cone, C., & Kroon, A., Eds.) p 3, North-Holland Publishing Co., Amsterdam.
Gelfand, R., & Attardi, G. (1981) *Mol. Cell. Biol. 1*, 497–511.
Groot, G., Van Harten-Loosbroek, N., Van Ommen, G., & Pijst, H. (1981) *Nucleic Acids Res. 9*, 6369–6377.
Kantharaj, G., Bhat, K., & Avadhani, N. (1983) *Biochemistry 22*, 3151–3156.
Lehrach, H., Diamond, J., Wozney, J., & Boedtker, H. (1977) *Biochemistry 16*, 4743–4751.
Martens, P., & Clayton, D. (1979) *J. Mol. Biol. 135*, 327–351.
Montoya, J., Ojala, D., & Attardi, G. (1981) *Nature (London) 290*, 465–470.
Murphy, W., Attardi, B., Tu, C., & Attardi, G. (1975) *J. Mol. Biol. 99*, 809–814.
Niranjan, B., & Avadhani, N. (1980) *J. Biol. Chem. 255*, 6575–6578.
Ojala, D. Montoya, J., & Attardi, G. (1981) *Nature (London) 290*, 470–474.
Ricca, G., Hamilton, R., McLean, J., Conn, A., Kalinyak, J., & Taylor, J. (1981) *J. Biol. Chem. 256*, 10362–10368.
Rose, K., Morris, H., & Jacob, S. (1975) *Biochemistry 14*, 1025–1032.
Thomas, P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.
Van Etten, R., Walberg, M., & Clayton, D. (1980) *Cell (Cambridge, Mass.) 22*, 156–170.
Van Etten, R., Michael, N., Bibb, M., Brennicke, A., & Clayton, D. (1982) in *Mitochondrial Genes* (Slonimski, P., Borst, P., & Attardi, G., Eds.) p 73, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Venetierner, P., & Leder, P. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3892–3897.

Integrated Function of a Kinetic Proofreading Mechanism: Steady-State Analysis Testing Internal Consistency of Data Obtained in Vivo and in Vitro and Predicting Parameter Values†

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ABSTRACT: Experimental measurements of the kinetic mechanism involving isoleucyl-tRNA synthetase proofreading valyl-tRNA^{Ile} in *Escherichia coli* have been incorporated into the conventional Michaelis–Menten model for this system. The model was subjected to a detailed mathematical analysis in the steady state. The results of this analysis provide an excellent illustration of the value of integrating fragmentary data into a model of the intact system. (1) Such integration provides a rigorous test for consistency of the individual measurements. For the above synthetase system, the published experimental data were found to be internally inconsistent. (2) Such integration predicts which experimental data are most suspect. In this case, one of the three most questionable measurements, the isoleucine pool size in vivo, was found upon reexamination to be in error by 10–15-fold. Correction of this error produced a self-consistent set of parameter values. (3) The integrated analysis provides predictions for various parameter values. In many cases, these predictions provide estimates for parameter values that are difficult to determine directly or that have yet to be measured experimentally. (4) A sensitivity analysis provides an indication of the relative importance of various parameter values and, hence, an indication of where future experimental effort might be focused most profitably.

In order to maintain biological accuracy and integrity, living systems expend a considerable fraction of their available free energy for proofreading, repair, and disposal of faulty components. These processes have a formal similarity, and we have developed a general theory for the analysis of such phenomena (Savageau & Freter, 1979a, Freter & Savageau, 1980). Although these general methods have been applied to the specific case of Michaelis–Menten mechanisms (Savageau & Lapointe, 1981) and although these methods have been used to test certain experimental measurements (Savageau & Freter, 1979a,b; Freter & Savageau, 1980), a complete characterization of a specific proofreading mechanism has never been presented. In this and the following paper (Okamoto & Savageau, 1984), we shall present such a characterization by focusing upon the single best studied proofreading mechanism—isoleucyl-tRNA synthetase proofreading valyl-tRNA^{Ile} in *Escherichia coli*. The isoleucyl-tRNA synthetase occasionally links the amino acid valine to tRNA^{Ile}. However, the synthetase is known to possess a second activity that allows it to preferentially hydrolyze valyl-tRNA^{Ile} and thereby prevent the initial error in amino acid recognition from being passed on to the finished protein product. This process, by which an enzyme "reexamines" its products and preferentially "rejects" those that are faulty before they can be utilized, has been called by various names, including verification, editing, and kinetic proofreading. We shall simply use the term *proofreading*.

We have adopted the accepted Michaelis–Menten model for this enzyme [e.g., see Yarus (1969), Eldred & Schimmel (1972), Holler & Calvin (1972), and Mulvey & Fersht (1977)], used experimental measurements made both in vitro and in vivo, and integrated these to obtain a complete characterization of the rate constants and steady-state concentrations in the model. The results of this analysis suggest three important conclusions. (a) The published experimental data are internally inconsistent. (b) The analysis predicts which experimental data are most suspect. (c) The analysis predicts values of key physiological and biochemical parameters in vivo, including amino acid step time, tRNA charging ratios, amino

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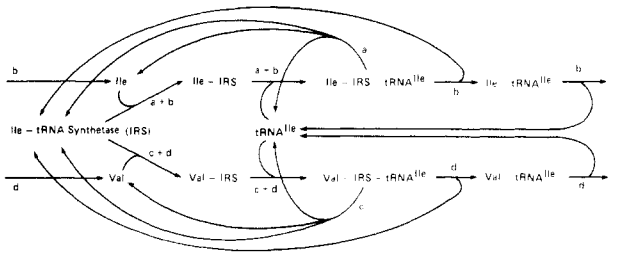

FIGURE 1: Isoleucyl-tRNA synthetase proofreading valyl-tRNA^{Ile}. Branching diagram that characterizes the pattern of macroscopic fluxes in a single-stage proofreading mechanism.

acid pool sizes, and parameters that previously have not been measured. Furthermore, the analysis used in this study identifies parameters for which a relatively small change in value produces the largest effect upon the behavior of the integrated system. This type of sensitivity analysis [see Cruz (1973) and Savageau (1976)] is performed by making a given percentage change in one of the parameters of the system and then calculating the resultant percentage change in the behavior of the system. By repeating the procedure for each of the parameters, one can identify those to which the system is most sensitive. Since experimental error in the measurement of these parameters will have the most profound influence upon the predicted behavior of the system, the analysis indicates that future experimental effort might be focused most profitably upon determination of these values.

Model

We shall consider a Michaelis–Menten model with a single stage of proofreading. But first, let us examine Figure 1, which illustrates the general branching structure of such proofreading mechanisms. There are four independent fluxes in such systems: a, b, c, and d. a is the net flux representing hydrolysis of correctly acylated tRNA, b is the net flux representing production of correctly acylated tRNA, c is the net flux representing hydrolysis of incorrectly acylated tRNA, and d is the net flux representing production of incorrectly acylated tRNA. The macroscopic behavior can be represented conveniently by a set of six macroscopic parameters defined in terms of these net fluxes (Savageau & Freter, 1979a; Freter & Savageau, 1980): initial discrimination ratio

$$I = (a + b)/(c + d) \tag{1}$$

proofreading discrimination ratio

$$P = (c/d)/(a/b) = bc/(ad) \tag{2}$$

net forward flux

$$F = b + d \tag{3}$$

net recycling flux

$$R = a + c \tag{4}$$

net error

$$E = d/(b + d) \tag{5}$$

cost of proofreading

$$C = R/F = (a + c)/(b + d) \tag{6}$$

These parameters, which have been measured macroscopically, also can be related to the underlying microscopic rate constants (Savageau & Lapointe, 1981), which in some cases have been determined in vitro by kinetic methods (see below).

