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Thermodynamic Linkages in Rabbit Muscle Pyruvate Kinase: Analysis of Experimental Data by a Two-State Model†

Robert W. Oberfelder,‡ B. George Barisas,§ and James C. Lee*

ABSTRACT: A concerted, allosteric model is developed, and equations are derived for quantitative interpretation of the kinetic and equilibrium binding data of rabbit muscle pyruvate kinase at pH 7.5 and 23 °C. The *simplest* model which seems likely to rationalize the experimental data involves two conformational states. In this model, two simplifying assumptions are made. First, the affinities of pyruvate kinase for both substrate and inhibitor are assumed to depend only upon the conformational state of the tetrameric enzyme. Second, the rate of product formation is also assumed to depend only upon the enzyme conformation. All types of experimental data are analyzed simultaneously to estimate the parameters which best predict the total body of experimental results. The fitted parameters indicate that the intrinsic allosteric equilibrium favors the active (R) state by 11 to 1. The substrate phosphoenolpyruvate binds preferentially, by a factor of 10, to the R state, whereas the inhibitor Phe has about 23 times higher affinity for the inactive (T) state. In all cases tested, the calculated values are in good agreement with the experimental data.

In the preceding paper (Oberfelder et al., 1984), it was shown that rabbit muscle pyruvate kinase (PK)[1] exhibits simple hyperbolic kinetics in the absence of Phe. The presence of Phe, however, induces sigmoidicity in the relation between activity and PEP concentration. This suggests a degree of cooperativity in PEP binding to PK which increases with Phe concentration. Results from equilibrium binding studies substantiate the kinetic observations in that the apparent affinity of PEP for PK is reduced in the presence of Phe which acts as a heterotropic effector. Difference sedimentation velocity and sulfhydryl titration experiments demonstrate unequivocally that Phe induces a gross structural change in PK and that completion of the protein structural change precedes the saturation of the binding sites in PK by Phe. Thus, it was proposed that the regulation of rabbit muscle PK by Phe can be best represented by the two-state model of Monod, Wyman, and Changeux (Monod et al., 1965). In this paper, methods are derived for quantitative interpretation of the kinetic and equilibrium binding data described in the preceding paper (Oberfelder et al., 1984). The results of such an analysis are presented, and their implications concerning the allosteric regulation of PK are discussed.

Theory

The simplest model which seemed likely to rationalize the experimental data satisfactorily was a two-state model. It is assumed that PK can only exist in two conformational states, designated R and T, each of which exhibits specific equilibrium binding and enzyme kinetic characteristics. Figure 1 illustrates the proposed regulatory model of PK, where E, S, and I are the enzyme, substrate, and inhibitor, respectively. Within each state, there are four possible molecular species, the enzyme alone (E), the enzyme–substrate complex (E·S), the enzyme–inhibitor complex (E·I), and the enzyme–substrate–inhibitor complex (E·S·I). Each of the liganded species can involve 1–4 mol of ligand bound per PK tetramer; thus, for instance, the term E·S represents the enzyme with 1, 2, 3, or 4 mol of substrate bound. The upper plane of the cube, as shown in Figure 1, contains all of those species which assume the R conformation, while the lower portion represents those species present in the T conformation. The species are interconnected by equilibrium constants (K). Superscripts denote the conformational state of the enzyme, and subscripts indicate either S or I binding. The intrinsic allosteric equilibrium constant (L) is the ratio of $[E^T]$ to $[E^R]$ in the absence of effectors. The catalyic rate constant for each state is designated k_2, and P is the product. The fundamental simplifying assumption of this model is that the symmetry of the tetrameric protein must be maintained in allosteric transitions. If one subunit undergoes a state change, the other subunits in the same enzyme molecule must also undergo the same transition. A single tetramer may not simultaneously contain some subunits in each of the two states. Since concerted conformational changes are required in this model, ligand-induced conformational changes must be completed and precede the saturation of the enzyme by ligand. Two simplifying assumptions were made in order to fit the experimental data to the *simplest* model. First, the affinities of PK for both substrate and inhibitor were assumed to depend only upon the conformational state of the tetramer; e.g., the constant for the binding of S to E^T is assumed to be the same as that of S to E^T·I. Similarly, the affinity of S for E^R and E^R·I is assumed to be the same. K^T_S and K^R_S may, however, assume different values. Second, the rate of product formation is also assumed to depend only upon the enzyme conformation. Thus, the rate of product formation by the E·S and E·S·I complexes in the R state, for example, is assumed to be equal; however, k^T_2 and k^R_2 may differ. A total of five equations were derived to model the data obtained in experimental studies of enzyme kinetics, ligand binding, and protein conformational changes. The derivations are shown in the Appendix. The expression for enzyme activity (V), expressed as moles of product formed per minute, is

$$V = \{(k^R_2[S]/K^R_S)(1 + [S]/K^R_S)^3(1 + [I]/K^R_I)^4 + (k^T_2[S]L/K^T_S)(1 + [S]/K^T_S)^3(1 + [I]/K^T_I)^4\}/\{(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^4 + L(1 + [I]/K^T_S)^4(1 + [I]/K^T_I)^4\} \quad (1)$$

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[1] Abbreviations: PK, pyruvate kinase; MWC, Monod–Wyman–Changeux; PEP, phosphoenolpyruvate.

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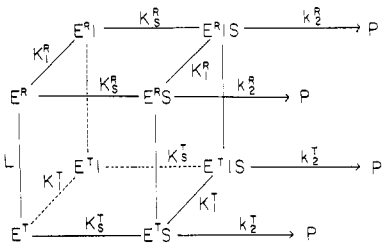

FIGURE 1: Proposed two-state model for the allosteric behavior of rabbit muscle PK. The various symbols are defined in the text.

