Integrated Function of a Kinetic Proofreading Mechanism: Dynamic Analysis Separating the Effects of Speed and Substrate Competition on Accuracy†

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ABSTRACT: All of the data relating to isoleucyl-tRNA synthetase and its proofreading of valyl-tRNA^{Ile} have been integrated into a single model whose dynamic behavior has been determined by numerical solution of the relevant kinetic equations. The results indicate that (1) the system normally operates in vivo with amino acid concentrations slightly above the apparent K_m of the system, (2) increases in the displacement of reactants from thermodynamic equilibrium increase the net reaction velocity when the appropriate nominal parameter values are selected, (3) the cost of proofreading decreases with an increase in displacement of reactants from equilibrium, (4) accuracy and reaction velocity tend to be inversely related when substrate competition is unchanged but directly related or unrelated when substrate competition is altered, (5) changes in substrate competition are about twice as effective as changes in reaction velocity in altering the overall accuracy of aminoacylation, and (6) simultaneous changes in substrate competition and reaction velocity have a cumulative but not additive effect upon accuracy. With regard to the temporal development of errors and proofreading costs, we have seen two different patterns. In one, errors or costs gradually change with time following an abrupt alteration; in the other, errors or costs change dramatically in one direction and then more slowly reverse themselves. In all cases, the system responds to change quickly (<0.02–0.2 s) but shows no tendency to oscillate.

The dynamic behavior of kinetic proofreading mechanisms has not been examined. Previous studies have discussed the relationship between accuracy and speed or flux through the system (Hopfield, 1974; Ninio, 1975; Gavlilova et al., 1976; Hopfield et al., 1976; Mulvey & Fersht, 1977; Savageau & Freter, 1979a; Yarus, 1979; Fersht, 1981). However, it is clear that these discussions concern the behavior and design of the system in a series of steady states and not true dynamic behavior. In related discussions concerning the effects of substrate competition (Yarus & Thompson, 1983), the coupling of speed and substrate competition has been discussed. For example, an increase in the cognate amino acid will decrease the competition with the noncognate amino acid for aminoacyl-tRNA synthetase but at the same time increase the flux through the system. What is the contribution of each of these effects to the net change in accuracy that is observed? Blomberg (1977) has used a linear approximation to explore the accuracy vs. speed question. His results suggest that accuracy and speed are inversely related in the Michaelis–Menten model examined.

In the previous paper (Okamoto & Savageau, 1984) we have used experimental measurements made in vitro and in vivo to completely characterize the kinetic parameters of a specific proofreading mechanism: the isoleucyl-tRNA synthetase of *Escherichia coli* proofreading valyl-tRNA^{Ile}. We are therefore in a position to examine the dynamic behavior of the intact system by numerical solution of the kinetic equations that characterize the system. By using these methods, we are able to separate the effects of substrate competition and speed upon the accuracy of the system. We also are able to demonstrate the temporal development of these effects in response to a sudden change in competition or speed of operation and to assess the stability of the system.

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Model

In the previous paper we employed the accepted Michaelis–Menten mechanism for single-stage proofreading of valyl-tRNA^{Ile} by the isoleucyl-tRNA synthease of *Escherichia coli*. This mechanism is shown in Figure 2 of the previous paper. The X's represent reactants (and their concentrations) and the k's represent elementary rate constants of the associated reactions.

The general macroscopic parameters that characterize such systems are defined in terms of the pattern of net fluxes (Savageau & Freter, 1979b; Freter & Savageau, 1980), and for the specific case shown in Figure 2 of the previous paper, these general macroscopic parameters can be related to the underlying macroscopic parameters as follows: initial discrimination ratio

$$I = (k_3X_1X_3 - k_{-3}X_5)/(k_4X_1X_4 - k_{-4}X_6) \quad (1)$$

proofreading discrimination ratio

$$P = [k_2X_8/(k_8X_8 - k_{-8}X_1X_{10})]/[k_1X_7/(k_7X_7 - k_{-7}X_1X_9)] \quad (2)$$

net recycling flux

$$R = k_1X_7 + k_2X_8 \quad (3)$$

net forward flux

$$F = k_9X_9 + k_{10}X_{10} \quad (4)$$

net error

$$E = k_{10}X_{10}/(k_9X_9 + k_{10}X_{10}) \quad (5)$$

cost of proofreading

$$C = R/F = (k_1X_7 + k_2X_8)/(k_9X_9 + k_{10}X_{10}) \quad (6)$$

The dynamic behavior of these macroscopic parameters thus will be determined by the kinetic equations of the system, which can be written as:

$$\dot{X}_1 = (k_1 + k_7)X_7 + (k_2 + k_8)X_8 + k_{-3}X_5 + k_{-4}X_6 - (k_3X_3 + k_4X_4 + k_{-7}X_9 + k_{-8}X_{10})X_1 \quad (7)$$

