Journal of Bioenergetics and Biomembranes, Vol. 16, Nos. 5/6, 1984

Synthesis and Degradation of Interconvertible Enzymes. Kinetic Equations of a Model System[1]

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Received April 17, 1984; revised June 18, 1984

Abstract

Steady-state and kinetic equations have been developed which characterize the rates of formation, interconversion, and degradation of an enzyme protein subject to reversible phosphorylation. The theoretical model system incorporates separate fractional degradative rate constants for the phosphorylated and dephosphorylated protein species. The classical models for interconvertible enzymes, and for protein turnover, are special limiting situations of the general model presented here.

Key Words: Protein turnover; interconvertible enzymes; protein degradation and phosphorylation state.

Introduction

Ordinarily the interconversion of an enzyme between its dephosphorylated and phosphorylated states is considered in the context of a closed reversible system where the ratio of the concentration of the dephosphorylated form [E] to the phosphorylated form [Ep] is uniquely determined, in steady-state operation, by the ratio of the activity of protein phosphatase, C, to the protein kinase, B:

$$[\mathrm{E}] \underset{C[\mathrm{Ep}]}{\overset{B[\mathrm{E}]}{\rightleftharpoons}} [\mathrm{Ep}] \tag{1}$$

$$[\mathrm{E}]/[\mathrm{Ep}] = C/B \tag{2}$$

Here, B is the catalytic activity of a protein kinase (including kinetic rate

[1]Dedicated to David E. Green, a man of happy memory, who, although he cautioned that one could not anticipate Nature, delighted in doing just that.

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0145-479X/84/1200-0433$03.50/0

constants) and C is a protein phosphatase (including its kinetic factors). This simplification of the steady-state equations is based on the assumption that [E] $< K_m$ for the B reaction and [Ep] $< K_m$ for the C reaction, and that any participating effectors or substrates, principally [ATP], are invariant. The total enzyme concentration does not vary. For a complete treatment of reversible phosphorylation kinetics see Stadtman and Chock (1977) and the review of Roach (1977).

Since the observations of Schoenheimer (1946) it is recognized that intracellular proteins in Metazoa are subject to degradation at rates that are met by replacement synthesis in steady-state operation. Endocrine signaling can engender self-limiting transitions between steady-state levels of certain enzymes through modulation of synthetic and/or degradative parameters. Equations (3)–(5) developed by Price *et al.* (1962), Segal and Kim (1963), and Schimke *et al.* (1964) have found wide application in describing adaptive transitions of enzyme concentrations in tissues, e.g., the coordinate net synthesis of lipogenic enzymes in liver in response to insulin (Gibson *et al.*, 1972).

$$\xrightarrow{A} [\mathrm{E}] \xrightarrow{D[\mathrm{E}]} \tag{3}$$

$$d[\mathrm{E}]/dt = A - D[\mathrm{E}] \tag{4}$$

$$[\mathrm{E}] = A/D - I\,e^{-Dt} = [\mathrm{E}]_s - ([\mathrm{E}]_s - [\mathrm{E}]_0)\,e^{-Dt} \tag{5}$$

In these equations A is the zero-order synthetic rate, D the fractional degradative rate parameter, D[E] the first-order rate of degradation of E, and I the integration constant which is equivalent here to the difference between the values of [E] at zero time and at the final steady state. The latter value is defined by A/D.

The above models do not address the turnover of interconvertible enzymes. In order to join the so-called "short-term" reversible phosphorylation determinants with the "long-term" kinetics of protein turnover, a more generalized model is proposed and its properties examined.