Figure 2 shows the specific Michaelis–Menten mechanism corresponding to the general branching model in Figure 1. The isoleucyl-tRNA synthetase (X_1) makes a single type of substrate recognition error (valine, X_4, for isoleucine, X_3), followed by acylation (X_5 or X_6) and ligation to tRNA^{Ile} (X_2). This sequence of events occurs on the surface of a single molecule of isoleucyl-tRNA synthetase. The enzyme-bound aminoacyl-tRNA^{Ile} (X_7 or X_8) is either released (X_9 or X_{10}) for eventual incorporation into a growing polypeptide chain or hydrolyzed to yield free amino acid and free tRNA in the proofreading reaction.

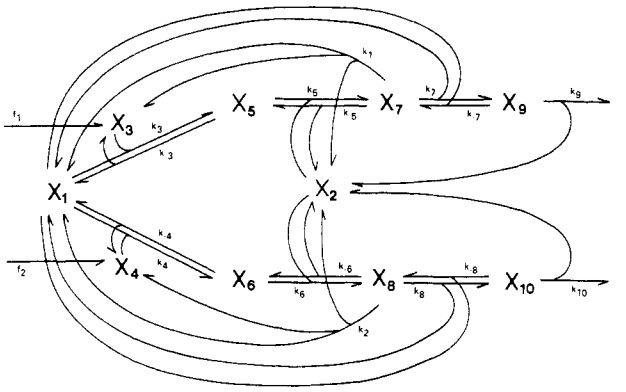

FIGURE 2: Isoleucyl-tRNA synthetase proofreading valyl-tRNA^{Ile}. Michaelis–Menten model corresponding to the branched diagram in Figure 1. Free isoleucyl-tRNA synthetase, X_1; free tRNA^{Ile}, X_2; free isoleucine, X_3; free valine, X_4; enzyme-bound isoleucyl adenylate, X_5; enzyme-bound valyl adenylate, X_6; enzyme-bound isoleucyl-tRNA^{Ile}, X_7; enzyme-bound valyl-tRNA^{Ile}, X_8; free isoleucyl-tRNA^{Ile}, X_9; free valyl-tRNA^{Ile}, X_{10}.

The mechanism is assumed to operate within *E. coli* cells growing exponentially in a glucose minimal medium with an average doubling time of 47 min. All of the elementary steps in the mechanism are reversible, although the amino acid biosynthetic pathways, the hydrolytic proofreading steps, and the uptake of aminoacyl-tRNA for protein synthesis are represented by their net forward rates. In practice, the hydrolytic steps are essentially irreversible under the conditions described above.

Experimental Procedures

Only some of the parameter values for the model have been experimentally determined; others, as we shall see, can be obtained by using the experimentally determined values together with the fundamental equations describing the mechanism.

The Initial Discrimination Ratio (I). This macroscopic parameter is given by the ratio $(V_{max}/K_m)_{Ile}[Ile]/[(V_{max}/K_m)_{Val}[Val]]$. The kinetic studies of Baldwin & Berg (1966), Loftfield & Eigner (1966), Fersht (1977), and Freist & Cramer (1983) have shown that the isoleucyl-tRNA synthetase of *E. coli* has a V_{max}/K_m ratio for isoleucine that is about 100–200 times that for valine. Following Freist & Cramer (1983), we shall assume a difference of 180. Raunio & Rosenqvist (1970) have determined that the concentration of valine in vivo is about 5 times that of isoleucine. Hence, the estimated value for the initial discrimination ratio is $I = 36$.

Proofreading Discrimination Ratio (P). The flux measurements of Hopfield et al. (1976) provide an estimate of this parameter. They found the c/d ratio to be 270 and the a/b ratio to be 0.5. However, the latter figure is an overestimate that should be corrected by 50% (Mulvey & Fersht, 1977), i.e., $a/b = 0.25$. Thus, the proofreading discrimination ratio for this system may be estimated to be $P = 1100$. This value appears to be relatively high, given the similarity of the molecules being recognized. However, the "double-sieve" mechanism of Fersht (1979) can produce such high values; alternatively, two stages of proofreading (each with a more

reasonable proofreading discrimination ratio) can produce similar results (Freter & Savageau, 1980).

Concentrations of Isoleucine and Valine in Vivo. By using gas–liquid chromatographic techniques, Raunio & Rosenqvist (1970) have determined the concentrations of these amino acids to be $X_3 = 41\ \mu M$ and $X_4 = 231\ \mu M$ in *E. coli* growing with a doubling time of 47 min.

a/b, c/d, E, and C Values. Mulvey & Fersht (1977) have estimated the a/b ratio to be 0.064, and with the value of P given above, one can estimate the corresponding c/d ratio to be 70.4. From the values of these two ratios and the value of I given above, one can calculate (from eq 1, 5, and 6) that the net error $E = 4.14 \times 10^{-4}$ and that the cost of proofreading $C = 0.093$. Other estimates of these parameters might be used [see Savageau & Freter (1979a,b)]; but, as will be shown later, the results are relatively insensitive to their values.

Concentration of Isoleucyl-tRNA Synthetase. This enzyme is a monomer having a molecular weight of 114 000 (Arndt & Berg, 1971). The monomer constitutes 2.45 mg/g of total cell protein (Pedersen et al., 1978). Total cellular mass (dry weight) consists of about 75% protein (Maaløe & Kjeldgaard, 1966), and since the cellular volume per gram dry weight of cell mass is about 3 mL, we can estimate that the total intracellular concentration of isoleucyl-tRNA synthetase is $X_S = 5.0\ \mu M$; i.e., $X_S = X_1 + X_5 + X_6 + X_7 + X_8 = 5.0\ \mu M$.

Concentration of $tRNA^{Ile}$. The total intracellular concentration of $tRNA^{Val}$ has been given by Lewis & Ames (1972) as 12 nmol/g dry weight of bacteria. If we adopt this value for $tRNA^{Ile}$ and convert it to molar concentration, we find a value of $X_T = 4.0\ \mu M$. However, since synthetases and their cognate tRNAs tend to be found in equimolar concentrations, we have changed this estimate to 5.0 μM; i.e., $X_T = X_2 + X_7 + X_8 + X_9 + X_{10} = 5.0\ \mu M$. As will be shown later, this parameter is quite insensitive, and thus, either value could have been used without significantly changing the results.

Aminoacylation Ratio for $tRNA^{Ile}$. Lewis & Ames (1972) have reported that exponentially growing cultures of wild-type enteric bacteria have about 77–88% of their $tRNA^{His}$ aminoacylated. We estimate a similar range of values for the aminoacylation ratio (A) of $tRNA^{Ile}$; namely, $A = (X_7 + X_9)/X_2 = 4.0$.