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$$\dot{X}_2 = (k_1 + k_{-5})X_7 + (k_2 + k_{-6})X_8 + k_9X_9 + k_{10}X_{10} - (k_5X_5 + k_6X_6)X_2 \quad (8)$$

$$\dot{X}_3 = f_1 + k_1X_7 + k_{-3}X_5 - k_3X_1X_3 \quad (9)$$

$$\dot{X}_4 = f_2 + k_2X_8 + k_{-4}X_6 - k_4X_1X_4 \quad (10)$$

$$\dot{X}_5 = k_3X_1X_3 + k_{-5}X_7 - (k_{-3} + k_5X_2)X_5 \quad (11)$$

$$\dot{X}_6 = k_4X_1X_4 + k_{-6}X_8 - (k_{-4} + k_6X_2)X_6 \quad (12)$$

$$\dot{X}_7 = k_5X_2X_5 + k_{-7}X_1X_9 - (k_1 + k_{-5} + k_7)X_7 \quad (13)$$

$$\dot{X}_8 = k_6X_2X_6 + k_{-8}X_1X_{10} - (k_2 + k_{-6} + k_8)X_8 \quad (14)$$

$$\dot{X}_9 = k_7X_7 - (k_{-7}X_1 + k_9)X_9 \quad (15)$$

$$\dot{X}_{10} = k_8X_8 - (k_{-8}X_1 + k_{10})X_{10} \quad (16)$$

Unless indicated otherwise, the numerical values of the kinetic parameters and steady-state concentrations will be those established in the previous paper (Okamoto & Savageau, 1984) and summarized there in Tables II and III. From those values and eq 1–6 given above, one can calculate the values for the six macroscopic parameters: $I = 36$, $P = 1100$, $R = 1.86$ μM s^{-1}, $F = 20$ μM s^{-1}, $C = 0.0931$, and $E = 4.14 \times 10^{-4}$.

Method of Numerical Solution. Although eq 7–16 can be solved directly by using available numerical routines, the cost is prohibitive because these equations are very "stiff". That is to say, with the parameter values determined in the previous paper, the "relaxation" time for the individual reactants varies over a wide spectrum; some reactants respond to change very quickly, while others respond very slowly. Even using numerical routines specifically designed to deal with such stiff systems of equations (Gear, 1971, 1981), we have found numerical solution of eq 7–16 to be costly.

We have therefore used an alterntive approach that has proved superior for our purposes. First, we have converted eq 7–16 into the corresponding equations in the power-law formalism (Savageau, 1976; Savageau & Voit, 1982):

$$\dot{X}_1 = \alpha_1X_5^{g_{15}}X_6^{g_{16}}X_7^{g_{17}}X_8^{g_{18}} - \beta_1X_1^{h_{11}}X_3^{h_{13}}X_4^{h_{14}}X_9^{h_{19}}X_{10}^{h_{1,10}} \quad (17)$$

$$\dot{X}_2 = \alpha_2X_7^{g_{27}}X_8^{g_{28}}X_9^{g_{29}}X_{10}^{g_{2,10}} - \beta_2X_2^{h_{22}}X_5^{h_{25}}X_6^{h_{26}} \quad (18)$$

$$\dot{X}_3 = \alpha_3X_5^{g_{35}}X_7^{g_{37}} - \beta_3X_1^{h_{31}}X_3^{h_{33}} \quad (19)$$

$$\dot{X}_4 = \alpha_4X_6^{g_{46}}X_8^{g_{48}} - \beta_4X_1^{h_{41}}X_4^{h_{44}} \quad (20)$$

$$\dot{X}_5 = \alpha_5X_1^{g_{51}}X_3^{g_{53}}X_7^{g_{57}} - \beta_5X_2^{h_{52}}X_5^{h_{55}} \quad (21)$$

$$\dot{X}_6 = \alpha_6X_1^{g_{61}}X_4^{g_{64}}X_8^{g_{68}} - \beta_6X_2^{h_{62}}X_6^{h_{66}} \quad (22)$$

$$\dot{X}_7 = \alpha_7X_1^{g_{71}}X_2^{g_{72}}X_5^{g_{75}}X_9^{g_{79}} - \beta_7X_7^{h_{77}} \quad (23)$$

$$\dot{X}_8 = \alpha_8X_1^{g_{81}}X_2^{g_{82}}X_6^{g_{86}}X_{10}^{g_{8,10}} - \beta_8X_8^{h_{88}} \quad (24)$$

$$\dot{X}_9 = \alpha_9X_7^{g_{97}} - \beta_9X_1^{h_{91}}X_9^{h_{99}} \quad (25)$$

$$\dot{X}_{10} = \alpha_{10}X_8^{g_{10,8}} - \beta_{10}X_1^{h_{10,1}}X_{10}^{h_{10,10}} \quad (26)$$

The apparent kinetic orders (g_{ij} and h_{ij}) and the apparent rate constants (α_i and β_i) are determined by the experimental data. It previously has been shown that this power-law formalism provides an accurate description of biochemical systems provided the excursions from the normal steady-state operating values are not too large (Savageau, 1976).