Results and Discussion

Phosphorylation of proteins is a posttranslational event, i.e., the dephosphorylated form of an interconvertible enzyme, [E], may be represented as the product of zero-order synthesis. In the model below [Eq. (6)] the reversible interconversion of [E] to [Ep] is designated as in Eq. (1) and (2) by the vectors B[E] and C[Ep]. However, this interconversion is influenced as well by the rates of degradation of [Ep] (vector D[Ep]) and of [E] (vector F[E]). All vectors are first order, except synthesis, A. In steady-state operation, as

represented in Eq. (6) and (9), there is continuous synthesis of E, and conversion of E to Ep, provided that D is greater than zero in the turnover of this protein. If the parameter B is zero, [Ep] would cease to exist and the classic turnover equations (3)–(5) would obtain. If A, D, and F are zero the isolated interconvertible system would become operational [Eqs. (1) and (2)].

$$\xrightarrow[F[\mathrm{E}]]{A} [\mathrm{E}] \xrightarrow[C[\mathrm{Ep}]]{B[\mathrm{E}]} [\mathrm{Ep}] \xrightarrow{D[\mathrm{Ep}]} \tag{6}$$

$$d[\mathrm{E}]/dt = A - F[\mathrm{E}] - B[\mathrm{E}] + C[\mathrm{Ep}] \tag{7}$$

$$d[\mathrm{Ep}]/dt = B[\mathrm{E}] - C[\mathrm{Ep}] - D[\mathrm{Ep}] \tag{8}$$

Steady state limiting values $[\mathrm{E}]_s$, $[\mathrm{Ep}]_s$, $[\mathrm{T}]_s$ (total enzyme) and the ratio $[\mathrm{E}]_s/[\mathrm{T}]_s$ are presented below in Eqs. (9)–(13). These identities are derived directly from the primary differential equations (7) and (8) set equal to zero. They are useful in gauging the extremes between the initial (defined) steady-state values ($[\mathrm{E}]_0$, $[\mathrm{Ep}]_0$, $[\mathrm{ET}]_0$, and $[\mathrm{E}]_0/[\mathrm{ET}]_0$) and the final steady-state concentrations which are determined by assigning values to the parameters A, B, C, D, and F at time zero.

$$A - F[\mathrm{E}]_s = B[\mathrm{E}]_s - C[\mathrm{Ep}]_s = D[\mathrm{Ep}]_s \tag{9}$$

$$[\mathrm{E}]_s = (AC + AD)/(BD + CF + DF) \tag{10}$$

$$[\mathrm{Ep}]_s = AB/(BD + CF + DF) \tag{11}$$

$$[\mathrm{T}]_s = (AB + AC + AD)/(BD + CF + DF) \tag{12}$$

$$[\mathrm{E}]_s/[\mathrm{T}]_s = (C + D)/(B + C + D) \tag{13}$$

Expressions for [E], [Ep], and [T] as functions of the transition time between initial and final states are presented in Eqs. (14)–(16). Progression depends on the initial values of these variables and the values of parameters A, B, C, D, and F imposed on the system at time zero.

$$[\mathrm{E}] = \frac{m_1 + C + D}{B} I_1 e^{m_1 t} + \frac{m_2 + C + D}{B} I_2 e^{m_2 t} + \frac{AC + AD}{BD + CF + DF} \tag{14}$$

$$[\mathrm{Ep}] = I_1 e^{m_1 t} + I_2 e^{m_2 t} + \frac{AB}{BD + CF + DF} \tag{15}$$

$$[\mathrm{T}] = \left(1 + \frac{m_1 + C + D}{B}\right) I_1 e^{m_1 t} + \left(1 + \frac{m_2 + C + D}{B}\right) I_2 e^{m_2 t} + \frac{AB + AC + AD}{BD + CF + DF} \tag{16}$$

As seen in Eqs. (17)–(20) below, the integration constants, I_1 and I_2, incorporate the initial and steady-state values of [E] and [Ep] which are defined in any transition. Similarly m_1 and m_2 (which are always negative) are functions of B, C, D, and F, but not of A.

$$m_1 = \tfrac{1}{2}\Big(-(B + C + D + F) + [(B + C + D + F)^2 - 4(BD + CF + DF)]^{1/2}\Big) \quad (17)$$

$$m_2 = \tfrac{1}{2}\Big(-(B + C + D + F) - [(B + C + D + F)^2 - 4(BD + CF + DF)]^{1/2}\Big) \quad (18)$$

$$I_1 = [\mathrm{Ep}]_0 - [\mathrm{Ep}]_s - I_2 \quad (19)$$

$$I_2 = \frac{B}{m_1 - m_2}\left(\frac{m_1 + C + D}{B}([\mathrm{Ep}]_0 - [\mathrm{Ep}]_s) - [\mathrm{E}]_0 + [\mathrm{E}]_s\right) \quad (20)$$