Flux of Isoleucine through the System. The amino acid isoleucine constitutes about 240 μmol/g dry weight of *E. coli* (Roberts et al., 1957). For *E. coli* growing with a doubling time of 47 min, approximately 0.059 μmol/s isoleucine must be acylated to tRNA in every gram dry weight of cells. Thus, the flux of isoleucine through the system in steady state is $f_1 = 20\ \mu M/s$.

k_3 and k_{-3} Values. Holler & Calvin (1972) have used stop-flow techniques to investigate the preequilibrium kinetics of the isoleucine activation reaction catalyzed by isoleucyl-tRNA synthetase. They found values of $2.2 \times 10^6\ M^{-1}\ s^{-1}$ and $15\ s^{-1}$ (pH 8.0, 25 °C) for the rate constants k_3 and k_{-3}, respectively. By assuming a $Q_{10} = 2$ for this reaction, we have estimated that $k_3 = 4.4 \times 10^6\ M^{-1}\ s^{-1}$ and $k_{-3} = 30\ s^{-1}$ (pH 8.0, 35 °C).

k_5 and k_{-5} Values. Eldred & Schimmel (1972) have reported the equilibrium constant for binding $tRNA^{Ile}$ and the cognate synthetase to be $K_5 = k_5/k_{-5} = 2.86 \times 10^6\ M^{-1}$ at 3 °C.

k_7 and k_{-7} Values. Yarus (1969) has reported the equilibrium constant for binding isoleucyl-$tRNA^{Ile}$ and the cognate synthetase to be $K_7 = k_7/k_{-7} = 7.1 \times 10^{-9}$ M at 17 °C in the presence of ATP and isoleucine. The forward rate constant $k_7 \geq t_s^{-1}$, where t_s is the amino acid step time. k_7 initially will be assumed to have a value of $20\ s^{-1}$.

Fundamental Equations. The kinetic equations that govern the system represented in Figure 2 are given below, and in the steady state (when the concentrations and fluxes are unchanging) the derivatives of each of the concentration variables must be set equal to zero:

$$\dot{X}_1 = k_{-3}X_5 + k_{-4}X_6 + (k_7 + k_1)X_7 + (k_8 + k_2)X_8 - k_3X_1X_3 - k_4X_1X_4 - k_{-7}X_1X_9 - k_{-8}X_1X_{10} = 0 \quad (7)$$

$$\dot{X}_2 = (k_{-5} + k_1)X_7 + (k_{-6} + k_2)X_8 + k_9X_9 + k_{10}X_{10} - k_5X_2X_5 - k_6X_2X_6 = 0 \quad (8)$$

$$\dot{X}_3 = f_1 + k_{-3}X_5 + k_1X_7 - k_3X_1X_3 = 0 \quad (9)$$

$$\dot{X}_4 = f_2 + k_{-4}X_6 + k_2X_8 - k_4X_1X_4 = 0 \quad (10)$$

$$\dot{X}_5 = k_3X_1X_3 + k_{-5}X_7 - k_{-3}X_5 - k_5X_2X_5 = 0 \quad (11)$$

$$\dot{X}_6 = k_4X_1X_4 + k_{-6}X_8 - k_{-4}X_6 - k_6X_2X_6 = 0 \quad (12)$$

$$\dot{X}_7 = k_5X_2X_5 + k_{-7}X_1X_9 - (k_{-5} + k_7 + k_1)X_7 = 0 \quad (13)$$

$$\dot{X}_8 = k_6X_2X_6 + k_{-8}X_1X_{10} - (k_{-6} + k_8 + k_2)X_8 = 0 \quad (14)$$

$$\dot{X}_9 = k_7X_7 - k_{-7}X_1X_9 - k_9X_9 = 0 \quad (15)$$

$$\dot{X}_{10} = k_8X_8 - k_{-8}X_1X_{10} - k_{10}X_{10} = 0 \quad (16)$$

Not all of these equations are independent, e.g.

$$\dot{X}_1 = -(\dot{X}_5 + \dot{X}_6 + \dot{X}_7 + \dot{X}_8)$$

$$\dot{X}_2 = -(\dot{X}_7 + \dot{X}_8 + \dot{X}_9 + \dot{X}_{10})$$

or, alternatively

$$X_S = X_1 + X_5 + X_6 + X_7 + X_8 \quad (17)$$

$$X_T = X_2 + X_7 + X_8 + X_9 + X_{10} \quad (18)$$

where X_S and X_T are the total synthetase and $tRNA^{Ile}$ concentrations.

We also shall use the following physical and algebraic constraints:

$$A = (X_7 + X_9)/X_2 \quad (19)$$

where A is the amino acylation ratio

$$S_{57} = k_{-5}/k_7 \quad (20)$$

where S_{57} is a "symmetry coefficient" with respect to the release of uncharged (k_{-5}) and isoleucyl-charged (k_7) tRNA that is initially taken as unity

$$S_{68} = k_{-6}/k_8 \quad (21)$$

where S_{68} is a symmetry coefficient with respect to the release of uncharged (k_{-6}) and valyl-charged (k_8) tRNA that is initially taken as unity

$$D_{34} = K_3/K_4 \quad (22)$$

where D_{34} is a "discrimination factor" between the corresponding cognate ($K_3 = k_3/k_{-3}$) and noncognate ($K_4 = k_4/k_{-4}$) steps that is initially taken as 180 (Loftfield & Eigner, 1966; Freist & Cramer, 1983)

$$D_{56} = K_5/K_6 \quad (23)$$

where D_{56} is a discrimination factor between the corresponding cognate ($K_5 = k_5/k_{-5}$) and noncognate ($K_6 = k_6/k_{-6}$) steps that should be greater than 1 for effective proofreading and is initially taken as 5

$$k_9 = k_{10} \quad (24)$$

when codon–anticodon recognition is unaffected by the amino acid residue bound to the tRNA

$$E = f_2/(f_1 + f_2) \quad (25)$$

$$a/b = k_1X_7/(k_7X_7 - k_{-7}X_1X_9) \quad (26)$$

and

$$c/d = k_2X_8/(k_8X_8 - k_{-8}X_1X_{10}) \quad (27)$$

The experimental data presented in the previous subsections, together with the constraints imposed by the above equations governing the system, allow one to estimate the remaining rate constants and concentration variables in the steady state. We shall make such estimates in two stages. First, we shall obtain conditions that ensure nonnegative concentration values. Second, these conditions will be refined so as to ensure nonnegative rate constants as well.

Results

Conditions for Nonnegative Concentration Values. From the experimentally determined values given in the previous section and the reasonable assumptions regarding the values of k_7 and S_{57}, the following independent parameters can be specified for the system in Figure 2:

$$f_1 = 20\ \mu M\ s^{-1} \quad k_3 = 4.4 \times 10^6\ M^{-1}\ s^{-1}$$
$$k_{-3} = 30\ s^{-1} \quad K_5 = k_5/k_{-5} = 2.86 \times 10^6\ M^{-1}$$
$$k_7 = 20\ s^{-1} \quad K_7 = 7.1 \times 10^{-9}\ M$$
$$E = 4.14 \times 10^{-4} \quad a/b = 0.064$$
$$X_3 = 41\ \mu M \quad X_S = 5.0\ \mu M \quad X_T = 5.0\ \mu M$$
$$A = 4.0 \quad S_{57} = 1.0 \quad (28)$$

Only this subset of independent parameters is involved in determining the allowable range of values for the concentration variables. When these values were entered into a program designed to find values for the rate constant k_9 that would guarantee nonnegative values for all of the concentration variables (see Appendix), no acceptable values of k_9 were found, which indicates that the values given in equation 28 are internally inconsistent. At least one of the values in eq 28 must be altered to produce a biologically meaningful estimate for the rate constant k_9.

We have used two criteria for identifying the parameters whose values are most suspect: effectiveness (sensitivity) in producing biologically meaningful estimates for k_9 and magnitude of experimental error inherent in measurement. For example, a parameter whose measurement was accurate to ±100% still might not be questioned if it were very insensitive and one with ±1% accuracy still might be questioned if it were extremely sensitive. However, one that was both accurately determined and very insensitive would not be questioned, while one that was known imprecisely and had a high sensitivity would certainly be suspect.

