cdots & h_{k-1,k-1} & -h_{kk} & \cdots & 0 & 0 \\ \vdots & & & & \ddots & \vdots & \vdots \\ 0 & \cdots & 0 & -h_{nk} & \cdots & g_{n,n-1} - h_{nn} & -h_{n,n+1} \\ 0 & \cdots & 0 & h_{nk} & \cdots & 0 \quad h_{nn} & -(h_{n+1,n+1} - h_{n,n+1}) \end{bmatrix} \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ \vdots \\ y_p \\ \vdots \\ y_k \\ \vdots \\ y_n \\ y_{n+1} \end{bmatrix} \quad (1)$$

where

$$\begin{aligned} y_i &= \log X_i \\ b_i &= \log (\beta_i/\alpha_i) \qquad i = 1, p, n \\ &= \log (\beta_i/\beta_{i-1}) \quad \text{all other } i. \end{aligned}$$

The dependent concentration variables in equation (2) can be solved for in terms of the independent variables (y_0, $y_{p'}$, $y_{n'}$) and the parameters characterizing the system. These solutions form the basis for further analysis as will be described in later sections.

The systems represented in Fig. 1 will be compared with an equivalent system in which X_{n-1} is the feedforward modifier. The criteria for functional effectiveness that will be used to make these comparisons have been discussed elsewhere (Savageau, 1976, 1979).

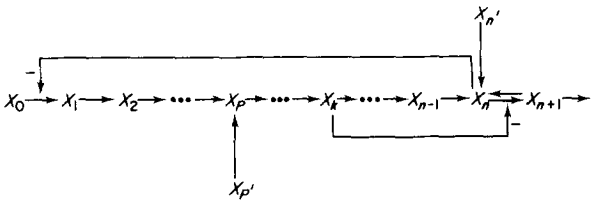

FIG. 1. Model of an unbranched pathway for the biosynthesis of an amino acid, including feedback inhibition of the first enzyme by the amino-acid end product and feedforward inhibition of the aminoacyl-tRNA synthetase by an intermediate. X_i represents the ith metabolite, $X_{p'}$ and $X_{n'}$ represent extracellular pools corresponding to the intracellular pools of an intermediate X_p and the end product X_n, respectively, and X_{n+1} represents aminoacyl tRNA, the activated end product. An arrow between symbols represents an enzyme-catalyzed reaction or transport process that is, except for the synthetase reaction, essentially irreversible for these kinetic purposes; an arrow from a symbol to the center of another arrow represents the influence of a modifier upon a regulatory enzyme. See Savageau (1979) for further discussion.

3. Response to Initial Substrate

From the steady-state solution of equation (2) one can calculate the percent change in the concentration of the activated end product (X_{n+1}) for a 1% change in the concentration of the initial substrate (X_0) by taking the partial derivative of y_{n+1} with respect to y_0. This quantity is defined as the overall logarithmic gain of the system ($L_{n+1,0} = \partial y_{n+1}/\partial y_0$). For the system in Fig. 1 with X_k as the feedforward modifier, the overall logarithmic gain is

$$L^k_{n+1,0} = g_{10} g_{p,p-1} g_{n,n-1} h^k_{nn} / \Delta^{pk}, \tag{3}$$

where

$$\Delta^{pk} = h_{p-1,p-1} h_{n-1,n-1} h^k_{nn} h_{n+1,n+1} + g_{1n}\left[g_{p,p-1} g_{n,n-1} (h^k_{n,n+1} - h_{n+1,n+1}) + g_{p,p-1} \frac{h_{nk}}{h_{kk}} h_{n-1,n-1} h_{n+1,n+1} \right], \tag{4}$$

when $p \leqslant k$ or

$$L^k_{n+1,0} = g_{10} g_{p,p-1} g_{n,n-1} h^k_{nn} / \Delta^{kp} \tag{5}$$

where

$$\Delta^{kp} = h_{p-1,p-1} h_{n-1,n-1} h^k_{nn} h_{n+1,n+1} + g_{1n}\left[g_{p,p-1} g_{n,n-1} (h^k_{n,n+1} - h_{n+1,n+1}) + h_{p-1,p-1} \frac{h_{nk}}{h_{kk}} h_{n-1,n-1} h_{n+1,n+1} \right], \tag{6}$$

when $p > k$.

Similarly for the system in which X_{n-1} is the feedforward modifier

$$L^{n-1}_{n+1,0} = g_{10} g_{p,p-1} g_{n,n-1} h^{n-1}_{nn} / \Delta^{n-1}, \tag{7}$$

where

$$\Delta^{n-1} = h_{p-1,p-1} h_{n-1,n-1} h^{n-1}_{nn} h_{n+1,n+1} + g_{1n}[g_{p,p-1} g_{n,n-1} (h^{n-1}_{n,n+1} - h_{n+1,n+1}) + g_{p,p-1} h_{n,n-1} h_{n+1,n+1}]. \tag{8}$$

For comparisons the systems are required to be identical in every respect except for the properties of the synthetase reaction. Parameters common to the two systems must have identical values wherever possible (internal equivalence). These parameters are represented without superscripts in equations (3) through (7). Parameters that characterize the synthetase reaction have different values for the two systems, and these are indicated with appropriate superscripts. The steady-state behavior of the two systems also is required to be identical from an external perspective (external equivalence), i.e. the systems will have the same steady-state level of activated end product ($X^k_{n+1} = X^{n-1}_{n+1}$), the same response to changes in initial substrate ($L^k_{n+1,0} = L^{n-1}_{n+1,0}$), and the same response to changes in demand

for the activated end product ($S_{L^k_{n+1,0}h_{n+1,n+1}} = S_{L^{n-1}_{n+1,0}h_{n+1,n+1}}$, see next section). This specification of both internal and external equivalence implies the following constraints among the parameters of the synthetase reactions:

For $p \leqslant k$,

$$h^k_{nn} = \left[\frac{h_{n-1,n-1}h_{nk} - g_{n,n-1}h_{kk}}{h_{n,n-1}h_{kk} - g_{n,n-1}h_{kk}}\right] h^{n-1}_{nn}, \tag{9}$$

$$h^k_{n,n+1} = \left[\frac{h_{n-1,n-1}h_{nk} - g_{n,n-1}h_{kk}}{h_{n,n-1}h_{kk} - g_{n,n-1}h_{kk}}\right] h^{n-1}_{n,n+1}, \tag{10}$$

$$\log \beta^k_n = \left[\frac{h_{n-1,n-1}h_{nk} - g_{n,n-1}h_{kk}}{h_{n,n-1}h_{kk} - g_{n,n-1}h_{kk}}\right] \times \left\{\log \beta^{n-1}_n - \left[\frac{h_{n-1,n-1}h_{nk} - h_{n,n-1}h_{kk}}{h_{n-1,n-1}h_{nk} - g_{n,n-1}h_{kk}}\right] \log \alpha_n\right\}. \tag{11}$$

For $p > k$,

$$h^k_{nn} = \left[\frac{h_{p-1,p-1}h_{n-1,n-1}h_{nk} - g_{p,p-1}g_{n,n-1}h_{kk}}{g_{p,p-1}h_{n,n-1}h_{kk} - g_{p,p-1}g_{n,n-1}h_{kk}}\right] h^{n-1}_{nn}, \tag{12}$$

$$h^k_{n,n+1} = \left[\frac{h_{p-1,p-1}h_{n-1,n-1}h_{nk} - g_{p,p-1}g_{n,n-1}h_{kk}}{g_{p,p-1}h_{n,n-1}h_{kk} - g_{p,p-1}g_{n,n-1}h_{kk}}\right] h^{n-1}_{n,n+1}, \tag{13}$$

$$\log \beta^k_n = \left[\frac{h_{p-1,p-1}h_{n-1,n-1}h_{nk} - g_{p,p-1}g_{n,n-1}h_{kk}}{g_{p,p-1}h_{n,n-1}h_{kk} - g_{p,p-1}g_{n,n-1}h_{kk}}\right] \times \left\{\log \beta^{n-1}_n - \left[\frac{h_{p-1,p-1}h_{n-1,n-1}h_{nk} - g_{p,p-1}h_{n,n-1}h_{kk}}{h_{p-1,p-1}h_{n-1,n-1}h_{nk} - g_{p,p-1}g_{n,n-1}h_{kk}}\right] \log \alpha_n\right\}. \tag{14}$$

4. Response to Demand for Activated End Product

A change in demand for activated end product can be represented by a change in the apparent kinetic order ($h_{n+1,n+1}$) for the degradation of X_{n+1}, and the response of the system to this change can be represented by the sensitivity of the overall logarithmic gain ($L_{n+1,0}$) with respect to $h_{n+1,n+1}$ (Savageau, 1976).

For the system in which X_k is the feedforward modifier

$$S_{L^k_{n+1,0}h_{n+1,n+1}} = \frac{\partial L^k_{n+1,0}}{\partial h_{n+1,n+1}} \frac{h_{n+1,n+1}}{L^k_{n+1,0}}$$

$$= \frac{-\left[h_{p-1,p-1} h_{n-1,n-1} h^k_{nn} h_{n+1,n+1} + g_{1n}\left(g_{p,p-1} \frac{h_{nk}}{h_{kk}} h_{n-1,n-1} h_{n+1,n+1} - g_{p,p-1} g_{n,n-1} h_{n+1,n+1} \right) \right]}{\Delta^{pk}}, \quad p \leqslant k, \quad (15)$$

$$= \frac{-\left[h_{p-1,p-1} h_{n-1,n-1} h^k_{nn} h_{n+1,n+1} + g_{1n}\left(h_{p-1,p-1} \frac{h_{nk}}{h_{kk}} h_{n-1,n-1} h_{n+1,n+1} - g_{p,p-1} g_{n,n-1} h_{n+1,n+1} \right) \right]}{\Delta^{kp}}, \quad p > k, \quad (16)$$

and for the system in which X_{n-1} is the feedforward modifier

$$S_{L^{n-1}_{n+1,0}h_{n+1,n+1}} = \frac{-[h_{p-1,p-1} h_{n-1,n-1} h^{n-1}_{nn} h_{n+1,n+1} + g_{1n}(g_{p,p-1} h_{n,n-1} h_{n+1,n+1} - g_{p,p-1} g_{n,n-1} h_{n+1,n+1})]}{\Delta^{n-1}}. \quad (17)$$

Equivalence of the sensitivities in equations (15) and (17), together with equivalence of the overall logarithmic gains in equations (3) and (7), specifies the constraints in equations (9) and (10). Equivalence of the steady-state levels for the two systems then yields the constraint relation in equation (11). Similarly, equivalence of equations (16) and (17) and of equations (5) and (7) specify the constraints in equations (12) and (13); equivalence of the steady-state levels then yields the constraint in equation (14).

5. Accumulation of Metabolic Intermediates

The response of the intermediate concentrations (X_i) in the pathway to changes in the steady-state concentration of the initial substrate (X_0) will be represented by the intermediate logarithmic gains $L_{i0} = \partial y_i / \partial y_0$.

First, the expressions for the system represented in Fig. 1 are calculated when $p \leqslant k$.

$$L^k_{i0} = g_{10} h_{p-1,p-1} h_{n-1,n-1} h^k_{nn} h_{n+1,n+1} / (h_{ii} \Delta^{pk}), \quad 1 \leqslant i < p, \quad (18)$$

$$= g_{10} g_{p,p-1} h_{n-1,n-1} h^k_{nn} h_{n+1,n+1} / (h_{ii} \Delta^{pk}), \quad p \leqslant i < n, \quad (19)$$

$$= -g_{10}g_{p,p-1}\left[g_{n,n-1}(h^k_{n,n+1} - h_{n+1,n+1}) + \frac{h_{nk}}{h_{kk}}h_{n-1,n-1}h_{n+1,n+1}\right]\Big/\Delta^{pk}, \quad i = n. \tag{20}$$

Similar expressions can be obtained for the system in which X_{n-1} is the feedforward modifier.

