Time course of the uridylylation and adenylylation states in the glutamine synthetase bicyclic cascade

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A kinetic analysis of the glutamine synthetase bicyclic cascade is presented. It includes the dependence on time from the onset of the reaction of both the uridylylation of Shapiro's regulatory protein and the adenylylation of the glutamine synthetase. The transient phase equations obtained allow an estimation of the time elapsed until the states of uridylylation and adenylylation

reach their steady-states, and therefore an evaluation of the effective sensitivity of the system. The contribution of the uridylylation cycle to the adenylylation cycle has been studied, and an equation relating the state of adenylylation at any time to the state of uridylylation at the same instant has been derived.

INTRODUCTION

Glutamine synthetase (GS) catalyses the production of glutamine from NH_3 and glutamic acid. The glutamine produced serves as a source of nitrogen atoms in the biosynthesis of all amino acids, purines and pyrimidine nucleotides, glucosamine 6-phosphate, *p*-aminobenzoic acid and nicotinamide derivatives. GS activity may be modulated by a closed bicyclic cascade (Stadtman and Chock, 1978), which is capable of simultaneously sensing changes in the concentration of many metabolites and of integrating their effects in such a way that the catalytic activity of the enzyme is adjusted to meet the ever-changing demand for glutamine.

Cyclic cascades have special characteristics which make them well suited to play a central role in cellular regulation. A cyclic cascade involves the reversible covalent modification of an interconvertible protein, i.e. the enzyme-catalysed transfer of a modifying group from a donor to the specific site on a receptor protein and its subsequent removal. If the modified form of an interconvertible protein influences the covalent modification of another interconvertible protein, then the two cycles are linked forming a so called bicyclic cascade. In the closed bicyclic cascades (Stadtman et al., 1980; Ottaway, 1988), both the modified and the unmodified forms of the interconvertible protein in the first cycle serve as converter enzymes in the second cycle.

The steady-state kinetics of a model (described in the next section) for the bicyclic cascade modulating the activity of GS has been studied extensively (Stadtman et al., 1979, 1981; Rhee et al., 1985, 1988, 1989; Ottaway, 1988; Chock et al., 1990). However, a complete kinetic analysis of this model, valid for both the transient phase and the steady state, has not yet appeared in the literature. The experimental contributions concerning the time course of the uridylylation and adenylylation of the regulatory protein P_{II} and the adenylylation of the GS respectively (Stadtman et al., 1980, 1981; Rhee et al., 1980) are only descriptive and therefore without any theoretical support. Hence an overall understanding of the modulation mechanism of the GS activity is lacking. Moreover, the kinetic analysis of the transient phase may reveal new features which are not emanating from a kinetic analysis of only the steady state, such as the time

the system needs to reach the steady state and the shape of the transition curve.

The aim of this paper is to present a kinetic analysis of a model of the closed bicyclic cascade regulating GS activity, the steady state of which has previously been analysed kinetically in the literature (Stadtman et al., 1981; Rhee et al., 1985), but covering the whole course of the reaction.

KINETIC ANALYSIS

Description of the closed bicyclic cascade which modulates the activity of GS

This cascade contains two inter-related enzyme cycles (Stadtman et al., 1981; Rhee et al., 1985). One of these describes the adenylylation and de-adenylylation of GS, and the other involves the uridylylation and de-uridylylation of a small regulatory protein known as Shapiro's P_{II} protein (Brown et al., 1971). The adenylylation of GS is carried out by the covalent attachment of an adenylyl group from ATP to each subunit of GS. As GS contains 12 identical subunits, a maximum of 12 mol of AMP can be bound per mol of enzyme. Under most physiological conditions, the adenylylation of a subunit leads to inactivation of that subunit only. Thus the global specific activity of GS is inversely proportional to the average number of adenylyl groups bound per mol of enzyme. There are two separate catalytic sites on the adenylyltransferase (ATase): one catalyses the adenylylation reaction (AT_a) and the other catalyses the de-adenylylation reaction (AT_d).

In the other cycle, the modification of the P_{II} protein involves the covalent attachment of a uridylyl group from UTP to each subunit of the regulatory protein. In this case too, the uridylylation and de-uridylylation of P_{II} are catalysed at separate catalytic sites (denoted UT_u and UT_d) on another bifunctional enzyme, uridylyltransferase (UTase).

The closed coupling of the two cycles arises from the fact that the unmodified form of the P_{II} protein stimulates the ATase-catalysed adenylylation of GS. Furthermore, the uridylylated form of the regulating protein stimulates the ATase-catalysed de-

Scheme 1

adenylation of GS. Therefore the specific activity of GS is determined by the relative catalytic activities of the converter enzymes (i.e. ATase and UTase). These activities are, in turn, determined by the specific rate constants of the reactions, by the dissociation constants of the protein-protein complexes and by the relative concentrations of allosteric effectors and other metabolites that control the activity of the converter enzymes.