As can be seen in Table I, a change in some of the parameter values is more effective than a change in others with regard to producing biologically realistic estimates for the rate constant k_9. According to the ranking in Table I, there might be reason to question the measurements of X_S, K_5, K_7, X_3, and k_3.

A reexamination of the inherent experimental error in X_S leads us to the conclusion that it is probably small. Although overestimates in the protein fraction and in cellular volume have probably been made, they would tend to be small and in opposite directions. Reexamination of the experimental conditions used for the measurement of K_5 suggests that this parameter value may be high because of the salt concentration and temperature used. The salt concentration has a marked effect upon the value of K_5 obtained in vitro, and Lövgren et al. (1976) have suggested that the value of K_5 in vivo may be considerably smaller than the value reported by Eldred & Schimmel (1972). Measurements of K_5 at physiological temperatures are difficult to make, and the survey of such measurements by Lövgren et al. (1975) did not include physiological values for isoleucyl-tRNA synthetase. However, the value they gave for seryl-tRNA synthetase at 25 °C [originally reported by Rigler et al. (1970)] suggests a K_5 value for isoleucyl-tRNA synthetase that could be about $^1/_5$ that reported by Eldred & Schimmel (1972). At any rate, these errors would be in the wrong direction to resolve the inconsistency noted above (see Table I). By the same reasoning, the value of K_7 may be somewhat low because of the conditions used for its measurement in vitro. Measurements of pool sizes such as X_3 are inherently difficult. In reexamining the data of Raunio & Rosenqvist (1970) we have found an error of about 10–15-fold in their conversion of micromoles per gram dry weight into micromolar units. This error alone is sufficient to produce the inconsistencies that we have observed among the published experimental data. Although the isoleucine and valine concentrations are in error, the ratio of their concentrations that was used in a previous section to estimate I may still be valid because these authors used the same conversion factor for calculating the molar concentration of each amino acid. There appears to be little reason to question the ex-

Table I: Effect of Change in Independent Parameter Values upon the Allowable Values for the Rate Constant k_9

parameter	value[a]	allowable values for k_9[a]
X_S	0.25	$>1.2 \times 10^{19}$
	5.00	$>4.9 \times 10^{18}$
	6.00	$>3.4 \times 10^{18}$
	7.00	389–1168
K_5	3.00×10^5	$>4.1 \times 10^{19}$
	2.86×10^6	$>4.9 \times 10^{18}$
	5.00×10^6	none
	7.10×10^6	226–270
K_7	3.6×10^{-10}	none
	7.1×10^{-9}	$>4.9 \times 10^{18}$
	5.0×10^{-8}	$>7.2 \times 10^{18}$
	5.7×10^{-8}	34.5–34.7
X_3	4.1	none
	41.0	$>4.9 \times 10^{18}$
	205.0	$>1.5 \times 10^{18}$
	410.0	2.7×10^{17} to 1.0×10^{18}
	550.0	32.9–33.4
k_3	4.4×10^5	$>1.0 \times 10^{20}$
	4.4×10^6	$>4.9 \times 10^{18}$
	6.0×10^7	32.5–33.1
k_7	1.0	none
	20.0	$>4.9 \times 10^{18}$
	400.0	8.5×10^{19} to 8.0×10^{20}
k_{-3}	1.5	$>2.4 \times 10^{17}$
	30.0	$>4.9 \times 10^{18}$
	999.0	$>2.2 \times 10^{20}$
f_1	1.0	4.3×10^{18} to 1.0×10^{19}
	20.0	$>4.9 \times 10^{18}$
	500.0	none
A	0.2	none
	4.0	$>4.9 \times 10^{18}$
	80.0	1.0×10^{20} to 8.0×10^{20}
S_{57}	0.01	$>3.4 \times 10^{20}$
	1.00	$>4.9 \times 10^{18}$
	20.00	$>2.0 \times 10^{18}$
E	1.00×10^{-5}	$>4.9 \times 10^{18}$
	4.14×10^{-4}	$>4.9 \times 10^{18}$
	8.18×10^{-3}	$>4.9 \times 10^{18}$
	8.28×10^{-3}	none
a/b	0.001	$>4.8 \times 10^{18}$
	0.064	$>4.9 \times 10^{18}$
	1.28	$>8.2 \times 10^{18}$
X_T	0.25	none
	5.00	$>4.9 \times 10^{18}$
	100.00	$>1.5 \times 10^{20}$

[a] See text eq 28 for units.

perimental value of k_3. Although k_3 is as sensitive as X_3, this simply reflects the fact that they occur as a product, k_3X_3, and thus, changes in k_3 mimic changes in X_3. In view of this reassessment and the fact that the revealed error in X_3 alone can account for the inconsistency, we have chosen to retain all the previous estimates in eq 28 except for X_3, which we have adjusted to 550 μM.

Conditions for Nonnegative Rate Constants. In addition to the parameters listed in eq 28, there are three parameters (S_{68}, D_{34}, and D_{56}) that are involved in determining the allowable ranges for the rate constants in the system. It should be emphasized that these three parameters have nothing to do with the conditions for nonnegative concentration values given in the previous section. Equations 9–27 and the equation $K_5 = k_5/k_{-5}$ provide a set of 20 equations with 20 unknown variables: f_2, k_1, k_2, k_4, k_{-4}, k_5, k_{-5}, k_6, k_{-6}, k_8, k_{-8}, k_{10}, X_1, X_2, X_5, X_6, X_7, X_8, X_9, and X_{10}. The eight concentration variables can be obtained in steady state by the procedure outlined in the Appendix. The unknown rate constants and the flux f_2 then can be sequentially related to other known quantities as follows:

$$f_2 = f_1E/(1 - E) \quad (29)$$

$$k_1 = (a/b)f_1/X_7 \quad (30)$$

$$k_2 = (c/d)f_2/X_8 \quad (31)$$

$$k_4 = (1 + c/d)f_2/(X_1X_4 - D_{34}X_6/K_3) \quad (32)$$

$$k_{-4} = D_{34}k_4/K_3 \quad (33)$$

$$k_5 = S_{57}K_5k_7 \quad (34)$$

$$k_{-5} = S_{57}k_7 \quad (35)$$

$$k_6 = (1 + c/d)f_2/(X_2X_6 - D_{56}X_8/K_5) \quad (36)$$

$$k_{-6} = D_{56}k_6/K_5 \quad (37)$$

$$k_8 = k_{-6}/S_{68} \quad (38)$$

$$k_{-8} = (k_8X_8 - f_2)/(X_1X_{10}) \quad (39)$$

$$k_{10} = k_9 \quad (40)$$

The values of the unknowns f_2, k_1, k_2, k_5, k_{-5}, and k_{10} are always positive if the concentration variables are positive. The values of the rate constants k_4 and k_{-4} will be positive if

$$D_{34} < K_3X_1X_4/X_6 \quad (41)$$

Similarly, k_6, k_{-6}, and k_8 will be positive if

$$D_{56} < K_5X_2X_6/X_8 \quad (42)$$

Finally, k_{-8} will be positive if

$$D_{56} > S_{68}K_5X_2X_6X_8^{-1}/[(1 + c/d) + S_{68}] \quad (43)$$

Thus, when the concentration variables are positive, eq 41–43 are necessary and sufficient conditions for nonnegative rate constants.

When the parameter values established in the previous subsection are inserted into the last three inequalities, one finds a threshold value of 745 for D_{34} and bounds of 0.070 and 5.04 for D_{56}. All these conditions are satisfied by the values we have used for these discrimination factors, namely, $D_{34} = 180$ and $D_{56} = 5$. Clearly, if we had used values in violation of these conditions, then negative rate constants would have been indicated, and the conditions in eq 41–43 would have suggested the direction in which to change the discrimination factors so as to establish consistency.