Second, we have explicitly evaluated the relaxation times for the concentration variables in our system, identified those with the shortest relaxation time, and assumed the corresponding equations in the set 17–26 to be in quasi steady state. This latter assumption allowed us to convert the differential equations in question into a corresponding subset of algebraic equations, leaving a smaller set of differential equations to solve that were less stiff than the original set.

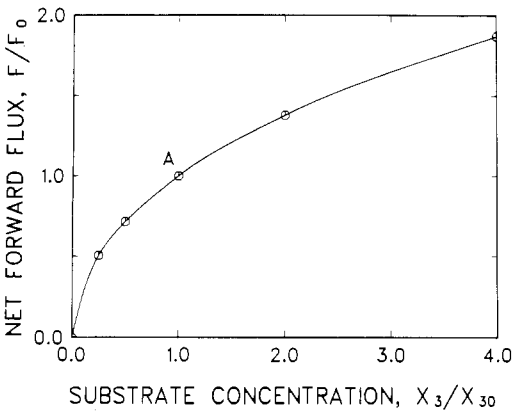

FIGURE 1: Flux through the system as a function of substrate concentration. The net forward flux F is normalized with respect to its nominal value ($F_0 = 20.0$ μM s^{-1}). The ratio of substrate concentrations X_4/X_3 is maintained at the nominal value of 5.64 while the concentrations are varied in parallel. The value of X_3 is shown normalized with respect to its nominal value ($X_{30} = 550$ μM). Thus, the nominal operating point for this system is (1, 1), the point marked A.

Third, the total concentration of synthetase and the total concentration of tRNA may be considered constants throughout the period of time during which we have examined the dynamic behavior. Finally, for convenience, we have considered the concentrations of the cognate and noncognate amino acids as independent or "input" variables of the system.

The consequences of this approach have been to reduce the number of differential equations from 10 to 4 typically. These remaining differential equations have been solved by using the Runge–Kutta–Fehlberg method as previously described (Savageau, 1976), with the modification that the quasi-steady-state algebraic equations are solved simultaneously with the differential equations. In comparison with the direct solution of eq 7–16, the above procedure is approximately 5 times faster (less costly) while maintaining absolute errors within 2%.

Results

Effect of Speed on Accuracy. Throughout this section on speed vs. accuracy, we shall maintain the ratio of noncognate to cognate amino acid concentrations at 5.6; any change in these concentrations will be in parallel so as to eliminate any effect due to changes in competition for substrates. As the substrate concentrations are varied, the steady-state flux through the system changes as shown in Figure 1. Point A is the standard value as given under Model. These data show that the system normally operates in the midrange of the rate vs. substrate curve, and since the enzyme is not saturated, we can fruitfully go on to examine the dynamic response to changes in rate. In the following sections, we shall consider perturbations about the standard point A, although qualitatively similar results have been obtained about alternative operating values.

(A) Effects of Changes in Substrate Concentrations. The effects upon the macroscopic fluxes in the system that result from doubling or halving the substrate concentrations are shown in Figure 2. The ordinate in each panel represents the relative change from the nominal value. As can be seen from this figure, an increase in substrate concentrations leads to rapid increases in several of the macroscopic parameters. The initial discrimination ratio I subsequently decreases to a steady-state value that is slightly above its predisturbance steady state. The proofreading discrimination ratio P also decreases subsequently, but to a steady-state value that is below