We have studied the glutamine synthetase bicyclic cascade pattern used for a steady-state analysis by Rhee et al. (1985), although we refer to the single subunits of the tetrameric protein P_{II} and those of the dodecameric enzyme GS, instead of the overall molecules of these proteins. The pattern of this system is illustrated in Scheme 1.

In Scheme 1, UR is activated by the allosteric effector e_1 . The active UR_{e_1} catalyses the cleavage of UMP from a uridylylated subunit of P_{II} , denoted in Scheme 1 as X_1 , to form the corresponding deuridylylated subunit, denoted in Scheme 1 as X_3 . The subunits X_3 in turn activate AT_a . The activated $AT_a X_3$ then catalyses the adenylation of the subunits of the dodecameric enzyme GS, denoted in Scheme 1 as X_5 . The regeneration cascade is triggered by the interaction of an allosteric effector, e_2 , with UT to form UT_{e_2} , which in turn catalyses the uridylylation of X_3 . The uridylylated subunit X_1 catalyses the de-adenylation of the subunits X_7 . The set of reactions, of which the system is composed, is presented in the more detailed Scheme 2.

At least 40 different metabolites are known which act as the allosteric effectors generally denoted by e_1 and e_2 in Scheme 2 (Stadtman et al., 1980; Rhee et al., 1985, 1988). Important examples of the effectors e_1 and e_2 are glutamine and α -oxoglutarate, which stimulate the de-uridylylation of X_1 and the uridylylation of X_3 , respectively (Rhee et al., 1985, 1988).

Notation and definitions

For ease of typing and so that the equations are not excessively long the following simplified notations are used:

$[E_{1i}]$, $[E_{2a}]$, $[R_{2i}]$, $[R_{2a}]$, concentrations of AT_a , $AT_a X_3$, AT_d and $AT_d X_1$ respectively.

Scheme 2

$[E_{2i}]$, $[E_{2a}]$, $[R_{2i}]$, $[R_{2a}]$, concentrations of AT_a , $AT_a X_3$, AT_d and $AT_d X_1$ respectively.

$[X_i]$ ($i = 1, 2, \dots, 8$), concentrations of X_i .

$[E_1]$, concentration of UR at $t = 0$. At any time we have:

$$[E_1] = [E_{1i}] + [E_{1a}] + [X_2] \quad (1)$$

$[R_1]$, concentration of UT at $t = 0$. At any time we have:

$$[R_1] = [R_{1i}] + [R_{1a}] + [X_4] \quad (2)$$

$[E_2]$, concentration of AT_a at $t = 0$. At any time we have:

$$[E_2] = [E_{2i}] + [E_{2a}] + [X_6] \quad (3)$$

$[R_2]$, concentration of AT_d at $t = 0$. At any time we have:

$$[R_2] = [R_{2i}] + [R_{2a}] + [X_8] \quad (4)$$

$[I_1]$, $[I_2]$, total concentration of the interconvertible protein subunits of P_{II} and GS respectively, i.e.:

$$[I_1] = [X_1] + [X_2] + [X_3] + [X_4] + [X_6] + [X_8] + [E_{2a}] + [R_{2a}] \quad (5)$$

$$[I_2] = [X_5] + [X_6] + [X_7] + [X_8] \quad (6)$$

K_i ($i = 1, 2, 3, 4$); dissociation constants equal to k_{-i}/k_{+i} .

K_{1f} , K_{1r} , K_{2f} , K_{2r} , dissociation constants k_{-5}/k_{+5} , k_{-6}/k_{+6} , k_{-7}/k_{+7} and k_{-8}/k_{+8} respectively.

α_{1f} , α_{1r} , α_{2f} , α_{2r} , parameters equal to k_{1f}/K_{1f} , k_{1r}/K_{1r} , k_{2f}/K_{2f} and k_{2r}/K_{2r} respectively.

β_{2f} and β_{2r} , parameters equal to $\alpha_{2f}[E_2]/K_3$ and $\alpha_{2r}[R_2]/K_4$.

u , state of uridylylation of the protein P_{II} at any time t . Since the protein P_{II} is tetrameric, we have:

$$u = 4[X_1]/[I_1] \quad (7)$$

and $0 \leq u \leq 4$.

u_0 and u_∞ , u values at $t = 0$ and in the steady state ($t \rightarrow \infty$) respectively.

n , state of adenylation of GS at any time t . Since GS is dodecameric, it is verified that

$$n = 12[X_7]/[I_2] \quad (8)$$

and $0 \leq n \leq 12$.

n_0 and n_∞ , n values at $t = 0$ and in the steady state ($t \rightarrow \infty$) respectively.