By adoption of the "standard" set of self-consistent, independent parameter values in Table II, one can solve for the remaining dependent rate constants and concentration variables in steady state (see Table III). There appear to be no unreasonable values among these dependent parameters.

Table II: Standard Values for the Independent Variables of the System

$k_3 = 4.40 \times 10^6$ M^{-1} s^{-1}	$X_3 = 550\ \mu$M
$k_{-3} = 30$ s^{-1}	$X_4 = 3.1$ mM
$k_7 = 20$ s^{-1}	$X_T = 5.0\ \mu$M
$K_7 = 7.1 \times 10^{-9}$ M	$X_S = 5.0\ \mu$M
$K_5 = 2.86 \times 10^6$ M^{-1}	$S_{57} = 1.0$
$f_1 = 20\ \mu$M s^{-1}	$S_{68} = 1.0$
$A = 4.0$	$D_{34} = 180$
a/b[a] $= 0.064$	$D_{56} = 5.0$
E[a] $= 4.14 \times 10^{-4}$	

[a] P and C values fixed at 1100 and 0.093, respectively.

Table III: Predicted Values for the Dependent Rate Constants and Concentration Variables in Steady State

$k_1 = 0.378$ s^{-1}	$X_1 = 2.81 \times 10^{-8}$ M
$k_2 = 60.0$ s^{-1}	$X_2 = 9.98 \times 10^{-7}$ M
$k_4 = 8.94 \times 10^3$ M^{-1} s^{-1}	$X_5 = 1.56 \times 10^{-6}$ M
$k_{-4} = 11.0$ s^{-1}	$X_6 = 1.72 \times 10^{-8}$ M
$k_{-5} = 20.0$ s^{-1}	$X_7 = 3.39 \times 10^{-6}$ M
$k_6 = 4.19 \times 10^9$ M^{-1} s^{-1}	$X_8 = 9.71 \times 10^{-9}$ M
$k_{-6} = 7.32 \times 10^3$ s^{-1}	$X_9 = 6.06 \times 10^{-7}$ M
$k_8 = 7.32 \times 10^3$ s^{-1}	$X_{10} = 2.51 \times 10^{-10}$ M
$k_{-8} = 1.01 \times 10^{13}$ M^{-1} s^{-1}	$f_2 = 8.28 \times 10^{-9}$ M s^{-1}
$k_9 = 33.0$ s^{-1}	
$k_{10} = 33.0$ s^{-1}	

Table IV: Effect of Change in Independent Parameter Values upon the Allowable Values for the Rate Constant k_9

parameter[a]	values[b]	acceptable values for k_9[b]
k_7[c]	<19.0	none
	19.5	33.34–33.35
	20.0	32.91–33.16
	22.0	31.80–32.50
	24.0	31.80–31.90
	24.5	31.80–31.80
	>25.0	none
S_{57}	<0.95	none
	0.96	33.10–33.11
	1.00	32.91–33.16
	1.20	32.70–33.30
	1.40	33.50–33.50
	>1.50	none

[a] Standard values for the other independent parameters are given in Table II. [b] See Tables II and III for units. [c] $K_7 = k_7/k_{-7}$ was fixed at 7.1×10^{-9} M.

Variations in the values of the parameters k_7 and S_{57} were shown in the previous subsection to have little effect upon the behavior of the system. However, because these values initially were chosen somewhat arbitrarily, we have specifically examined alternative values for these parameters. The results in Table IV show that values of k_7 less than 19 s^{-1} are inconsistent with the other experimental measurements. Below this limit, it is impossible to find values for k_9 that will prevent the concentration variables in the system from becoming negative. On the other hand, k_7 can be no greater than about 25 s^{-1}. Beyond this limit, negative rate constants began to arise. Thus, the allowable range for k_7 appears to be 19–25 s^{-1}. Similar results for S_{57} are shown in Table IV; below 0.95, negative concentration variables are indicated, and above 1.50, negative rate constants arise. Thus, the allowable range for S_{57} appears to be 0.95–1.50.

Discussion

The postulation of a specific model for a biochemical mechanism places severe constraints upon the values of the

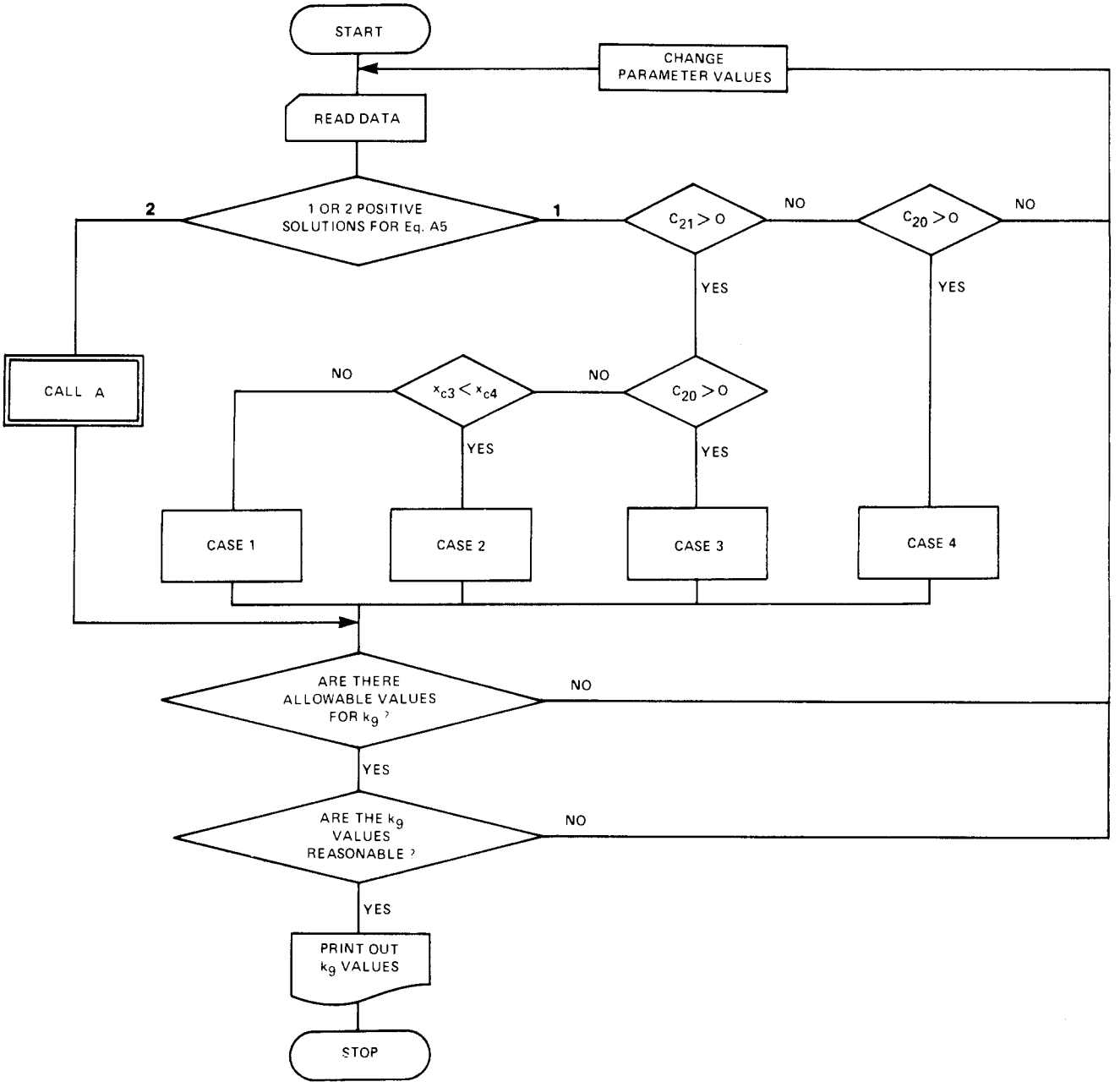

FIGURE A1: Flow diagram illustrating the procedures used to exhaustively test for acceptable values of the rate constant k_9. Acceptable values of k_9 are those that yield a steady-state solution with nonnegative concentration values.

parameters that characterize the mechanism. For example, in the previous section we have shown that the experimentally determined parameter values in eq 28 cannot all be correct. Of course, the discrepancy might be with the model, in which case the meaning of the various measurements themselves is unclear. Alternatively, one or more of the experimental measurements might be in error. In this latter situation, one can make reasonable inferences as to the parameter values in question.