Employing Eqs. (14)–(20), we plot an example of a theoretical specific transition in Fig. 1. The parameters and variables of the initial and final steady states are defined in the legend. In this particular transition which was initiated at time zero by a 10-fold fall in C (phosphatase enzyme activity) and a 2.5-fold rise in D (degradation parameter for Ep) there was relatively little change in [E]/[T] (percent of dephosphorylated enzyme) even though total enzyme concentration [T] fell by 56%.

This model for the turnover of an interconvertible enzyme may be further characterized by the following relationships[3]: (a) The system is operational regardless of the relative values of the degradative parameters D and F (as long as $D >$ zero). (b) Changes in protein kinase (B) and phosphatase (C) do affect the level of total enzyme [T] in shifting from one steady state to another. Major changes in [T], however, are more readily accomplished by adjusting the synthetic parameter A and/or the degradative parameters D and F [see Eq. (12)]. (c) The steady-state extremes of the ratio [E]/[T] are independent of the synthetic rate A and of F [see Eq. (13)]. Consequently, wide variations of total enzyme concentration brought about by induction and repression of enzyme synthesis would have no effect on the value of this ratio *at its final steady state.* (d) If vectors B[E] and C[Ep] in Eq. (6) are quite large relative to the others, a near-equilibrium state would exist between [E] and [Ep]. In steady-state operation toward the right (which would obtain if D[Ep] > 0) the difference B[E] $-$ C[Ep] would nevertheless be equal to

[3]Within the framework of the simplifying assumptions: [E] $< K_m$ for the B reaction and [Ep] $< K_m$ for the C reaction.

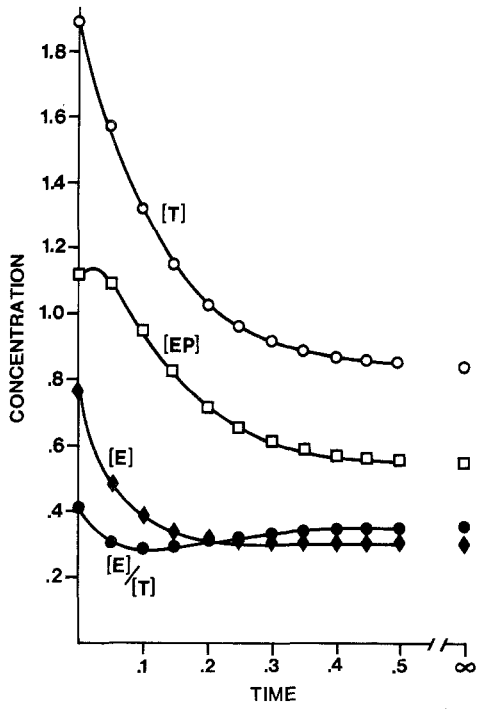

Fig. 1. Variation with time of [T], [Ep], [E], and [E]/[T]. The initial steady state (left) is defined by the parameters ($A = 6$, $B = 20$, $C = 10$, $D = 4$, and $F = 2$) to give the following initial values: $[E]_0 = 0.778$, $[Ep]_0 = 1.111$, $[T]_0 = 1.889$, and $[E]_0/[T]_0 = 0.412$. At time zero the value of C is changed from 10 to 1.0 and D from 4 to 10. The system then progresses to a new steady state as shown: $[E]_s = 0.297$, $[Ep]_s = 0.540$, $[T]_s = 0.838$, and $[E]_s/[T]_s = 0.354$. The integration constants for this transition are $I_1 = 1.185$ and $I_2 = -0.614$.

D[Ep] and to $A - F$[E] however small these latter values may be. This kind of relationship is probably typical for most interconvertible enzyme systems wherein rates of interconversion are much more rapid than enzyme synthesis and degradation.