$$L^{n-1}_{i0} = g_{10}h_{p-1,p-1}h_{n-1,n-1}h^{n-1}_{nn}h_{n+1,n+1}/(h_{ii}\Delta^{n-1}), \quad 1 \leqslant i < p, \tag{21}$$

$$= g_{10}g_{p,p-1}h_{n-1,n-1}h^{n-1}_{nn}h_{n+1,n+1}/(h_{ii}\Delta^{n-1}), \quad p \leqslant i < n, \tag{22}$$

$$= -g_{10}g_{p,p-1}[g_{n,n-1}(h^{n-1}_{n,n+1} - h_{n+1,n+1}) + h_{n,n-1}h_{n+1,n+1}]/\Delta^{n-1}, \quad i = n, \tag{23}$$

and when the parameters with superscripts are replaced by the relations in equations (9) and (10), one finds that

$$L^k_{i0} = L^{n-1}_{i0}, \quad 1 \leqslant i \leqslant n. \tag{24}$$

Next, the corresponding expressions for the system in Fig. 1 are calculated when $p > k$.

$$L^k_{i0} = g_{10}h_{p-1,p-1}h_{n-1,n-1}h^k_{nn}h_{n+1,n+1}/(h_{ii}\Delta^{kp}), \quad 1 \leqslant i < p, \tag{25}$$

$$= g_{10}g_{p,p-1}h_{n-1,n-1}h^k_{nn}h_{n+1,n+1}/(h_{ii}\Delta^{kp}), \quad p \leqslant i < n, \tag{26}$$

$$= -g_{10}\left[g_{p,p-1}g_{n,n-1}(h^k_{n,n+1} - h_{n+1,n+1}) + h_{p-1,p-1}\frac{h_{nk}}{h_{kk}}h_{n-1,n-1}h_{n+1,n+1}\right]\Big/\Delta^{kp}, \quad i = n, \tag{27}$$

and when the parameters with superscripts in equations (21) through (23) are replaced by the relations in equations (12) and (13), one finds that again

$$L^k_{i0} = L^{n-1}_{i0}, \quad 1 \leqslant i \leqslant n. \tag{28}$$

The response of the intermediate concentrations in the pathway to change in the steady-state demand for activated end product will be represented by the sensitivities of the intermediate logarithmic gains with respect to changes in $h_{n+1,n+1}$. For the system represented in Fig. 1, when $p \leqslant k$,

$$S_{L^k_{i0}h_{n+1,n+1}} = \frac{\partial L^k_{i0}}{\partial h_{n+1,n+1}}\frac{h_{n+1,n+1}}{L^k_{i0}} = g_{1n}g_{p,p-1}g_{n,n-1}h^k_{n,n+1}/\Delta^{pk}, \quad 1 \leqslant i < n, \tag{29}$$

$$= \frac{h_{p-1,p-1}h_{n-1,n-1}h^k_{nn}h_{n+1,n+1}g_{n,n-1}h^k_{n,n+1}}{\left[g_{n,n-1}(h^k_{n,n+1}-h_{n+1,n+1})+\dfrac{h_{nk}}{h_{kk}}h_{n-1,n-1}h_{n+1,n+1}\right]\Delta^{pk}}, \quad i=n. \tag{30}$$

The corresponding expressions for the system in which X_{n-1} is the feedforward modifier are

$$S_{L^{n-1}_{i0}h_{n+1,n+1}}$$

$$= g_{1n}g_{p,p-1}g_{n,n-1}h^{n-1}_{n,n+1}/\Delta^{n-1}, \quad 1 \leqslant i < n, \tag{31}$$

$$= \frac{h_{p-1,p-1}h_{n-1,n-1}h^{n-1}_{nn}h_{n+1,n+1}g_{n,n-1}h^{n-1}_{n,n+1}}{[g_{n,n-1}(h^{n-1}_{n,n+1}-h_{n+1,n+1})+h_{n,n-1}h_{n+1,n+1}]\Delta^{n-1}}, \quad i=n, \tag{32}$$

and when the parameters with superscripts are replaced by the expressions in equations (9) and (10), one finds that

$$S_{L^k_{i0}h_{n+1,n+1}} = S_{L^{n-1}_{i0}h_{n+1,n+1}}, \quad 1 \leqslant i \leqslant n. \tag{33}$$

When $p < k$, the sensitivities for the system represented in Fig. 1 are:

$$S_{L^k_{i0}h_{n+1,n+1}}$$

$$= g_{1n}g_{p,p-1}g_{n,n-1}h^k_{n,n+1}/\Delta^{kp}, \quad 1 \leqslant i < n, \tag{34}$$

$$= \frac{h_{p-1,p-1}h_{n-1,n-1}h^k_{nn}h_{n+1,n+1}g_{p,p-1}g_{n,n-1}h^k_{n,n+1}}{\left[g_{p,p-1}g_{n,n-1}(h^k_{n,n+1}-h_{n+1,n+1})+h_{p-1,p-1}\dfrac{h_{nk}}{h_{kk}}h_{n-1,n-1}h_{n+1,n+1}\right]\Delta^{kp}},$$

$$i=n, \tag{35}$$

and when the parameters with superscripts in equations (31) and (32) are replaced by the constraint relations in equations (12) and (13), one finds that again

$$S_{L^k_{i0}h_{n+1,n+1}} = S_{L^{n-1}_{i0}h_{n+1,n+1}}, \quad 1 \leqslant i \leqslant n. \tag{36}$$

The results in equations (24), (28), (33) and (36) show that the accumulation of metabolic intermediates, either in response to a change in the concentration of initial substrate or in response to a change in demand for the activated end product, is the same regardless of which intermediate is the feedforward modifier.

6. Response to Addition of Exogenous Product

The responses of the dependent concentrations to a change in the steady-state level of exogenous end product ($X_{n'}$) are given by the following logarithmic gains when $p \leqslant k$:

$$L^k_{in'} = -g_{nn'}g_{1n}h_{p-1,p-1}h_{n-1,n-1}(h^k_{n,n+1} - h_{n+1,n+1})/(h_{ii}\Delta^{pk}), \quad 1 \leqslant i < p, \tag{37}$$

$$= -g_{nn'}g_{1n}g_{p,p-1}h_{n-1,n-1}(h^k_{n,n+1} - h_{n+1,n+1})/(h_{ii}\Delta^{pk}), \quad p \leqslant i < n, \tag{38}$$

$$= -g_{nn'}h_{p-1,p-1}h_{n-1,n-1}(h^k_{n,n+1} - h_{n+1,n+1})/\Delta^{pk}, \quad i = n, \tag{39}$$

$$= g_{nn'}h_{n-1,n-1}\left(h_{p-1,p-1}h^k_{nn} + g_{1n}g_{p,p-1}\frac{h_{nk}}{h_{kk}}\right)\Big/\Delta^{pk}, \quad i = n+1. \tag{40}$$