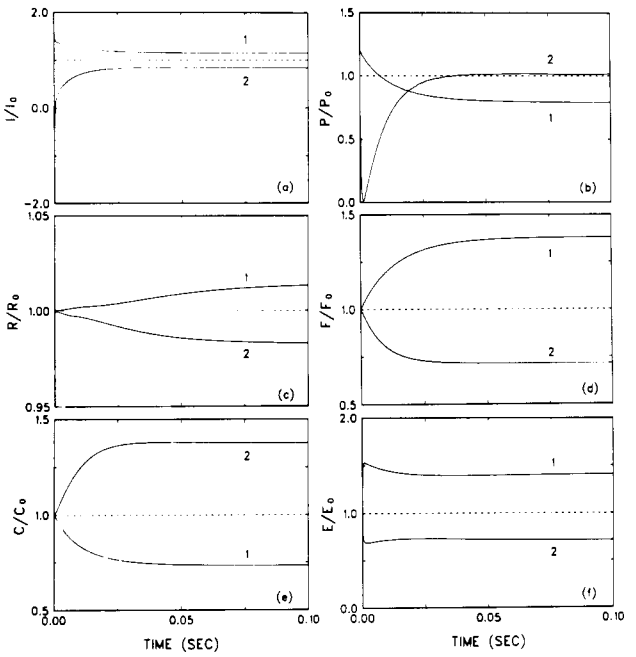

FIGURE 2: Effects of speed on accuracy: changes in speed produced by alterations in substrate concentrations that leave substrate competition unaffected. (Case 1) Speed of operation is increased by doubling the nominal substrate concentration of both cognate (X_3) and noncognate (X_4) amino acids at time zero. (Case 2) Speed of operation is decreased by halving the nominal substrate concentrations of both cognate and noncognate amino acids. The following macroscopic parameters of the system are plotted as a function of time following the change in substrate concentrations: initial discrimination ratio I (panel a), proofreading discrimination ratio P (panel b), net recycling flux R (panel c), net forward flux F (panel d), cost of proofreading C (panel e), and net error E (panel f). The macroscopic parameters are normalized with respect to their nominal values (see Model).

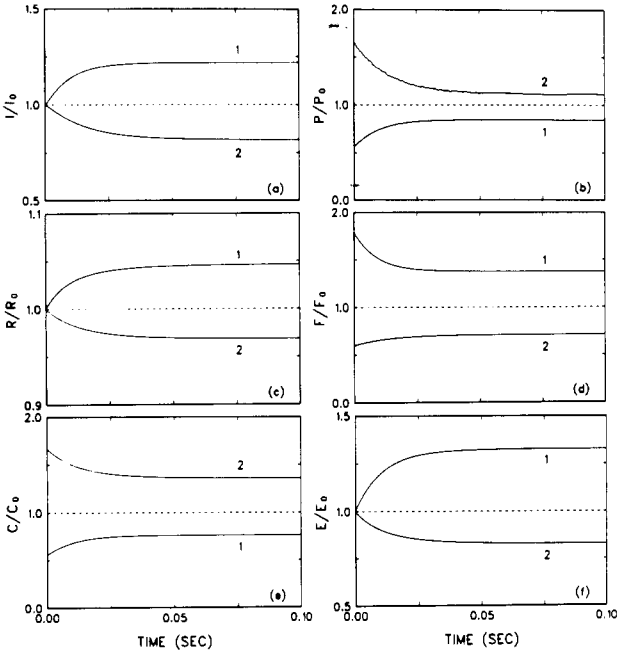

FIGURE 3: Effects of speed on accuracy: changes in speed produced by alterations in demand for product that leave substrate competition unaffected. (Case 1) Speed of operation increased by a 79% increase in both the rate constants k_9 and k_{10} at time zero. (Case 2) Speed of operation decreased by a 40% decrease in both the rate constants k_9 and k_{10}. Panels a–f are as described in Figure 2.

its predisturbance steady state. The net recycling flux R and net forward flux F increase at a slower rate and asymptotically approach steady-state values that are higher than their nominal steady state. Since F increases more than R, the cost of proofreading C decreases as shown in Figure 2e. This reduction in the cost or extent of proofreading, together with the reduction in the proofreading discrimination ratio, causes the error to increase as shown in Figure 2f.

The responses to a decrease in substrate concentrations are nearly the mirror images of the responses to an increase; quantitatively, the magnitudes of the responses differ because of the nonlinearity of the system. From these results, we conclude that speed and accuracy are inversely related: The higher the value of F (net forward flux), the lower the accuracy (the higher the error E).

(*B*) *Effects of Changes in Demand for Product.* Flow through the system also can be influenced by the demand for product. Hence, we have examined the pattern of macroscopic fluxes in response to an increase or decrease of the rate constants k_9 and k_{10} (where $k_9 = k_{10}$). Although the time course of the changes is different from that seen in Figure 2, the steady-state results are much the same. When demand is increased, the initial discrimination ratio I and the recycling flux R increase continuously and asymptotically approach elevated steady-state values. The proofreading discrimination ratio P decreases rapidly and then slowly returns to a new steady state that is lower than its original value. The net forward flux F rapidly increases and then decreases to a new steady state that is higher than its predisturbance value. Since F increases more than R, the cost of proofreading C decreases as shown in Figure 3e. This reduction in the cost or extent of proofreading, together with the reduction in the proofreading discrimination ratio P, causes the error to increase as shown in Figure 3f. The error increases despite an increase in the initial discrimination ratio (Figure 3a).