Time-course equation

The set of differential equations which describes Scheme 2 cannot be solved analytically because it is not linear. However, analytical expressions may be obtained by making the same assumptions as those already used in the literature for a steady-state analysis of this type of bicyclic cascade (Chock and Stadtman, 1977, 1980). These assumptions are: (a) an equilibrium is rapidly established by the formation of the enzyme–enzyme and enzyme–effector complexes; (b) the concentrations of the enzyme–enzyme complexes are negligible compared with those of the modified and unmodified (i.e. active and inactive) enzymes; (c) the concentrations of the allosteric effectors, e_1 and e_2 , are maintained at a constant level in any given metabolic state.

Using these assumptions, the time-course equations for the state of uridylation, u , of the protein P_{II} as well as the state of adenylation, n , of the glutamine synthetase can be derived as shown in the Appendix. The result is:

$$u = u_\infty + (u_0 - u_\infty)e^{-\lambda_1 t} \quad (9)$$

and

$$n = n_\infty e^{-\lambda_2 t} \left[1 + \left(\frac{n_0}{n_\infty} e^{\lambda_2 t} - 1 \right) e^{-\lambda_1 t} + \sum_{j=0}^{\infty} \frac{B_j}{(j+1)!} \frac{B - (j+1)(D_1/C_2)}{1 - (j+1)(\lambda_1/\lambda_2)} (e^{-(j+1)\lambda_1 t} - e^{-\lambda_2 t}) \right] \quad (10)$$

where λ_1 , D_1 , C_2 , B and λ_2 are given by eqns. (A32), (A35) and (A37)–(A39) in the Appendix and u_∞ and n_∞ are the state of uridylation of P_{II} and adenylation of GS in the steady state ($t \rightarrow \infty$), their expression being:

$$u_\infty = 4 \left[\frac{\alpha_{11}[e_1][E_1](K_2 + [e_2])}{\alpha_{11}[e_2][R_1](K_1 + [e_1])} + 1 \right]^{-1} \quad (11)$$

and

$$n_\infty = 12 \left[\frac{\beta_{2r} u_\infty}{\beta_{2r}(4 - u_\infty)} + 1 \right]^{-1} \quad (12)$$

RESULTS AND DISCUSSION

We have obtained the time-course equations, valid for the whole course of the reaction, for uridylation and adenylation states in the bicyclic cascade system which modulates the activity of glutamine synthetase. Previous kinetic analysis (Stadtman et al., 1980, 1981; Rhee et al., 1985) had furnished expressions for the above states in their steady-state.

Eqns. (9) and (10) give the state of uridylation of the protein P_{II} and the state of adenylation of the enzyme GS, respectively, at any time from the onset of the reaction and at any initial values of the initial states of uridylation, u_0 (range 0–4), and adenylation, n_0 (range 0–12). Therefore, they are wide of applicability. The kinetic study presented here could be performed under less restrictive conditions, e.g. by not considering the concentrations of the enzyme–enzyme complexes negligible in the first cycle. However, we preferred to undertake this study under exactly the same simplifying conditions as those made in the previous steady-state study of this cascade, so as to add the information available from the transient phase.

The results for the steady state (eqns. 11 and 12) coincide with those obtained in previous kinetic studies confined to the steady

Figure 1 Plots of eqns. (9) and (10) for an arbitrary set of values of the kinetic parameters and the concentrations which yield a global view of the kinetics of the system

Curve (i) corresponds to u and curve (ii) corresponds to n . The values of T_u and T_n are 5.91 s and 19.9 s respectively. Values used: $u_0 = 2$, $n_0 = 1$, $[E_1] = [R_1] = [e_2] = 5.91 \times 10^{-7}$ M, $[E_2] = [R_2] = 2 \times 10^{-6}$ M, $[I_1] = 4 \times 10^{-6}$ M, $[I_2] = 1.2 \times 10^{-4}$ M, $[e_1] = 10^{-7}$ M, $K_3 = K_4 = 5 \times 10^{-6}$ M, $K_{11} = K_{1r} = 2 \times 10^{-6}$ M, $K_{2r} = 2 \times 10^{-5}$ M, $k_{11} = 4$ s $^{-1}$, $k_{1r} = k_{2r} = 10$ s $^{-1}$ and $k_{2r} = 5$ s $^{-1}$.

state (Rhee et al., 1985). Note that u_∞ is independent of u_0 and that n_∞ is independent of n_0 , i.e. both the state of uridylation of protein P_{II} and that of adenylation of GS in the steady state are independent of the corresponding uridylation and adenylation states at the onset of the reaction. Obviously, u_∞ can be less than, equal to or higher than u_0 . Likewise, n_∞ can be less than, equal to or higher than n_0 . Nevertheless, the u_0 and n_0 values influence the shape of the progress curves obtained from plots of eqns. (9) and (10) as well as the period that the system elapses to reach the steady state, as we shall see in the following.