The corresponding expressions for the system in which X_{n-1} is the feedforward modifier are

$$L^{n-1}_{in'} = -g_{nn'}g_{1n}h_{p-1,p-1}h_{n-1,n-1}(h^{n-1}_{n,n+1} - h_{n+1,n+1})/(h_{ii}\Delta^{n-1}), \quad 1 \leqslant i < p, \tag{41}$$

$$= -g_{nn'}g_{1n}g_{p,p-1}h_{n-1,n-1}(h^{n-1}_{n,n+1} - h_{n+1,n+1})/(h_{ii}\Delta^{n-1}), \quad p \leqslant i < n, \tag{42}$$

$$= -g_{nn'}h_{p-1,p-1}h_{n-1,n-1}(h^{n-1}_{n,n+1} - h_{n+1,n+1})/\Delta^{n-1}, \quad i = n, \tag{43}$$

$$= g_{nn'}h_{n-1,n-1}\left(h_{p-1,p-1}h^{n-1}_{nn} + g_{1n}g_{p,p-1}\frac{h_{n,n-1}}{h_{n-1,n-1}}\right)\Big/\Delta^{n-1}, \quad i = n+1. \tag{44}$$

One can compare these expressions with those in equations (37) through (40) by replacing the parameters with superscripts in equations (41) through (44) by the constraint relations in equations (9) and (10) and then taking the ratio of the corresponding expressions.

For $1 \leqslant i \leqslant n$:

$$\frac{L^k_{in'}}{L^{n-1}_{in'}} = 1, \quad h_{n-1,n-1}h_{nk} = h_{n,n-1}h_{kk},$$
$$< 1, \quad h_{n-1,n-1}h_{nk} < h_{n,n-1}h_{kk}, \tag{45}$$
$$> 1, \quad h_{n-1,n-1}h_{nk} > h_{n,n-1}h_{kk}.$$

For $i = n+1$:

$$\frac{L^k_{in'}}{L^{n-1}_{in'}} = 1, \quad h_{n-1,n-1}h_{nk} = h_{n,n-1}h_{kk},$$
$$> 1, \quad h_{n-1,n-1}h_{nk} < h_{n,n-1}h_{kk}, \tag{46}$$
$$< 1, \quad h_{n-1,n-1}h_{nk} > h_{n,n-1}h_{kk}.$$

When $p > k$, the expressions for the system in Fig. 1 are

$$L^k_{in'} = -g_{nn'}g_{1n}h_{p-1,p-1}h_{n-1,n-1}(h^k_{n,n+1} - h_{n+1,n+1})/(h_{ii}\Delta^{kp}), \quad 1 \leqslant i < p, \tag{47}$$

$$= -g_{nn'}g_{1n}g_{p,p-1}h_{n-1,n-1}(h^k_{n,n+1} - h_{n+1,n+1})/(h_{ii}\Delta^{kp}), \quad p \leqslant i < n, \tag{48}$$

$$= -g_{nn'}h_{p-1,p-1}h_{n-1,n-1}(h^k_{n,n+1} - h_{n+1,n+1})/\Delta^{kp}, \quad i = n, \tag{49}$$

$$= g_{nn'}h_{n-1,n-1}\left(h_{p-1,p-1}h^k_{nn} + g_{1n}h_{p-1,p-1}\frac{h_{nk}}{h_{kk}}\right)/\Delta^{kp}, \quad i = n+1. \tag{50}$$

Again, one can compare these expressions with those in equations (41) through (44) by making the appropriate substitution of parameter values and taking the ratios of the corresponding expressions.

For $1 \leqslant i \leqslant n$:

$$\begin{aligned} \frac{L^k_{in'}}{L^{n-1}_{in'}} &= 1, \quad h_{p-1,p-1}h_{n-1,n-1}h_{nk} = g_{p,p-1}h_{n,n-1}h_{kk}, \\ &< 1, \quad h_{p-1,p-1}h_{n-1,n-1}h_{nk} < g_{p,p-1}h_{n,n-1}h_{kk}, \\ &> 1, \quad h_{p-1,p-1}h_{n-1,n-1}h_{nk} > g_{p,p-1}h_{n,n-1}h_{kk}. \end{aligned} \tag{51}$$

For $i = n+1$:

$$\begin{aligned} \frac{L^k_{in'}}{L^{n-1}_{in'}} &= 1, \quad h_{p-1,p-1}h_{n-1,n-1}h_{nk} = g_{p,p-1}h_{n,n-1}h_{kk}, \\ &> 1, \quad h_{p-1,p-1}h_{n-1,n-1}h_{nk} < g_{p,p-1}h_{n,n-1}h_{kk}, \\ &< 1, \quad h_{p-1,p-1}h_{n-1,n-1}h_{nk} > g_{p,p-1}h_{n,n-1}h_{kk}. \end{aligned} \tag{52}$$

Thus, if the strengths of the feedforward inhibitions are further constrained by the relations

$$h_{nk} = \left[\frac{h_{kk}}{h_{n-1,n-1}}\right]h_{n,n-1}, \quad p \leqslant k, \tag{53}$$

and

$$h_{nk} = \left[\frac{g_{p,p-1}h_{kk}}{h_{p-1,p-1}h_{n-1,n-1}}\right]h_{n,n-1}, \quad p > k, \tag{54}$$

then the responses of the dependent concentrations to an addition of exogenous end product are unaffected by the position of the feedforward modifier in the pathway.

7. Responses to Perturbations in the Structure of the System

The structure of the system is specified by the parameter values in equation (1). The response of the system to perturbations in the structure of the system itself can be determined by calculating the sensitivity of X_{n+1} or the overall logarithmic gain with respect to the parameter of interest. For example, the

sensitivity of the system represented in Fig. 1 to a change in the parameter h_{kk} is

$$S_{L^k_{n+1,0}h_{kk}} = g_{1n}g_{p,p-1}h_{n-1,n-1}h_{n+1,n+1}h_{nk}/(h_{kk}\Delta^{pk}), \qquad p \leqslant k, \quad (55)$$

$$= g_{1n}h_{p-1,p-1}h_{n-1,n-1}h_{n+1,n+1}h_{nk}/(h_{kk}\Delta^{kp}), \qquad p > k. \quad (56)$$

For the corresponding system in which X_{n-1} is the feedforward modifier

$$S_{L^{n-1}_{n+1,0}h_{kk}} = 0. \quad (57)$$

Thus, a system in which X_k is the feedforward modifier will be more sensitive to changes in the parameter h_{kk} than will systems in which other intermediates are the feedforward modifier.