Table I: Substrate Concentrations That Maintain the Same Net Forward Flux through the System

substrate concn (μM)			
cognate (X_3)	noncognate (X_4)	ratio X_4/X_3	net forward flux (F) (μM s^{-1})
550.05	1550.0	2.8179	20.008
550.00	3100.0	5.6364	20.008
549.85	6200.0	11.2757	20.008

The responses to a decrease in demand for product are nearly the mirror images of the responses to an increase. Again, speed and accuracy are inversely related.

Effect of Substrate Competition on Accuracy. In order to separate these effects from those due to changes in flux through the system, we shall vary the ratio of noncognate to cognate substrate concentrations in such a way as to maintain the same net forward flux F. Table I shows the values of substrate concentrations that will approximately double or halve their ratio while maintaining the nominal value for the net forward flux.

The dynamic response to a sudden increase in the ratio of noncognate to cognate substrate concentrations is shown in Figure 4. The initial discrimination ratio I drops precipitously and then slowly increases to a steady-state value that is about half its original value. The proofreading discrimination ratio P initially increases, then decreases below its original value, and finally increases to a steady-state value that is slightly below its original value. The net recycling flux R increases continually to its new steady-state value, while the net forward flux F increases transiently and then returns to its original steady-state value. As a result of the increase in recycling flux with constant forward flux, the cost of proofreading increases (Figure 4e). Although the cost or extent of proofreading is

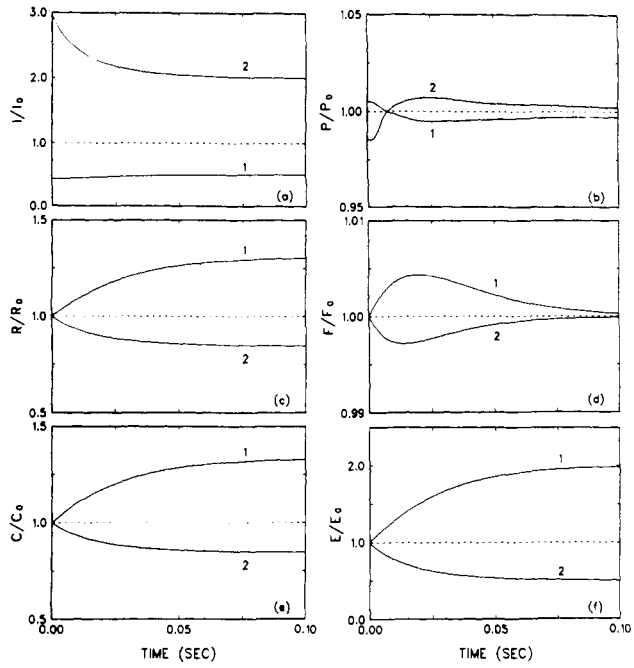

FIGURE 4: Effects of substrate competition on accuracy. Changes in substrate competition are produced by altering the relative concentrations of the substrates in such a manner that the net forward flux remains unchanged in the steady state. (Case 1) At time zero the concentration of the noncognate amino acid (X_4) is doubled and that of the cognate amino acid (X_3) decreased by an amount that leaves the net forward flux unchanged in steady state. The ratio X_4/X_3 = 11.28. (Case 2) At time zero the concentration of the noncognate amino acid is halved and that of the cognate amino acid increased by an amount that leaves the net forward flux unchanged in steady state. The ratio X_4/X_3 = 2.82. Panels a–f are as described in Figure 2.