The quantities D and F are defined simply as degradative parameters since they represent the first step in the *irreversible* removal of Ep and E from the system. Depending on specific situations, this would include spontaneous disruption of noncovalent bonds, sequestration of protein (e.g., in autophagosomes), direct action of proteolytic enzymes, or other irreversible covalent modification (e.g., oxidation). It is known that the dephosphorylated form of protein can be handled differently from the phosphorylated species. Berg-

strom *et al.* (1978) have reported that phosphorylated pyruvate kinase from liver (E.C. 2.7.1.40) is ten times more susceptible to proteolysis by bacterial subtilisin (E.C. 3.4.21.14) than the dephosphorylated enzyme substrate. Hall *et al.* (1979) found that pyruvate kinase obtained from liver of fasted rats (in contrast to carbohydrate-fed rats) consisted of two subunits of 56,000 and 51,000 daltons. Only the former could be phosphorylated by cAMP-dependent protein kinase *in vitro*. These authors suggested that phosphorylation of the 56,000 dalton subunit *in vivo* may have marked the protein for irreversible proteolytic modification to yield the 51,000 dalton subunit. At least two other proteins seem to fall into this category: yeast fructose-1,6-bisphosphatase (Müller and Holzer, 1981) and troponin I from cardiac muscle (Toyo-Oka, 1982). Nevertheless, there is no *a priori* reason to believe that the phosphorylated forms of other interconvertible enzymes are more readily degraded. The only criterion for application of the turnover equations presented here is that the phosphorylated species can be degraded at a measurable rate (regardless of the value of the degradation parameter for the dephosphorylated species).

The turnover model for interconvertible enzymes may find application in defining the regulation of the microsomal enzyme HMG CoA reductase (E.C. 1.1.1.34). The enzyme is rapidly inactivated *in vitro* by phosphorylation with a cAMP-independent protein kinase and restored to full activity with protein phosphatase (Ingebritsen and Gibson, 1980; Beg and Brewer, 1981). Further, this system is acutely regulated (<60 min) in suspensions of intact hepatocytes by glucagon or mevalonate (an initial fall in [E]/[T] as well as a decrease in [T] (Ingebritsen *et al.*, 1979; Parker *et al.*, 1983). Preincubation of hepatocytes with insulin has the opposite effect. Early studies with intact animals subjected to long-term endocrine and nutritional manipulation showed that although the total HMG CoA reductase activity in liver, [T], varied widely over a period of hours the [E]/[T] ratio appeared to remain invariant (Nordstrom *et al.*, 1977; Brown *et al.*, 1979). More recent evidence, however, has demonstrated that rat liver HMG CoA reductase does go through a change in activation state ([E]/[T] and [E]/[Ep]) during the diurnal feeding cycle provided that microsomes are separated rapidly from livers of anesthetized animals (Easom and Zammit, 1984). During periods of feeding these ratios rose to a maximal value which is indicative of progressive dephosphorylation of reductase in response to a rise in the insulin/glucagon ratio. Equation (13), or its equivalent forms in Eq. (21),

$$\frac{C + D}{B} = \frac{[E]_s/[T]_s}{1 - ([E]_s/[T]_s)} = \frac{[E]_s}{[Ep]_s} \tag{21}$$

offer a means for evaluating experimental data of this kind. If the $[E]_s/[T]_s$ and $[E]_s/[Ep]_s$ ratios at the extreme steady states of the diurnal cycle are in

fact invariant, then the value of D must vary dramatically to offset the changes in B and C. An overall increase in the rate of liver protein degradation is engendered by glucagon (Mortimore, 1982). On the other hand, if these ratios in Eq. (21) are elevated in response to feeding (insulin) and depressed in starvation (glucagon), they would for the most part, respectively, depend on the expected rise in C relative to B, and B relative to C, with little interference from the values of D in these extremes.

Acknowledgments

Research cited from this laboratory was supported by grants from the National Institutes of Health (AM21278, GM 24623) and the Grace M. Showalter Foundation. The authors are indebted to Drs. Peter Roach and Howard Edenberg for helpful discussions.

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