Similar calculations show that, when equations (53) and (54) are satisfied, the systems are equally sensitive to changes in all the parameters they have in common, except h_{kk} and $h_{n-1,n-1}$ if $p \leqslant k$, and h_{kk}, $h_{n-1,n-1}$, $g_{p,p-1}$ and $h_{p-1,p-1}$ if $p > k$.

8. Stability

The stability of the system represented in Fig. 1 will be examined with respect to small disturbances about a steady state. As in the previous paper (Savageau, 1979), the descriptive equations (1) can be linearized and the kinetic parameters made identical. In particular,

$$\begin{aligned} F_i &= F, \quad \text{for all } i, \\ h_{ii} &= h, \quad \text{for all } i < n, \\ h^0_{nn} &= h, \quad \text{and} \\ (h_{n+1,n+1} - h^0_{n,n+1}) &= h. \end{aligned} \quad (58)$$

The parameters with a zero superscript refer to the equivalent system without feedforward inhibition. When $p \leqslant k$, they are related to the corresponding parameters of the systems with feedforward inhibition:

$$h^0_{nn} = \left[\frac{g_{n,n-1}h_{kk}}{g_{n,n-1}h_{kk} - h_{nk}h_{n-1,n-1}}\right] h^k_{nn}, \quad (59)$$

$$h^0_{n,n+1} = \left[\frac{g_{n,n-1}h_{kk}}{g_{n,n-1}h_{kk} - h_{nk}h_{n-1,n-1}}\right] h^k_{n,n+1}. \quad (60)$$

When $p > k$,

$$h^0_{nn} = \left[\frac{g_{p,p-1}g_{n,n-1}h_{kk}}{g_{p,p-1}g_{n,n-1}h_{kk} - h_{p,p-1}h_{nk}h_{n-1,n-1}}\right] h^k_{nn}, \quad (61)$$

$$h^0_{n,n+1} = \left[\frac{g_{p,p-1}g_{n,n-1}h_{kk}}{g_{p,p-1}g_{n,n-1}h_{kk} - h_{p,p-1}h_{nk}h_{n-1,n-1}}\right] h^k_{n,n+1}. \quad (62)$$

Finally, if we define

$$g = g_{1n} g_{p,p-1} g_{n,n-1} h^{-3}, \tag{63}$$

then the resulting characteristic equation for the system in which X_k is the feedforward modifier can be written:

$$\begin{aligned}[(\lambda+Fh)^n - g(Fh)^n] + Fh^0_{n,n+1} Fh(\lambda+Fh)^{n-2} \\ - \frac{h_{nk} Fh}{g_{n,n-1}} [(\lambda+Fh)^{n-1} - g(Fh)^k(\lambda+Fh)^{n-k-1}] \\ + Fh^0_{n,n+1} \frac{h_{nk} Fh}{g_{n,n-1}} (\lambda+Fh)^{n-2} \left[\frac{(\lambda+Fh)^{n-k}}{(Fh)^{n-k-2}} - 2 \right] = 0, \end{aligned} \tag{64}$$

when $p \leqslant k$. The same equation, except with $g_{n,n-1}$ replaced by $(g_{p,p-1} g_{n,n-1}/h)$, applies when $p > k$.

There is no obvious solution to equation (64). However, solutions can be obtained by a perturbation analysis based upon the solution for a system without feedforward inhibition ($h_{nk} = 0$) and with an irreversible synthetase reaction ($h_{n,n+1} = 0$). Under these latter conditions, equation (64) reduces to

$$(\lambda+Fh)^n - g(Fh)^n = 0 \tag{65}$$

and has solutions

$$\lambda_0 = (Fh)[(-1)^{1/n}(-g)^{1/n} - 1]. \tag{66}$$

The solutions for equation (64) are assumed to have the form of a power series in two variables

$$\lambda = \lambda_0 + \lambda_{11}\varepsilon + \lambda_{12}\delta + \lambda_{21}\varepsilon^2 + \lambda_{22}\delta^2 + \lambda_{23}\varepsilon\delta + \ldots, \tag{67}$$

where

$$\varepsilon = -\frac{Fh^0_{n,n+1}}{n}$$

and

$$\begin{aligned} \delta &= -\frac{h_{nk} Fh}{n g_{n,n-1}}, \quad \text{when } p \leqslant k, \\ &= \frac{h_{nk} Fh^2}{n g_{p,p-1} g_{n,n-1}}, \quad \text{when } p > k. \end{aligned}$$

Substituting these solutions into equation (64) and setting the coefficients for each power of ε and δ to zero yields the following series of equations:

$$(\lambda_0 + Fh)^n - g(Fh)^n = 0. \tag{68}$$

$$\lambda_{11} - (Fh)(\lambda_0 + Fh)^{-1} = 0, \tag{69}$$

$$\lambda_{12}+1-g(Fh)^k(\lambda_0+Fh)^{-k}=0. \tag{70}$$

$$\vdots$$

These equations give the zero-order and first-order terms in the approximation, and if ε and δ are small, the higher-order terms can be neglected. The approximate solutions then are given by

$$\lambda=\lambda_0+\lambda_{11}\varepsilon+\lambda_{12}\delta. \tag{71}$$

If for convenience the reference system is chosen to be on the boundary of stability, i.e. $\mathrm{Re}\,(\lambda_0)=0$ for the root with the largest real part, then $g=-\sec^n(\pi/n)$ and for the solution obtained by perturbation analysis

$$\begin{aligned}\mathrm{Re}\,(\lambda)&=\varepsilon\,\mathrm{Re}\,(\lambda_{11})+\delta\,\mathrm{Re}\,(\lambda_{12})\\&=\varepsilon\cos^2(\pi/n)+\delta\,\mathrm{Re}\,\{g/[(-1)^{1/n}(-g)^{1/n}]^k-1\}\\&=\varepsilon\cos^2(\pi/n)+\delta\,\mathrm{Re}\,\{(-1)^{(n-k)/n}(-g)^{(n-k)/n}-1\}\\&=\varepsilon\cos^2(\pi/n)+\delta\{\sec^{n-k}(\pi/n)\cos[(n-k)\pi/n]-1\}\end{aligned} \tag{72}$$

for the root with the largest real part.