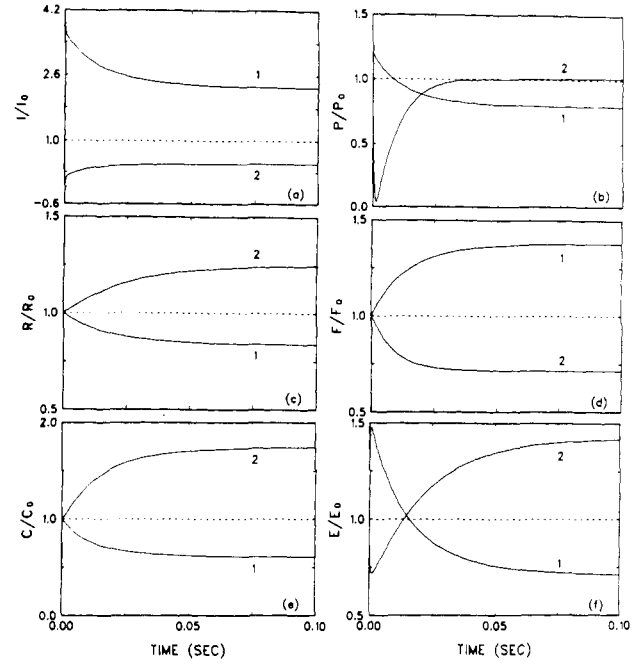

FIGURE 5: Combined effects of speed and substrate competition on accuracy. Changes in speed of operation and substrate competition are produced by altering the concentration of cognate amino acid (X_3) while the concentration of the noncognate amino acid (X_4) remains fixed. (Case 1) At time zero the concentration of the cognate amino acid is doubled; this increases the speed of operation and decreases the competition by the noncognate amino acid. (Case 2) At time zero the concentration of the cognate amino acid is halved; this decreases the speed of operation and increases the competition by the noncognate amino acid. Panels a–f are as described in Figure 2.

increased and although the proofreading discrimination ratio is little changed, the large decrease in the initial discrimination ratio leads to a corresponding increase in the net error E (Figure 4f).

The responses to a decrease in the noncognate to cognate ratio are nearly the mirror images of those we have just discussed; the magnitudes of the responses are of course different because of the nonlinearity of the system. From these results, we conclude that substrate competition is a critical determinant of accuracy, independent of any changes in net forward flux.

Combined Effects of Speed and Substrate Competition on Accuracy. One sees in Figure 2 that error is decreased 30% by a 50% reduction in substrate concentration while one sees in Figure 4 that error is descreased 50% by a 50% reduction in noncognate competition. Alternatively, one sees in Figure 2 that error is increased 40% by a 2-fold increase in substrate concentration, while one sees in Figure 4 that error is increased 100% by a 2-fold increase in noncognate competition. Thus, when considered separately, substrate competition is on the average about twice as effective as substrate concentration (speed) in influencing accuracy.

The combined effects of a change in substrate competition and a change in substrate concentrations are difficult to predict. The results of such a combined challenge are shown in Figure 5. The concentration of the cognate amino acid is suddenly doubled at time zero and then maintained at the elevated level; the concentration of the noncognate amino acid is unchanged. The result is an increase in flux through the system and a decrease in noncognate competition. The responses of P, F, and C in Figure 5 are much the same as they were in Figure 2 (when the concentration of both substrates was changed in parallel). However, the initial discrimination ratio I in Figure 5a is affected to a greater extent than it was in Figure 2a, and the recycling flux R behaves in exactly the opposite fashion in Figures 5c and 2c. The result of these differences is in turn manifested in the net error E (compare Figure 5f with Figure 2f).

The effects of speed and substrate competition on the steady-state value of E appear to be cumulative but not additive. For example, doubling the concentration of substrates increases the error by a factor of 1.4 (Figure 2f), and halving the competition by noncognate substrate decreases the error by a factor of 0.5 (Figure 4f), making both changes simultaneously decreases the error to 0.7 of its nominal value (Figure 5f), which is the cumulative result one would predict by making the two changes sequentially in either order ($1 \times 1.4 \times 0.5 = 1 \times 0.5 \times 1.4 = 0.7$).

Alternative Operating Points on the Cost–Accuracy Curve. In the previous paper (Okamoto & Savageau, 1984), we have shown that the steady-state behavior of the isoleucyl-tRNA synthetase system is relatively insensitive to variations in accuracy, E, and to variations in the ratio of cognate flux recycled to cognate flux into protein, a/b (or, equivalently, proofreading cost C). Nevertheless, the dynamic behavior of this system might still be strongly affected by the values of these parameters. To examine this possibility, we have altered the nominal values of these two parameters within the allowable range determined in the previous paper and thereby determined alternative operating points on the cost–accuracy curve [see Savageau & Freter (1979a)]. Aside from the difference in operating point, equivalence in all other respects was maintained to achieve well-controlled comparisons.

The previous experiment, in which we suddenly doubled the concentration of both cognate and noncognate amino acids and then observed the dynamic response of the system (Figure 2), was repeated for each of the operating points. The results