The fact that eqns. (9) and (10) for the time-dependence of the states of uridylation and adenylation, respectively, permit any value of u_0 and n_0 renders our analysis of wide generality and utility, because it can be applied to experimental data of u and n for any experimental values of the initial states of uridylation and adenylation.

In Figure 1 we have plotted the time course of the state of uridylation, u , of the regulatory protein P_{II} , and of the state of adenylation, n , of GS, for an arbitrary set of values of the kinetic parameters, concentrations, and u_0 and n_0 . For the acquisition of the points necessary to plot eqn. (10) we elaborated a computer program using the QBASIC language, which is available to interested readers on request. This program permits any number of terms to be chosen in the infinite series in eqn. (10). We progressively increased this number from 1, and, in all cases, the differences between the successive results were negligible for less than 30 steps. We employed 30 terms.

Time needed by the system to reach the steady state

The transient phase equations of the bicyclic cascade of GS permit us to estimate the time needed by the system to reach the steady state. This is an important parameter, because a cascade of high steady-state sensitivity but possessing a long transient time may, in real time, behave as a low-sensitivity system. Conversely, a cascade of moderate steady-state sensitivity could,

in real time, display considerable sensitivity if it has a short transient phase.

We arbitrarily assume that the steady state for uridylylation has already been reached when $|u - u_\infty| = 10^{-4}u_\infty$ and we denote the corresponding time by T_u . From the above considerations and from eqn. (9) we obtain:

$$T_u = \frac{1}{\lambda_1} \ln \left(10^4 \left| 1 - \frac{u_0}{u_\infty} \right| \right) \quad (13)$$

Similarly, we consider the steady state of the adenylylation as having been reached when $|n - n_\infty| = 10^{-4}n_\infty$, and we denote this time as T_n . If the same procedure as that used in deriving T_u is applied to obtain T_n by using eqn. (10), then no explicit expression for T_n can be obtained. The value of T_n for a given set of the kinetic parameters and the initial concentrations can be obtained from eqn. (10) by elaborating a list of related t and n values and taking the t values which correspond to such a n value that $|n - n_\infty| = 10^{-4}n_\infty$. Then an approximate expression for T_n may be obtained from eqn. (A36) because, at high t values (such as those corresponding to the steady state of the adenylylation), the exponential terms in eqn. (A36) may be ignored. The result is the following simple differential equation:

$$\frac{dn}{dt} + \lambda_2 n = 12\beta_{2t}C_2 \quad (\text{at high } t \text{ values}) \quad (14)$$

Table 1 T_u and T_n values corresponding to different u_0 and n_0 values

The T_n values in the penultimate column have been obtained by the use of a set of related t and n values and those in the last column from eqn. (16). In all the cases the values of the constants and initial concentrations are those used in Figure 1.

Case	u_0	n_0	T_u (s)	T_n (s)	T_n (s) (eqn. 16)
1	0	0	6.50	20.6	22.2
2	0	12	6.50	23.6	24.5
3	4	0	5.08	20.6	21.4
4	4	12	5.08	23.6	23.4
5	2	1	5.91	19.9	19.5

Figure 2 Dependence of T_u and T_n on u_0 and n_0 respectively

The values of the kinetic parameters and the initial concentrations are those used in Figure 1.

the integration of which yields:

$$n = n_\infty + (n_0 - n_\infty)e^{-\lambda_2 t} \quad (\text{at high } t \text{ values}) \quad (15)$$

From eqn. (15), one easily obtains:

$$T_n = \frac{1}{\lambda_2} \ln \left(10^4 \left| 1 - \frac{n_0}{n_\infty} \right| \right) \quad (16)$$

In Table 1, the T_n values obtained by using the set of related t and n values corresponding to different cases as well as those obtained from eqn. (16) are listed.