The first term in equation (72) represents the destabilizing effect of adding a reverse component to the synthetase reaction; this effect is the same for all systems regardless of which metabolite is the feedforward modifier.

The second term in equation (72) represents the stabilizing effect of feedforward inhibition. This term is plotted in Fig. 2, where the degree of stabilization is seen to increase as the position of the feedforward modifier becomes more proximal to the initial substrate. The stabilizing effect of

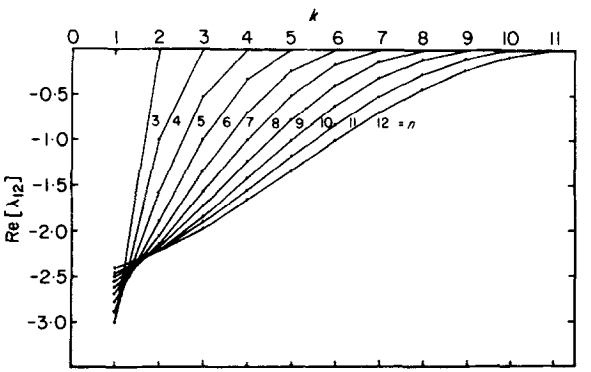

FIG. 2. Stabilizing effect of feedforward inhibition. The real part of λ_{12}, the contribution of feedforward inhibition to the root of the characteristic equation, is plotted as a function of k, the position of the feedforward modifier in the pathway, for pathways of different length n. The stabilizing influence increases as this contribution becomes more negative. See text for further discussion.

feedforward inhibition is as significant as that produced by shortening the path length either by physically reducing the number of reactions or by kinetically reducing the effective path length (Savageau, 1975, 1976). This is evident from a comparison of Re (λ_0) for shorter path lengths $(m \leqslant n)$

$$\begin{aligned} \text{Re}\,(\lambda_0) &= (-g)^{1/m}(-1)^{1/m} - 1 \\ &= \sec^{n/m}\left(\frac{\pi}{n}\right)\cos\left[\frac{n}{m}\left(\frac{\pi}{n}\right)\right] - 1 \end{aligned} \tag{73}$$

and Re (λ_{12}) for more proximal feedforward modifiers $(k \leqslant n-1)$

$$\text{Re}\,(\lambda_{12}) = \sec^{n-k}\left(\frac{\pi}{n}\right)\cos\left[\left(\frac{n-k}{n}\right)\pi\right] - 1. \tag{74}$$

In each case,

$$g = -\sec^{n}\left(\frac{\pi}{n}\right).$$

Equivalent effects are produced when

$$m = n/(n-k). \tag{75}$$

If we assume a marginally stable system of path length n, then the stabilizing effect of reducing the kinetically important steps to $m = 3$ is equivalent to that achieved by a feedforward modifier that is 2/3 of the way along the pathway $(k = 2n/3)$. That produced when $m = 2$ is equivalent to that achieved by having the feedforward modifier located in the middle of the pathway $(k = n/2)$. The stabilizing effect of having a single kinetically

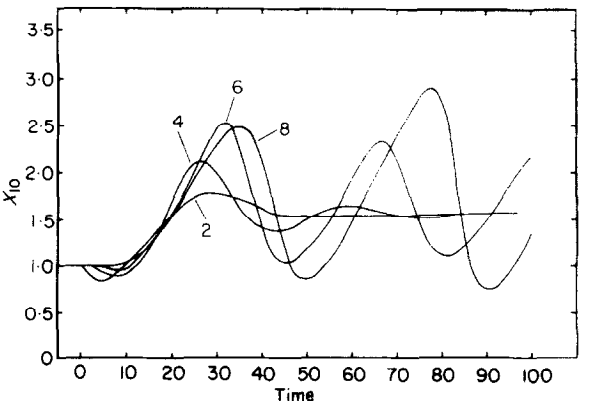

FIG. 3. Stabilizing effect of feedforward inhibition. The model in Fig. 1 is simulated with $n = 9, g = -1{\cdot}8, h_{9k} = -1{\cdot}0$, and various positions (k) of the feedforward modifier, as indicated. The independent concentration variable X_0 is perturbed at time zero. See text for further discussion.

important step ($m = 1$) is about the same as that achieved when the first intermediate in a long pathway is the feedforward modifier ($k = 1$).

These conclusions based on analysis also are supported by computer simulation of the non-linear systems. The techniques are those discussed elsewhere (Savageau, 1976). The systems represented by Fig. 1 are initially in a steady state with concentrations normalized to unity. At time zero the concentration of the initial substrate for each system is suddenly increased and maintained at a constant elevated level for the subsequent time period. The response of the concentration of the activated end product (X_{n+1}) is shown as a function of time in Fig. 3. The stabilizing effect of feedforward inhibition shown in Fig. 3 is very similar to that shown by computer simulation of systems with shorter path lengths (see Fig. 7 in Savageau, 1975, or Fig. 11–7 in Savageau, 1976).

9. Temporal Responsiveness

Although there is no analytical method for determining the temporal responsiveness of these non-linear systems, we can gain an appreciation for their behavior according to this criterion by using the computer simulation techniques referred to in section 8. We have observed that the "response time" generally increases as the position of the feedforward metabolite approaches that of the penultimate product. The differences in response time can vary from slight to marked, depending upon the particular parameter values. A typical comparison is shown in Fig. 4. When the feedforward

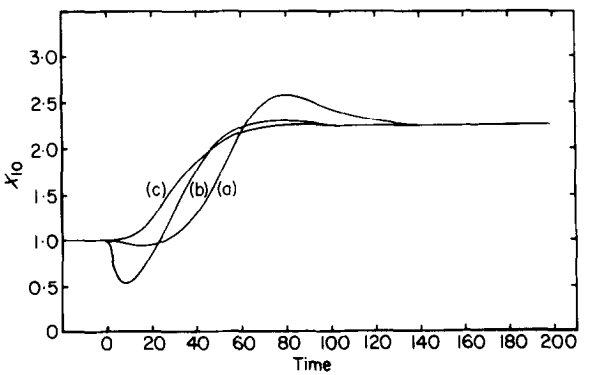

FIG. 4. Temporal responsiveness of equivalent systems having the feedforward modifier located in different positions. The model in Fig. 1 is simulated with (a) $n = 9$, $g = -0{\cdot}2$, $h_{98} = -1{\cdot}0$, i.e. the penultimate product X_8 is the feedforward modifier; (b) $n = 9$, $g = -0{\cdot}2$, $h_{92} = -1{\cdot}0$, i.e. the second intermediate is the feedforward modifier, and (c) without feedforward inhibition. See text for further discussion.