We have used two criteria to suggest which parameter values are most suspect when a set of parameter values is inconsistent: parameter sensitivity and experimental uncertainty. The parameter values that are most suspect will be those that are most sensitive, i.e., for which the smallest percentage change in value would produce consistency, and those that have the highest intrinsic experimental uncertainty.

Our sensitivity analysis lead us to a reexamination of the isoleucine pool size as reported by Raunio & Rosenqvist (1970). It appears that these authors have used an erroneous conversion factor to generate micromolar values from micromoles per gram dry weight. The measurements themselves might exhibit errors that we have not considered, but the error in the conversion factor alone is sufficient to account for the inconsistency in the original set of data.

The parameter change that is most effective in producing consistency among the concentration values (nonnegative concentration values) is an increase in the concentration of isoleucyl-tRNA synthetase (X_S). A 40% increase over the experimentally determined value of 5.0 μM would be required if this error were responsible for the inconsistency; this seems unreasonable. The next most sensitive parameter is the equilibrium constant for binding $tRNA^{Ile}$ to the cognate synthetase bound with isoleucyl adenylate (K_5). An increase of 2.5-fold over the experimental value of 2.86×10^6 M^{-1} would be required to account for the inconsistency. This magnitude of error may not be unreasonable because the conditions used in vitro were not physiological. (However, see the remarks concerning K_5 at the end of the subsection Con-

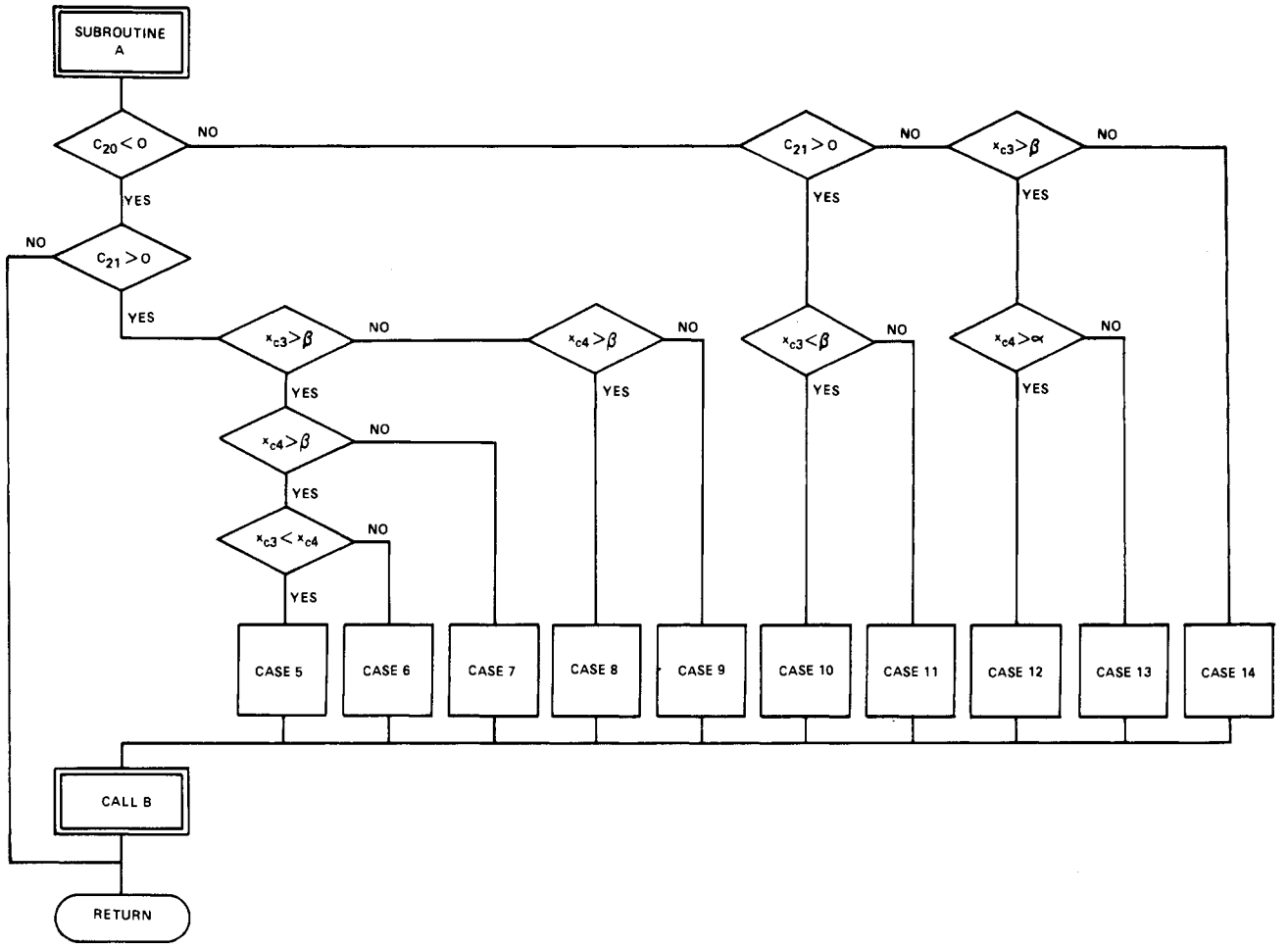

FIGURE A2: Expanded view of subroutine A in Figure A1. The two real solutions of eq A5 are denoted α and β, where $\alpha < \beta$.

ditions for Nonnegative Concentration Values.) Somewhat lower in sensitivity is the equilibrium constant for binding isoleucyl-tRNA^{Ile} to the cognate synthetase (K_7). An increase of 8-fold is required to account for the inconsistency in the original set of data. This magnitude of error seems less reasonable but still might be attributable to differences in conditions in vitro and in vivo. The rate constant for binding isoleucine (k_3) has been measured directly, and there is little reason to question its value; it seems certain that the errors in this measurement would be less than the 13.6-fold that would be necessary to account for the inconsistency. Thus, although we have identified the inconsistency with the revealed error in the concentration of isoleucine (X_3) and although this error is sufficient to remove the inconsistency among the original data, there might well be other parameter values with significant error. In this regard, our analysis leads us to emphasize the need for closer examination of the parameters K_5 and K_7.