Eqns. (13) and (A32) show the dependence of T_u on the variables $[e_1]$, $[e_2]$, $[E_1]$ and $[R_1]$ and u_0 . Analogously, eqns. (16), (A39), (A34), (A37) and (A32) give the dependence of T_n on the variables $[e_1]$, $[e_2]$, $[E_1]$, $[R_1]$, $[E_2]$, $[R_2]$ and n_0 . Note that T_u is n_0 -independent and that T_n is u_0 -independent. In Figure 2, the dependence of both T_u and T_n on u_0 and n_0 , respectively, is shown. As is to be expected, the nearer u_0 is to u_∞ , the smaller the value of T_u . Similarly, the nearer n_0 is to n_∞ , the smaller the value of T_n . Although the curves in Figure 2 have been obtained for an arbitrary set of values of the concentrations and kinetic parameters, the results are of general validity. In Figure 3, the dependence of both T_u and T_n on $[e_1]$ and $[e_2]$ is shown schematically. The dependence of T_u and T_n on the other variables could be obtained similarly.

Reciprocal influence of the uridylylation and adenylylation cycles

Note that, according to eqn. (9), the uridylylation state at any time is independent of the kinetic parameters of the second cycle of the cascade, i.e. the one yielding adenylylation and de-adenylylation of GS, and it is a consequence of the assumptions made. Therefore, the same eqn. (9) could be obtained from a transient-phase kinetic study of the uridylylation cycle alone. Conversely, the time course of the state of adenylylation depends on all the kinetic parameters of the system, except $[I_1]$ and $[I_2]$.

Eqn. (12) shows the influence of u_∞ on n_∞ . Moreover, the study presented here shows the influence of the state of uridylylation at any time on the state of adenylylation at the same instant. From eqn. (9), we obtain:

$$e^{-\lambda_1 t} = (u - u_\infty)/(u_0 - u_\infty) \quad (17)$$

Figure 3 (a) Schematic dependence of T_u on $[e_1]$ and $[e_2]$ and (b) schematic dependences of T_n on $[e_1]$ and $[e_2]$

In (a), when the concentration axis is e_j ($j = 1$ or 2), then L and M result by setting $[e_j] = 0$ and $[e_j] \rightarrow \infty$ respectively into eqn. (13). In (b), if the concentration axis is $[e_1]$, then N is $1/\beta_{2t}$ and curves (i), (ii) and (iii) correspond to the cases in which β_{2t} is less than, equal to or larger than β_{2t} , respectively. In each case P may be obtained by setting $[e_1] \rightarrow \infty$ into eqn. (16). If the concentration axis is $[e_2]$, then N is $1/\beta_{2t}$ and curves (i), (ii) and (iii) correspond to the cases in which β_{2t} is less than, equal to or larger than β_{2t} , respectively. In each case P may be obtained by setting $[e_2] \rightarrow \infty$ into eqn. (16).

and, subsequently:

$$t = -\frac{1}{\lambda_1} \ln \frac{u - u_\infty}{u_0 - u_\infty} \quad (18)$$

Then the insertion of eqns. (17) and (18) into eqn. (10) yields:

$$n = n_\infty \exp \left[B \left(\frac{u_\infty - u}{u_0 - u_\infty} \right) \right] \left\{ 1 + \left(\frac{n_0}{n_\infty} e^B - 1 \right) \exp \left[\frac{\lambda_2}{\lambda_1} \ln \left(\frac{u - u_\infty}{u_0 - u_\infty} \right) \right] + \sum_{j=0}^{\infty} \frac{B^j}{(j+1)!} \frac{B - (j+1)(D_1/C_2)}{1 - (j+1)(\lambda_1/\lambda_2)} \left[\left(\frac{u - u_\infty}{u_0 - u_\infty} \right)^{j+1} - \exp \left[\frac{\lambda_2}{\lambda_1} \ln \left(\frac{u - u_\infty}{u_0 - u_\infty} \right) \right] \right] \right\} \quad (19)$$

which explicitly relates n with u .

The kinetic data analysis which we suggest is based on the experimental progress curves of the state of uridylylation and adenylylation and on the explicit solutions in eqns. (9) and (15).

Evaluation of α_{1r} , α_{1r} , K_1 and K_2

The u_∞ value, i.e. the u value at infinite time, is directly obtained from the experimental progress curve of the state of uridylylation. Then, according to eqn. (9), a plot of $\ln |u - u_\infty|$ versus t gives a straight line with slope λ_1 (the absolute value is needed if $u_0 < u_\infty$).