modifier is the penultimate product, the concentration of activated end product exhibits a slight decrease, then increases, overshoots the new steady-state value and finally decreases slowly to that value. By comparison, the equivalent system in which the second intermediate is the feedforward modifier exhibits a more pronounced "false start" but then recovers more rapidly and achieves the final steady state without an exaggerated overshoot. The response time, as measured by the time to reach one-half the final value, is 25% faster for this system. If the response time is measured as the time to reach 90% of the final value and remain within 10% of it, then this latter system has a response time 53% faster than that of the system in which the penultimate product is the feedforward modifier.

Although the response time would improve if the feedforward modifier were more proximal to the initial substrate of the pathway, the response time can approach but be no better than that of the equivalent system without feedforward inhibition. The response of the equivalent system without feedforward inhibition is also shown in Fig. 4.

10. Response to Addition of Exogenous Intermediates

The responses of the dependent concentrations to a change in concentration of an exogenous intermediate ($X_{p'}$) are given by the following expressions when $p \leqslant k$:

$$L^k_{ip'} = \frac{-g_{pp'}g_{1n}h_{p-1,p-1} \times \left[g_{n,n-1}(h^k_{n,n+1} - h_{n+1,n+1}) + h_{n-1,n-1}\dfrac{h_{nk}}{h_{kk}}h_{n+1,n+1} \right]}{h_{ii}\Delta^{pk}},$$

$$1 \leqslant i < p, \quad (76)$$

$$= \frac{g_{pp'}h_{p-1,p-1}h_{n-1,n-1}h^k_{nn}h_{n+1,n+1}}{h_{ii}\Delta^{pk}}, \qquad p \leqslant i < n, \quad (77)$$

$$= \frac{-g_{pp'}h_{p-1,p-1}\left[g_{n,n-1}(h^k_{n,n+1} - h_{n+1,n+1}) + h_{n-1,n-1}\dfrac{h_{nk}}{h_{kk}}h_{n+1,n+1} \right]}{\Delta^{pk}},$$

$$i = n, \quad (78)$$

$$= \frac{g_{pp'}g_{n,n-1}h_{p-1,p-1}h^k_{nn}}{\Delta^{pk}}, \qquad i = n+1, \quad (79)$$

All the concentrations preceding X_p decrease, whereas all those following X_p increase, in response to an increase in $X_{p'}$.

The same set of equations, but with k replaced by $n-1$ and Δ^{pk} replaced by Δ^{n-1}, describes the equivalent system in which X_{n-1} is the feedforward modifier. By utilizing the constraints in equations (9) and (10), one finds that

$$L^{k}_{ip'} = L^{n-1}_{ip'}, \quad 1 \leqslant i \leqslant n+1. \tag{80}$$

Thus feedforward inhibition has no effect upon the system's response to additions of exogenous intermediates, provided the position of the added intermediate is before that of the feedforward modifier. In particular, the concentration of activated end product must increase in response to an addition of exogenous intermediate, contrary to the behavior that has been attributed to this type of model of feedforward inhibition in the arginine biosynthetic pathway of *Chlamydomonas reinhardi* (Sussenbach and Strijkert, 1969).

However, if the model in Fig. 1 is modified by the inclusion of an alternative reaction for the utilization of X_n, one obtains a model that is capable of explaining the experimental results of Sussenbach and Strijkert (1969). By following the arguments presented in the previous paper (Savageau, 1979), one finds that the change in the concentration of activated end product resulting from an addition of exogenous intermediate ($p \leqslant k$) is given by

$$\begin{aligned} L^{k}_{n+1,p'} &= \partial y_{n+1}/\partial y_{p'} \\ &= \frac{g_{pp'}h_{p-1,p-1}\left[g_{n,n-1}g_{n+1,n} + \dfrac{h_{n-1,n-1}}{h_{kk}}\left(h_{nn}g_{n+1,k} - g_{n+1,n}h_{nk}\right)\right]}{h_{p-1,p-1}h_{n-1,n-1}[h_{nn}(h_{n+1,n+1} - g_{n+1,n+1}) - h_{n,n+1}g_{n+1,n}] \\ &\qquad + g_{1n}g_{p,p-1}g_{n,n-1}(g_{n+1,n+1} - h_{n+1,n+1}) \\ &\qquad + g_{1n}g_{p,p-1}\frac{h_{n-1,n-1}}{h_{kk}}[g_{n+1,k}h_{n,n+1} - h_{nk}(g_{n+1,n+1} - h_{n+1,n+1})]} \end{aligned} \tag{81}$$

Thus, if

$$g_{n,n-1} > \frac{h_{n-1,n-1}}{h_{kk}}\left[h_{nk} - \frac{h_{nn}}{g_{n+1,n}}g_{n+1,k}\right], \tag{82}$$

then

$$L^{k}_{n+1,p'} = \frac{\partial y_{n+1}}{\partial y_{p'}} > 0 \tag{83}$$

and the concentration of activated end product will increase. However, if

$$g_{n,n-1} < \frac{h_{n-1,n-1}}{h_{kk}}\left[h_{nk} - \frac{h_{nn}}{g_{n+1,n}}g_{n+1,k}\right], \tag{84}$$

then

$$L^k_{n+1,p'} = \frac{\partial y_{n+1}}{\partial y_{p'}} < 0 \tag{85}$$

and the concentration of activated end product will decrease in response to an addition of $X_{p'}$. [See Savageau (1979) for further discussion.]