There are a number of other parameter values with considerable experimental uncertainty. However, their sensitivities are generally so low that an inordinately large experimental error (>10^2-fold in many cases) would be required to account for the observed inconsistency. For example, the net error E as determined from the measurements of Hopfield et al. (1976) and Mulvey & Fersht (1977) in vitro is somewhat higher than the value reported by Loftfield & Vanderjagt (1972) in vivo. The measurements in vitro might not reflect the true conditions in vivo. Alternatively, recent studies (Bouadloun et al., 1983; Parker et al., 1983) have suggested amino acid error rates for translation that exceed those reported by Loftfield & Vanderjagt (1972) and Edelmann & Gallant (1977) for extracellular proteins. The clearance of erroneous protein, which in effect provides another stage of proofreading, or the inability to excrete erroneous protein might account for the lower error rates seen in extracellular proteins [see also Yarus (1979)]. At any rate, in spite of such uncertainty in the value of E, the sensitivity of this parameter is so low that a ±20-fold change has no effect in producing nonnegative concentration values (Table I). Thus, errors in the estimate of E are unlikely to account for the inconsistencies in the set of experimental measurements noted in the previous section.

With the exception of the above change in X_3, all other experimentally determined parameter values were left unchanged (Table II). This represents a minimal change sufficient to bring about consistency in the experimental data. Under these conditions, a reasonable and consistent solution was obtained for all rate constants and concentration values in steady state. The values in Table II might be interpreted as predictions for a number of biochemically and physiologically important parameters. For example, the concentration of isoleucine in vivo is predicted to be 550 μM and the aminoacylation ratio for tRNA^{Ile} is predicted to be 4. From Table IV, the symmetry coefficient S_{57} ($=k_{-5}/k_7$) is predicted to be between 0.95 and 1.50, and the minimum value for the amino acid step time (t_s) is predicted to be 0.0526 s. This latter prediction results because the smallest apparent first-order rate constant in the forward pathway must be greater than the inverse of the average amino acid step time (t_s^{-1}). From our analysis, the smallest of these rate constants is k_7, which has a lower limit of 19 s^{-1}; hence, $t_s^{-1} \leq 19$ s^{-1}. This value compares favorably with experimentally determined values reported in the literature (Maaløe, 1979). In addition to the predictions

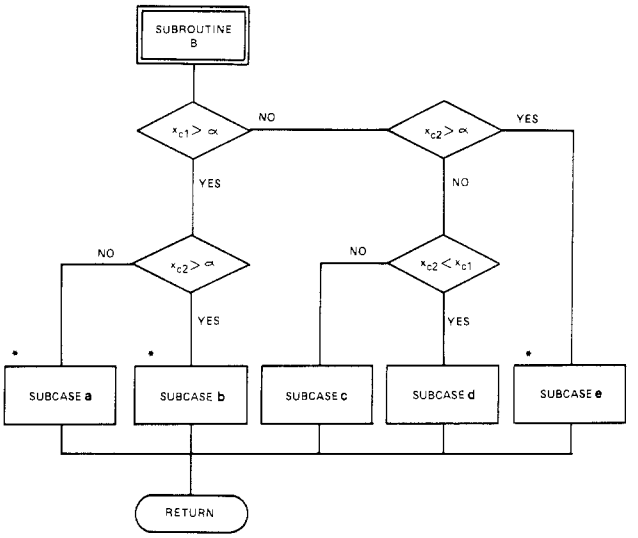

FIGURE A3: Expanded view of subroutine B in Figure A2. The two real solutions of eq A5 are denoted α and β, where $\alpha < \beta$. The subcases marked by the asterisks do not apply to cases 7–10 and 14.

Table AI: Summary of Conditions

name	condition	name	condition
A	$C_{12}/f_1 < 0$	M	$f(X_{c1}) > 0$
B	$C_{11}^2 - 4C_{10}C_{12} \geq 0, C_{12}/f_1 > 0$	N	$f(X_{c1}) < 0$
C	$C_{21} > 0$	O	$f(X_{c2}) > 0$
D	$C_{21} < 0$	P	$f(X_{c2}) < 0$
E	$C_{20} < 0$	Q	$-C_{11}/(2C_{10}) < X_{c3}$
F	$C_{20} > 0$	R	$-C_{11}/(2C_{10}) < X_{c4}$
G	$X_{c3} < X_{c4}$	S	$-C_{11}/(2C_{10}) > X_{c4}$
H	$X_{c3} > X_{c4}$	T	$-C_{11}/(2C_{10}) > X_{c1}$
I	$f(X_{c3}) > 0$	U	$-C_{11}/(2C_{10}) > X_{c2}$
J	$f(X_{c3}) < 0$	V	$X_{c2} < X_{c1}$
K	$f(X_{c4}) > 0$	W	$X_{c2} > X_{c1}$
L	$f(X_{c4}) < 0$		

Table AII: Conditions That Define Each of the Cases in Figures A1-A3

case	combination of conditions[a]
1	A + C + E + H + K + N + P
2	A + C + E + G + I + N + P
3	A + C + F + I + N + P
4	A + D + F + I + L + N + P
5	B + C + E + G + I + Q
6	B + C + E + H + K + R
7	B + C + E + I + L + Q
8	B + C + E + J + K + R
9	B + C + E + J + L
10	B + C + F + J
11	B + C + F + I + Q
12	B + D + F + I + L + Q
13	B + D + F + I + K + Q + S
14	B + D + F + J + K + S
subcases[b]	**combination of conditions**
a	N + O + U
b	N + P
c	O + U + W
d	M + T + V
e	M + T + P

[a] Individual conditions are summarized in Table AI. [b] Subcases do not apply to cases 1-4.

mentioned above, Table III may be viewed as predicting the values for a number of *dependent* rate constants and concentration values that have yet to be measured experimentally.

It should be emphasized that the standard values in Table II are not unique in producing consistent behavior for the system. As indicated above, one could have errors in parameter values other than X_3 and thus produce a consistent solution that is different from the one given in Tables II and III. However, to substantiate such solutions, it would be necessary to identify appropriate errors in the experimentally determined parameter values. The sensitivities shown in Table I provide a useful guide for identifying such parameters.

Acknowledgments

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Appendix

Algebraic Solution and Test for Consistency of Kinetic Equations. Equations 9–27 can be reduced to a set of four simultaneous algebraic equations:

$$C_3X_1 - C_2X_5 = C_1 \quad \text{(A1)}$$

$$C_5X_1 + C_4X_2X_5 - C_6X_7 = C_7 \quad \text{(A2)}$$

$$C_8X_7 - C_5X_1 = C_9 \quad \text{(A3)}$$

$$X_7 = AX_2 - f_1/k_9 \quad \text{(A4)}$$

where the constant coefficients are given by

$$C_1 = (1 + a/b)f_1 \qquad C_2 = k_{-3} \qquad C_3 = k_3X_3$$

$$C_4 = K_5S_{57}k_7 \qquad C_5 = f_1k_{-7}/k_9 \qquad C_6 = (1 + S_{57})k_7$$

$$C_7 = (a/b)f_1 \qquad C_8 = k_7 \qquad C_9 = f_1$$

Equations A1–A4 can be further reduced to a quadratic equation in X_2:

$$C_{10}X_2^2 + C_{11}X_2 + C_{12} = 0 \quad \text{(A5)}$$

where

$$C_{10} = C_4C_3AC_8/(C_2C_5)$$

$$C_{11} = A(C_8 - C_6) - C_4[C_1C_5 + C_3(C_9 + C_8f_1k_9^{-1})]/(C_2C_5)$$

$$C_{12} = (C_6 - C_8)f_1/k_9 - C_7 - C_9$$

The concentration variables X_1, X_5, X_7, X_9, and X_{10} in turn can be obtained from the known values of X_2:

$$X_1 = C_{13}X_2 + C_{14} \quad \text{(A6)}$$

$$X_5 = C_{15}X_2 + C_{16} \quad \text{(A7)}$$

$$X_7 = AX_2 + C_{17} \quad \text{(A8)}$$

$$X_9 = f_1/k_9 \quad \text{(A9)}$$

$$X_{10} = f_1k_9^{-1}E/(1 - E) \quad \text{(A10)}$$

where

$$C_{13} = AC_8/C_5$$

$$C_{14} = -(C_9 + C_8f_1k_9^{-1})/C_5$$

$$C_{15} = AC_3C_8/(C_2C_5)$$

$$C_{16} = -[C_1C_5 + C_3(C_9 + C_8f_1k_9^{-1})]/(C_2C_5)$$

$$C_{17} = -f_1/k_9$$

Substitution of eq A8–A10 with eq 18 yields

$$X_8 = C_{18}X_2 + C_{19} \quad \text{(A11)}$$

where

$$C_{18} = -(1 + A)$$

$$C_{19} = X_T - f_1k_9^{-1}E/(1 - E)$$

Similarly, substitution of eq A6–A8 and A11 into eq 17 yields

$$X_6 = C_{20}X_2 + C_{21} \quad \text{(A12)}$$

where

$$C_{20} = 1 - AC_8(C_2 + C_3)/(C_2C_5)$$

$$C_{21} = [C_1C_5 + (C_2 + C_3)(C_9 + C_8f_1k_9^{-1})]/(C_2C_5) + f_1k_9^{-1}/(1 - E) + X_S - X_T$$

The values of X_3, X_4, X_9, and X_{10} are always positive. The solution for X_1 in eq A1 always will be positive if $X_5 \geq 0$, since C_1, C_2, and C_3 are all positive coefficients. Thus, necessary and sufficient conditions for all the concentration variables to be nonnegative are given by the following: (a) At least one of the solutions for X_2 in eq A5 must be positive real. (b) The solution for X_5 in eq A7 must be positive. (c) The solution for X_7 in eq A8 must be positive. (d) The solution for X_8 in eq A11 must be positive. (e) The solution for X_6 in eq A12 must be positive. There are four critical values of X_2 corresponding to the last four conditions:

$$X_{c1} = -C_{16}/C_{15} = [C_1C_5 + C_3(C_9 + C_8f_1k_9^{-1})]/(AC_3C_8)$$

$$X_{c2} = -C_{17}/A = f_1k_9^{-1}/A$$

$$X_{c3} = -C_{19}/C_{18} = [X_T - f_1k_9^{-1}E/(1 - E)]/(1 + A)$$

$$X_{c4} = -C_{21}/C_{20} = [C_1C_5 + (C_2 + C_3)(C_9 + C_8f_1k_9^{-1}) + C_2C_5f_1k_9^{-1}/(1 - E) + C_2C_5(X_S - X_T)]/[AC_8(C_2 + C_3) - C_2C_5]$$

The critical values of X_2, X_{ci}, and the functions $f(X_{ci}) = C_{10}X_{ci}^2 + C_{11}X_{ci} + C_{12}$ ($i = 1, 2, 3, 4$) are in turn functions of a single unknown variable, namely, k_9. Therefore, the necessary and sufficient conditions for positive concentrations become conditions on the allowable values for the rate constant k_9. These latter conditions can be tested in a systematic and exhaustive manner as outlined in Figure A1 when eq A5 has one positive real root. When eq A5 has two positive real roots, exhaustive testing of the above conditions requires the additional procedures given in Figures A2 and A3. However, the most appropriate biological results are obtained when there is only a single positive real root for eq A5. Tables AI and AII list the specific conditions that are tested in each of the cases shown in Figures A1–A3.

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

References

Arndt, D. J., & Berg, P. (1971) *J. Biol. Chem. 245*, 665–667.

Baldwin, A. N., & Berg, P. (1966) *J. Biol. Chem. 25*, 839–845.

Bouadloun, F., Donner, D., & Kurland, C. G. (1983) *EMBO J. 2*, 1351–1356.

Cruz, J. B., Ed. (1973) *System Sensitivity Analysis*, Dowden, Hutchinson and Ross, Stroudsburg, PA.

Edelmann, P., & Gallant, J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3396–3398.

Eldred, E. W., & Schimmel, P. R. (1972) *Biochemistry 11*, 17–23.

Fersht, A. R. (1977) *Enzyme Structure and Mechanism*, W. H. Freeman, San Francisco.

Fersht, A. R. (1979) in *Transfer RNA: Structure Properties and Recognition* (Schimmel, P. R., Söll, D., & Abelson, J. N., Eds.) pp 247–254, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Freist, W., & Cramer, F. (1983) *Eur. J. Biochem. 131*, 65–80.

Freter, R. R., & Savageau, M. A. (1980) *J. Theor. Biol. 85*, 99–123.

Holler, E., & Calvin, M. (1972) *Biochemistry 11*, 3741–3752.

Hopfield, J. J., Yamane, T., Yue, V., & Coutts, S. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1164–1168.

Lewis, J. A., & Ames, B. N. (1972) *J. Mol. Biol. 66*, 131–142.

Loftfield, R. B., & Eigner, E. A. (1966) *Biochim. Biophys. Acta 130*, 426–448.

Loftfield, R. B., & Vanderjagt, D. (1972) *Biochem. J. 128*, 1353–1356.

Lövgren, T. N. E., Heinonen, J., & Loftfield, R. B. (1975) *J. Biol. Chem. 250*, 3854–3860.

Lövgren, T. N. E., Pastuszyn, A., & Loftfield, R. B. (1976) *Biochemistry 15*, 2533–2540.

Maaløe, O. (1979) in *Biological Regulation and Development* (Goldberger, R. F., Ed.) pp 487–542, Plenum, New York.

Maaløe, O., & Kjeldgaard, N. O. (1966) *Control of Macromolecular Synthesis*, W. A. Benjamin, New York.

Mulvey, R. S., & Fersht, A. R. (1977) *Biochemistry 16*, 4731–4737.

Okamoto, M., & Savageau, M. A. (1984) *Biochemistry* (following paper in this issue).

Parker, J., Johnston, T. C., Borgia, P. T., Holtz, G., Remaut, E., & Fiers, W. (1983) *J. Biol. Chem. 258*, 10007–10012.

Pedersen, S., Bloch, P. L., Reeh, S., & Neidhardt, F. C. (1978) *Cell (Cambridge, Mass.) 14*, 179–190.

Raunio, R., & Rosenqvist, H. (1970) *Acta Chem. Scand. 24*, 2737–2744.

Rigler, R., Cronvall, E., Hirsch, R., Pachmann, U., & Zachau, H. (1970) *FEBS Lett. 11*, 320–323.

Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T., & Britten, R. J. (1957) *Studies of Biosynthesis in Escherichia coli*, Publication No. 607, Carnegie Institute of Washington, Washington, D.C.

Savageau, M. A. (1976) *Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology*, Addison-Wesley, Reading, MA.

Savageau, M. A., & Freter, R. R. (1979a) *Biochemistry 18*, 3486–3493.

Savageau, M. A., & Freter, R. R. (1979b) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4507–4510.

Savageau, M. A., & Lapointe, D. S. (1981) *J. Theor. Biol. 93*, 157–177.

Schreier, A. A., & Schimmel, P. R. (1972) *Biochemistry 11*, 1582–1589.

Yarus, M. (1969) *J. Mol. Biol. 42*, 171–189.

Yarus, M. (1979) in *Transfer RNA: Structure Properties and Recognition* (Schimmel, P. R., Söll, D., & Abelson, J. N., Eds.) pp 501–515, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.