The λ_1 values are determined in assays in which $[e_1] = 0$ but $[e_2]$ differs from assay to assay. According to eqn. (A32), we have:

$$\frac{[R_1]}{\lambda_1} = \frac{K_2}{\alpha_{1r}[e_2]} + \frac{1}{\alpha_{1r}} \quad ([e_1] = 0) \quad (20)$$

Hence a plot of $[R_1]/\lambda_1$ versus $1/[e_2]$ gives a straight line with the ordinate intercept $1/\alpha_{1r}$ and the slope K_2/α_{1r} . Therefore both α_{1r} and K_2 can be evaluated. Analogously, the λ_1 values are determined at $[e_2] = 0$ and different $[e_1]$ values. Then $[E_1]/\lambda_1$ is plotted versus $1/[e_1]$ to yield a straight line with the ordinate intercept $1/\alpha_{1r}$ and the slope K_1/α_{1r} , from which both α_{1r} and K_1 can be evaluated. This procedure does not require the initial-rate method to be followed to evaluate α_{1r} and α_{1r} , unlike in the procedure based on the steady-state analysis (Chock and Stadtman, 1980).

Evaluation of α_{2r}/K_3 and α_{2r}/K_4

The n_∞ value is obtained from the experimental time-progress curve of the state of adenylylation, n , by extrapolation to infinite time. Moreover, such a curve fits eqn. (15) at high t values. Therefore, the points of a such curve at high t values may be used to plot $\ln |n - n_\infty|$ versus t , and the parameter λ_2 is obtained as the slope of the resulting straight line.

According to eqns. (A39), (A34), (A38) and (A32) and the definitions of β_{2r} and β_{2r} , we have that if $[e_1] = 0$, then:

$$\lambda_2 = \frac{\alpha_{2r}}{K_4} \frac{[I_1]}{[R_2]} \quad ([e_1] = 0) \quad (21)$$

and if $[e_2] = 0$:

$$\lambda_2 = \frac{\alpha_{2r}}{K_3} \frac{[I_1]}{[E_2]} \quad ([e_2] = 0) \quad (22)$$

Two series of assays were carried out. In one of them, the $[e_1]$ value at $t = 0$ is fixed and in each assay a different $[I_1]/[R_2]$ value is used. The λ_2 value for each series is determined as explained above. In the other series, with $[e_2] = 0$ in all the assays, the $[I_1]/[E_2]$ value differs from assay to assay. The λ_2 value is determined for each assay. In the first series, λ_2 is plotted versus $[I_1]/[R_2]$, to yield a straight line, the slope of which, according to eqn. (21), is α_{2r}/K_4 . Analogously, we obtain α_{2r}/K_3 from the results of the second series and by using eqn. (22).

Validity of the analysis

The model here presented coincides with that proposed in the literature (Stadtman et al., 1980, 1981; Rhee et al., 1985) for an

analysis of the steady state of the bicyclic cascade modulating the activity of GS. One of the facts proving the correctness of the transient-phase analysis of this model is that eqns. (9) and (10) simplify to those of the steady state (eqns. 11 and 12) at infinite time.

The transient phase of the uridylylation of P_{II} protein and of the adenylylation of GS has only been studied experimentally and descriptively. The time-dependence of changes in the states of uridylylation of P_{II} and adenylylation of GS have been measured at various concentrations of glutamine (which is an effector, e_1 , in Scheme 1) and α -oxoglutarate (one of the possible metabolites having the function of the effector e_2). In Figure 4,

Figure 4 Time-dependence of the state of uridylylation of P_{II} at different $[e_1]$ and $[e_2]$ values: (a) $[e_1]$ constant; (b) $[e_2]$ constant

Values used: (a) $[e_1] = 10^{-6}$ M, $[e_2] = 10^{-5}$ M, 10^{-6} M and 10^{-7} M in cases (i), (ii) and (iii) respectively; (b) $[e_2] = 10^{-3}$ M, $[e_1] = 10^{-6}$ M, 1.1×10^{-6} M and 10^{-5} M in curves (i), (ii) and (iii), respectively. In all cases $u_0 = 0$ and the values of the kinetic parameters and the initial concentrations are those used in Figure 1.

Figure 5 Time-dependence of the state of adenylylation of GS at different $[e_1]$ and $[e_2]$ values: (a) $[e_1]$ constant; (b) $[e_2]$ constant

Values used: (a) $[e_1] = 10^{-6}$ M, $[e_2] = 10^{-5}$ M, 10^{-6} M and 10^{-7} M in cases (i), (ii) and (iii) respectively; (b) $[e_2] = 10^{-3}$ M, $[e_1] = 10^{-6}$ M, 1.1×10^{-6} M and 10^{-5} M in curves (i), (ii) and (iii) respectively. In all cases $n_0 = 2$ and the values of the kinetic parameters and the initial concentrations are those used in Figure 1.

we have plotted eqn. (9) for different $[e_1]$ and $[e_2]$ values. Note that at a fixed value of $[e_1]$ the value of u increases as $[e_2]$ increases, and that at a fixed value of $[e_2]$ the value of u increases as $[e_1]$ decreases. In Figure 5, we have plotted eqn. (10) for different $[e_1]$ and $[e_2]$ values. Note that at a fixed value of $[e_1]$ the value of n increases as $[e_2]$ decreases, and that at a fixed value of $[e_2]$ the value of n increases as $[e_1]$ increases. The shape of the curves in Figures 4 and 5, as well as the variation of u and n upon $[e_1]$ and $[e_2]$, coincide with those obtained experimentally (Rhee et al., 1988).