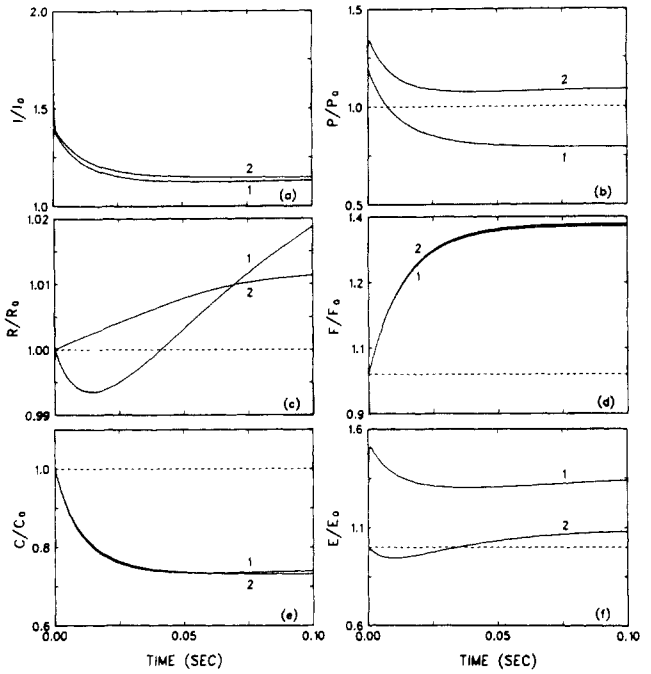

FIGURE 6: Behavior of systems with alternative operating points on the steady-state cost–accuracy curve. (Case 1) The system represented by the operating point with the lowest allowable value on the cost–accuracy curve. (Case 2) The system represented by the operating point with the highest allowable value on the cost–accuracy curve. At time zero the concentrations of both substrates of each system are doubled. Panels a–f are as described in Figure 2. (See text for further discussion.)

represented in Figure 6 show that the dynamic behavior of the systems operating at these points on the cost–accuracy curve is qualitatively similar in most respects. However, the response time with increased accuracy is somewhat faster, as can be seen most clearly in Figure 6c when the time course is extended (data not shown).

All the nominal values in Figure 6, with the exception of those for R, C, and E, are identical for cases 1 and 2. In case 1, $R_0 = 1.12\ \mu M\ s^{-1}$, $C_0 = 0.056$, and $E_0 = 8.86 \times 10^{-4}$; in case 2, $R_0 = 2.00\ \mu M\ s^{-1}$, $C_0 = 0.100$, and $E_0 = 3.78 \times 10^{-4}$. Because these differences in nominal values for R, C, and E do not appear in panels c, e, and f of Figure 6, care must be taken in interpreting these panels.

The recycling flux R in case 1 increases (after a transient reversal) to a relative value that is greater than that in case 2 (Figure 6c), but the absolute value of R in case 2 is initially 80% greater than that in case 1. The relative changes in proofreading costs are about the same for the two cases shown in Figure 6e; hence, the values of C for case 1 are always about 44% less than the corresponding values for case 2. On the other hand, the relative error in case 1 is significantly greater than that in case 2 (Figure 6f), and this is primarily due to differences in the proofreading discrimination ratios (Figure 6b). The absolute error in case 1 also is significantly greater than that in case 2 because the nominal value in case 1 is greater than that in case 2. Thus, systems operating toward the high cost–high accuracy end of the cost–accuracy curve appear to have a faster temporal response and an accuracy that is less influenced by changes in flux through the system.

Discussion

The value of integrating into a single model all of the data relating to a biochemical system such as the isoleucyl-tRNA synthetase proofreading mechanism has been clearly demonstrated with regard to steady-state behavior (Okamoto & Savageau, 1984). First, such integration provides a rigorous test for the consistency of the data. Indeed, the data for the isoleucine-tRNA synthetase mechanism were not self-consistent. Second, the analysis indicates which data are most suspect, and for the mechanism in question, the analysis revealed an error of 10–15-fold in a published measurement. Third, the analysis predicts values for rate constants and intermediate concentrations. These predictions provide estimates for parameter values that are difficult to determine directly or simply have yet to be measured. Finally, the analysis provides an indication of the relative importance of various parameters in determining the overall behavior of the system. This information is valuable as a guide in determining where future experimental effort might be focused most profitably.

In this paper, we have examined the dynamic behavior of the isoleucyl-tRNA synthetase mechanism and obtained information that bears on the following questions. (1) Where on the substrate–velocity curve does this mechanism normally operate in vivo? (2) How does the degree of displacement from equilibrium effect speed and accuracy of the reaction and cost of proofreading? (3) What is the relationship between speed of operation and accuracy? (4) How important is substrate competition in determining the overall accuracy of aminoacylation? (5) When in the period following a challenge (stress) is the system most vulnerable to error or heavy proofreading costs? (6) Is the system dynamically stable and temporally responsive to change?

Theoretical arguments suggest that enzymes in the cell would tend to operate in the midrange of their velocity vs. substrate curves; for a Michaelis–Menten mechanism, this would be the K_m value where the apparent kinetic order is 0.5 (Savageau, 1976). At lower substrate values the catalytic potential of the enzyme would be wasted, while at saturating levels of substrate the velocity of the reaction would be unresponsive to change. The normal steady-state operating values of the isoleucyl-tRNA synthetase system were determined in the previous paper, and the results in Figure 1 show that this system is in fact operating slightly above the apparent K_m for this system with an apparent kinetic order equal to 0.41. Having established that the normal operating values are reasonable, we could meaningfully inquire into the dynamic behavior of the system.