Now let us return to the situation in which X_k is the feedforward modifier and in which $p > k$. The expressions corresponding to equations (76) through (79) are

$$L^k_{ip'} = \frac{-g_{pp'}g_{1n}g_{n,n-1}h_{p-1,p-1}(h^k_{n,n+1} - h_{n+1,n+1})}{h_{ii}\Delta^{kp}}, \quad 1 \leqslant i < p, \tag{86}$$

$$= \frac{g_{pp'}h_{p-1,p-1}h_{n-1,n-1}h_{n+1,n+1}\left[h^k_{nn} + g_{1n}\dfrac{h_{nk}}{h_{kk}}\right]}{h_{ii}\Delta^{kp}}, \quad p \leqslant i < n, \tag{87}$$

$$= \frac{-g_{pp'}g_{n,n-1}h_{p-1,p-1}(h^k_{n,n+1} - h_{n+1,n+1})}{\Delta^{kp}}, \quad i = n, \tag{88}$$

$$= \frac{g_{pp'}g_{n,n-1}h_{p-1,p-1}\left[h^k_{nn} + g_{1n}\dfrac{h_{nk}}{h_{kk}}\right]}{\Delta^{kp}}, \quad i = n+1. \tag{89}$$

When these expressions are compared with those for the equivalent system having X_{n-1} for the feedforward modifier, by using the constraints in equations (12) and (13), one finds

$$L^k_{ip'} > L^{n-1}_{ip'}, \quad 1 \leqslant i < p, \tag{90}$$

$$L^k_{ip'} > L^{n-1}_{ip'}, \quad p \leqslant i < n, \tag{91}$$

$$L^k_{ip'} < L^{n-1}_{ip'}, \quad i = n, \tag{92}$$

$$L^k_{ip'} > L^{n-1}_{ip'}, \quad i = n+1. \tag{93}$$

Thus, if the position of the added intermediate is after that of the feedforward metabolite, then (1) the synthesis of intermediates is less effectively spared and (2) the increase in X_{n+1} relative to that in X_n is greater when compared to that of systems in which the position of the added intermediate is before that of the feedforward modifier.

Now, even if the model in Fig. 1 is modified by the inclusion of an alternative reaction for the utilization of X_n, the concentration of activated end product will increase in response to an addition of exogenous intermediates ($p > k$). This can be seen from the appropriate logarithmic gain expression:

$$L^{k}_{n+1,p'} = \frac{g_{pp'}h_{p-1,p-1}g_{n,n-1}[g_{n+1,n}+(g_{1n}/h_{kk})g_{n+1,k}]}{h_{p-1,p-1}h_{n-1,n-1}}$$
$$\times\left[h_{nn}(h_{n+1,n+1}-g_{n+1,n+1})+\frac{h_{n-1,n-1}}{h_{kk}}(h_{nn}g_{n+1,k}-g_{n+1,n}h_{nk})\right]$$
$$+g_{1n}g_{p,p-1}[g_{n,n-1}(g_{n+1,n+1}-h_{n+1,n+1})]$$
$$+g_{1n}h_{p-1,p-1}\frac{h_{n-1,n-1}}{h_{kk}}[g_{n+1,k}h_{n,n+1}-h_{nk}(g_{n+1,n+1}-h_{n+1,n+1})] \quad (94)$$

which is always positive for a stable system.

11. Discussion

The results in the previous section show that feedforward inhibition of the aminoacyl-tRNA synthetase by intermediates in the pathway could perform the two functions discussed in the previous paper (Savageau, 1979): (1) The system is able to distinguish the exogenous addition of a metabolite that follows the feedforward modifier in the pathway from the exogenous addition of one that precedes it, and from various endogenous influences. (2) The system preferentially diverts toward protein synthesis the increment in end product produced by the addition of a metabolite that follows the feedforward modifier, whereas it preferentially diverts to the alternative fate the increment produced by the addition of a metabolite that precedes the feedforward modifier. [See Savageau (1979) for further discussion.]

In contrast to these rather speculative possibilities, feedforward inhibition of a synthetase by an intermediate in the pathway can perform the *essential* functions of stabilizing the system and improving its temporal responsiveness. In section 8, we found that the stability of the system increased as the position of the feedforward modifier approached that of the initial substrate. This stabilizing effect is comparable to that produced by other well-known stabilizing influences in biosynthetic pathways controlled by end-product inhibition (Savageau, 1975). In section 9, we found that temporal responsiveness also improved as the position of the feedforward modifier approached that of the initial substrate. According to all the other criteria for functional effectiveness listed in section 2 of the preceding paper (Savageau, 1979), the performance of these biosynthetic systems is essentially unaffected by the position of the feedforward modifier in the pathway.

These results suggest that the optimal position in the pathway for the feedforward modifier is the first, i.e. the product of the first enzyme in the pathway. This would result in a special relationship between the first enzyme in the pathway and the corresponding aminoacyl-tRNA synthetase—

reactant(s) of the first enzyme would be (feedforward) modifiers of the synthetase and reactant(s) of the synthetase would be (feedback) modifiers of the first enzyme. One might expect that the performance of these essential functions would be further improved if these two enzymes were located in a common, multienzyme complex, thereby minimizing the time for the reactant(s) of the first enzyme to reach the synthetase by diffusion.

Feedforward inhibition of synthetases by intermediates has been reported in the arginine pathway of *Escherichia coli* (Williams *et al.*, 1973), although Charlier & Gerlo (1976) were unable to confirm these findings by using partially purified synthetase *in vitro*. Barthelmess *et al.* (1974) also found no evidence of such inhibition *in vivo* in *Neurospora crassa*, whereas Spurgeon & Matchett (1977) reported such inhibition for the histidyl-tRNA synthetase of *Neurospora* both *in vivo* and *in vitro*. In none of these reports has an effect, or the absence of an effect, of the product of the first enzyme in the pathway been mentioned.

Evidence suggesting some relation, direct or indirect, between the first enzyme and the synthetase of the isoleucine pathway recently has been reported by Williams *et al.* (1977): the physical stability of the isoleucyl-tRNA synthetase is decreased by mutations in the structural gene for the first enzyme or by deletion of this structural gene. However, it is not clear whether the normal *in vivo* stability of the synthetase is provided by the first enzyme itself, the reaction product(s) of the first enzyme, or a subsequent intermediate in the pathway.

There is no *direct* experimental evidence for or against feedforward inhibition of the aminoacyl-tRNA synthetase by the product of the first enzyme in a biosynthetic pathway undoubtedly because it has never been looked for. The results of the analysis in this paper are the first to provide a rationale for such inhibition and hopefully some motivation for a systematic experimental search.

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