Final remarks

The explicit solutions derived here for the time course of the states of uridylylation of P_{11} and adenylylation of GS describe the kinetics of the cascade and its response, at any time from the onset of the reaction, to variations of the primary variables, i.e. initial states of uridylylation and/or adenylylation and the concentrations of the allosteric effectors and the converter enzymes. These equations add to the existing kinetic analysis of the steady state of this system the knowledge of its kinetics during the approach to the steady state, under exactly the same assumptions. Hence the kinetics of the cascade in the entire time range from $t = 0$ to infinite time is now available. Moreover, the analysis presented here gives a theoretical support to the experimental data on the time-dependence of the states of uridylylation and adenylylation in the GS cascade.

The kinetics of the model used here can, for a chosen set of the values of the concentrations and kinetic parameters, be described by numerical solution of the corresponding set of differential equations. However, any such solution would only pertain to the set of values chosen, and no generalization can be made. The explicit equations derived here have the following advantages over numerical simulations of the kinetics of the system. (a) They allow an experimental design and a kinetic data analysis leading to the evaluation of the kinetic parameters. (b) They permit the prediction of the influence of the variables on the state of

uridylylation of P_{11} , u , and that of adenylylation of GS, n . (c) They explain previously uninterpretable experimental data, e.g. the time-dependence of u and n . (d) They permit the definition of new regulatory properties of the system, such as the elapse of time until u and n reach their steady-state levels, i.e. T_u and T_n , respectively. (e) They reveal conditions under which the cycles show particular kinetic features, e.g., we have delimited the relative values of the parameters β_{2r} and β_{2r} (Figure 3b) leading to an increase, decrease or constancy of the transient time T_n . Moreover, the explicit solutions allow the calculation of the dependence of the transient phase of the uridylylation on the u_0 value and the dependence of the state of adenylylation on the n_0 values (Figure 2).

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APPENDIX

Derivation of the time-progress equations of the states of uridylylation and adenylylation of the interconvertible proteins in Schemes 1 and 2

From Scheme 2 one obtains:

$$d[R_{2a}]/dt = k_{+4}[R_{2i}][X_1] - k_{-4}[R_{2a}] - k_{+8}[R_{2a}][X_7] + (k_{-8} + k_{2r})[X_8] \quad (A1)$$

$$d[X_1]/dt = -(k_{+5}[E_{1a}] + k_{+4}[R_{2i}])[X_1] + k_{-5}[X_2] + k_{1r}[X_4] + k_{-4}[R_{2a}] \quad (A2)$$

$$d[X_2]/dt = k_{+5}[E_{1a}][X_1] - (k_{-5} + k_{1r})[X_2] \quad (A3)$$

$$d[X_4]/dt = k_{+6}[R_{1a}][X_3] - (k_{-6} + k_{1r})[X_4] \quad (A4)$$

$$d[X_7]/dt = k_{2r}[X_6] - k_{+8}[R_{2a}][X_7] + k_{-8}[X_8] \quad (A5)$$

$$d[X_8]/dt = k_{+8}[R_{2a}][X_7] - (k_{-8} + k_{2r})[X_8] \quad (A6)$$

The assumptions in the text must be made to obtain analytical expressions of the progress curves from this non-linear set of differential equations. Assumption (a) allows us to write:

$$[E_{1a}] = ([E_{1i}] + [E_{1a}])[e_1]/(K_1 + [e_1]) \quad (A7)$$

$$[R_{1a}] = ([R_{1i}] + [R_{1a}])[e_2]/(K_2 + [e_2]) \quad (A8)$$

$$[E_{2a}] = [X_3][E_{2i}]/K_3 \quad (A9)$$

$$[R_{2a}] = [X_1][R_{2i}]/K_4 \quad (A10)$$

$$[X_2] = [X_1][E_{1a}]/K_{1r} \quad (A11)$$

$$[X_4] = [X_3][R_{1a}]/K_{1r} \quad (A12)$$

$$[X_6] = [X_5][E_{2a}]/K_{2r} \quad (A13)$$

$$[X_8] = [X_7][R_{2a}]/K_{2r} \quad (A14)$$

Assumption (b) allows the simplification of eqns. (1)–(6) in the main paper to:

$$[E_{1i}] + [E_{1a}] \approx [E_1] \quad (A15)$$

$$[R_{1i}] + [R_{1a}] \approx [R_1] \quad (A16)$$

$$[E_{2i}] \approx [E_2] \quad (A17)$$

$$[R_{2i}] \approx [R_2] \quad (A18)$$

$$[X_1] + [X_2] \approx [I_1] \quad (A19)$$

$$[X_5] + [X_7] \approx [I_2] \quad (A20)$$

If eqns. (A15) and (A16) are taken into account in eqns. (A7)–(A10), then it follows that:

$$[E_{1a}] \approx [E_1][e_1]/(K_1 + [e_1]) \quad (A21)$$

$$[R_{1a}] \approx [R_1][e_2]/(K_2 + [e_2]) \quad (A22)$$

$$[E_{2a}] \approx ([I_1] - [X_1])[E_2]/K_3 \quad (A23)$$

$$[R_{2a}] \approx [X_1][R_2]/K_4 \quad (A24)$$

If now eqns. (A19)–(A24) are taken into account in eqns. (A11)–(A14), then:

$$[X_2] \approx [X_1] \frac{[E_1][e_1]}{K_{1r}(K_1 + [e_1])} \quad (A25)$$

$$[X_4] \approx ([I_1] - [X_1]) \frac{[R_1][e_2]}{K_{1r}(K_2 + [e_2])} \quad (A26)$$

$$[X_6] \approx ([I_2] - [X_7])([I_1] - [X_1])[E_2]/(K_{2r}K_3) \quad (A27)$$

$$[X_8] \approx [X_1][X_7][R_2]/(K_{2r}K_4) \quad (A28)$$

Other relationships arising from assumption (b) are:

$$[R_{2a}] + [X_1] + [X_2] + [X_8] \approx [X_1] \quad (A29)$$

$$[X_7] + [X_8] \approx [X_7] \quad (A30)$$

The assumption (c) and eqns. (A21) and (A22) ensure that $[E_{1a}]$ and $[R_{1a}]$ remain constant from the onset of the reaction.

If eqns. (A1), (A2), (A3) and (A6) are added up and eqns. (A19), (A20), (A11) and (A12) and the definitions of α_{1r} and α_{1r} are taken into account, then the result is, after rearrangement:

$$\frac{d[X_1]}{dt} + \lambda_1[X_1] = \alpha_{1r}[I_1][R_1][e_2]/(K_2 + [e_2]) \quad (A31)$$

where:

$$\lambda_1 = \{\alpha_{1r}[E_1][e_1]/(K_1 + [e_1])\} + \{\alpha_{1r}[R_1][e_2]/(K_2 + [e_2])\} \quad (A32)$$

Eqn. (A31) is a linear differential equation, since at $t = 0$, if $[X_1] = u_0[I_1]/4$ ($0 \leq u_0 \leq 4$), the solution is:

$$[X_1] = C_1 + D_1 e^{-\lambda_1 t} \quad (A33)$$

where:

$$C_1 = \frac{\alpha_{1r}[R_1][e_2][I_1]}{\lambda_1(K_2 + [e_2])} \quad (A34)$$

$$D_1 = -C_1 + (u_0/4)[I_1] \quad (A35)$$

If we multiply both sides of eqn. (A33) by $4/[I_1]$ and take eqn. (7) into account, then eqn. (9) in the main text for the state of uridylylation of the protein P_{II} is obtained.

If eqns. (A5) and (A6) are added and eqns. (A20), (A23), (A24) and (8) and the definitions of β_{2r} and β_{2r} are taken into account, then, after rearrangement, the result is:

$$\frac{dn}{dt} + \lambda_2\{1 - (B\lambda_1/\lambda_2)e^{-\lambda_1 t}\}n = 12\beta_{2r}C_2\left(1 - \frac{D_1}{C_2}e^{-\lambda_1 t}\right) \quad (A36)$$

where:

$$C_2 = \frac{\alpha_{1r}[E_1][e_1][I_1]}{\lambda_1(K_1 + [e_1])} \quad (A37)$$

$$B = (\beta_{2r} - \beta_{2r})D_1/\lambda_1 \quad (A38)$$

$$\lambda = \beta_{2r}C_1 + \beta_{2r}C_2 \quad (A39)$$

Eqn. (A36) is a linear differential equation, the integration of which yields eqn. (10) in the main text.