We have examined the dynamic behavior of the system in response to changes in substrate concentration or changes in product concentration, which we represent simply as a change in the apparent first-order rate constant for product removal. We have assumed throughout these studies that the levels of the adenylate compounds remain constant; in fact, their values have been absorbed into the relevant rate constants. Although it is often believed that an increase in displacement of reactants from thermodynamic equilibrium will lead to an increase in reaction velocity, this is not always true. An increased displacement in systems subject to feed-forward inhibition can lead to a reduction in reaction velocity (Savageau & Jacknow, 1979). In fact, an alternative set of parameter values for the Michaelis–Menten mechanism examined in this paper can produce a system in which an increased displacement of reactants from equilibrium produces no change or even a reduction in reaction velocity (data not shown). However, with the values established in the previous paper, this mechanism does respond to increased displacements with an increase in reaction velocity (Figures 1, 2d, 3d, and 5d). It also is widely assumed that increases in accuracy and proofreading costs require increases in the displacement of reactants from thermodynamic equilibrium. However, it is clear in the original

treatment of Hopfield (1974) that these results depend upon the values of the rate constants in the mechanism. Again, with specific reference to the Michaelis–Menten model and the parameter values estalished in the previous paper, we find that increased displacements lead to a decrease in accuracy and cost of proofreading when substrate competition is unchanged (Figures 2e,f and 3e,f) but to an increase in accuracy with a decrease in cost of proofreading when substrate competition is concomitantly diminished (Figure 5e,f).

An inverse relationship between accuracy and speed of operation has been suggested by many authors [e.g., Hopfield (1974), Ninio (1975), Blomberg (1977), and Savageau & Freter (1979a)]. For the system in this paper, we have found that speed and accuracy tend to be inversely related when the effects of substrate competition are eliminated (Figures 2d,f, 3d,f, and 6d,f). Speed and accuracy are transiently correlated in an inverse fashion when substrate competition is altered (Figures 4d,f and 5d,f), but this correlation is eliminated (Figure 4d,f) or reversed (Figure 5d,f) by the time a new steady state is reached.

The importance of substrate (tRNA as well as amino acid) competition in determining accuracy has been stressed consistently by Yarus (Yarus, 1972, 1979; Yarus & Thompson, 1984). In a recent review, Yarus & Thompson (1984) have explained some of the effects of substrate competition in terms of its effects upon the speed of the subsequent reaction. In this paper, we have taken pains to separate the effects of speed (Figures 2 and 3) and substrate competition (Figure 4). From these separate results, we have been able to show that changes in substrate competition are about twice as effective as changes in speed of operation when it comes to altering the overall accuracy of aminoacylation. Furthermore, when simultaneously changed, speed and substrate competition appear to have a cumulative but nonadditive effect upon accuracy (Figure 5), which can be predicted from the results of separate changes in speed and substrate competition.

There have been no detailed dynamic studies of proofreading mechanisms reported in the literature. One study, using linear approximations to examine the temporal response, produced qualitative results indicating an inverse relation between accuracy and response time (Blomberg, 1977). However, in general, it is difficult to directly compare the results of that study with the results reported in this paper.

With regard to the dynamic development of errors and proofreading costs, we have seen two different patterns. In one case, errors or costs gradually change with time following an abrupt alteration (Figures 2e, 3f, 4e,f, 5e, and 6e); in the other, errors or costs change dramatically in one direction following an abrupt alteration and then slowly reverse themselves (Figures 2f, 3e, 5f, and 6f). The extent of the gradual changes can be predicted from a steady-state analysis, but only a detailed dynamic analysis can reveal the dramatic transients that a system might experience. The transient changes can be as great as twice the steady-state changes, which indicates that the transient period immediately following the onset of a stress, such as amino acid depletion or excess, may be crucial for the cell. The need to buffer the cell against sudden changes in amino acid flux and relative amino acid levels so as to diminish errors and proofreading costs is one of the most important but little recognized functions of the regulatory systems governing amino acid biosynthetic pathways.

The system analyzed in this paper is clearly dynamically stable and temporally responsive. In all cases, the system responds to change quickly (<0.02–0.2 s) but shows no tendency to oscillate. At most, there is a single "overshoot" or "false start" in the response. This is the behavior one would expect for an effectively designed biochemical system.

Acknowledgments

We thank Dr. M. Yarus and an anonymous reviewer of the manuscript for their helpful criticism of this work.

Registry No. Isoleucyl-tRNA synthetase, 9030-96-0.

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