The numbers of molecules of substrate ($\bar{Y}_S$) and inhibitor ($\bar{Y}_I$) bound per tetramer are expressed as

$$\bar{Y}_S = \{(4[S]/K^R_S)(1 + [S]/K^R_S)^3(1 + [I]/K^R_I)^4 + (4[S]/K^T_S)L(1 + [S]/K^T_S)^3(1 + [I]/K^T_I)^4\}/\{(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^4 + L(1 + [S]/K^T_S)^4(1 + [I]/K^T_I)^4\} \quad (2)$$

$$\bar{Y}_I = \{(4[I]/K^R_I)(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^3 + (4[I]L/K^T_I)(1 + [S]/K^T_S)^4(1 + [I]/K^T_I)^3\}/\{(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^4 + L(1 + [S]/K^T_S)^4(1 + [I]/K^T_I)^4\} \quad (3)$$

The fraction of PK molecules existing in each of the two conformational states is expressed as

$$\bar{R} = \{(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^4\}/\{(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^4 + L(1 + [S]/K^T_S)^4(1 + [I]/K^T_I)^4\} \quad (4)$$

$$\bar{T} = \{L(1 + [S]/K^T_S)^4(1 + [I]/K^T_I)^4\}/\{(1 + [S]/K^R_S)^4(1 + [I]/K^R_I)^4 + L(1 + [S]/K^T_S)^4(1 + [I]/K^T_I)^4\} \quad (5)$$

Materials and Methods

Inspection of Figure 1 shows that, in this two-state model, seven independent parameters, five equilibrium constants and two rate constants, are required to define the ligand binding and enzyme kinetic properties at constant temperature. There are three kinds of principal data in this study: rates of product formation by PK at various substrate and inhibitor concentrations, binding isotherms of PEP and of Phe to PK, and conformational changes in PK. Clearly, the kinetic data reflect in some measure all seven parameters while the ligand binding isotherms are determined by the equilibrium constants only. The goal is to analyze *simultaneously* all types of experimental data so as to estimate the parameters which best predict the total body of experimental results. This type of analysis has been applied to the trout hemoglobin system (Barisas & Gill, 1979).

The goal in processing the data is to obtain the model parameters which have the highest probability of yielding the observed data sets. It is assumed that, in all experiments, the standard errors in the substrate and inhibitor concentrations are much less than those in the observed reaction velocities (σ_V) and extents of substrate or inhibitor binding (σ_{Y_S} and σ_{Y_I}, respectively). Moreover, it is assumed that errors in V, Y_S, and Y_I are normally distributed. The parameter χ^2 must be minimized with respect to the seven adjustable parameters, and it is defined as

$$\chi^2 = \sum_m \sum_p (V_{obsd} - V_{calcd})^2/\sigma_V^2 + \sum_n \sum_p (Y_{S,obsd} - Y_{S,calcd})^2/\sigma_{Y_S}^2 + \sum_o \sum_p (Y_{I,obsd} - Y_{I,calcd})^2/\sigma_{Y_I}^2 \quad (6)$$

where m, n, and o are the number of experiments for kinetic studies, binding of substrate, and binding of inhibitor, respectively. p represents the number of experimental points in each of these experiments.

Table I: Parameters for the Two-State Model of PK Allosteric Behavior at pH 7.5, 23 °C, As Evaluated by Least-Squares Analysis of Experimental Data

parameter	fitted constant	$\Delta G°$ [a] (kcal/mol)
L	0.094 ± 0.03	+1.4
K^R_S (M)	(44 ± 2) × 10^{-6}	−5.9
K^T_S (M)	(440 ± 40) × 10^{-6}	−4.6
K^R_I (M)	(14 ± 3) × 10^{-3}	−2.5
K^T_I (M)	(0.60 ± 0.07) × 10^{-3}	−4.4
k^R_2	0.96 ± 0.11	
k^T_2	0.089 ± 0.07	

[a] The free energies given are for the R → T transition (as kilocalories per mole of tetramer) and for the binding of ligands to PK (as kilocalories per mole of ligand).

The first problem is the evaluation of the statistical weights. For each data set, the standard error σ^2 of observed points about a fitted curve changes little whether a data set was analyzed by itself or along with other data. Therefore, each data set was individually subjected to a preliminary analysis, and the value of σ^2 was noted. This enables χ^2 to be evaluated. The test of the validity of the assigned weights is that the contribution of each data set to χ^2 is approximately the number of points in that data set. χ^2 should, in turn, total the number of degrees of freedom in the system, namely, the total number of points in all data sets minus seven, the number of independently adjustable parameters.

Once the weights have been evaluated for each data set, χ^2 must be minimized with respect to the seven model parameters. The nonlinear least-squares fitting algorithm of Marquardt (1963) is extremely efficient for such minimization. By interpolation between parabolic and gradient searches in parameter spaces, the algorithm converges rapidly and monotonically on the minimum χ^2 values in the particular depression in parameter space where the procedure is initiated. The Marquardt algorithm was implemented in BASIC for execution on a NOVA 3/12 computer. Linear estimates of uncertainties in values of fitted parameters were obtained in the course of data analysis.

Results and Discussion

The model parameters obtained by least-squares analysis of the experimental data are summarized in Table I. From these parameters, calculated curves were generated and compared with the corresponding experimental data sets. A comparison between the calculated and experimental kinetic data is shown in Figure 2, while the binding isotherms of PEP and Phe to PK are shown in Figures 3 and 4, respectively. The standard error σ_V of the normalized kinetic data is 0.10 for all data sets, while the standard errors in PEP and Phe binding are about 0.2 mol of ligand per mol of tetrameric PK.

The value of L indicates that the intrinsic equilibrium favors the R state by a factor of 11 to 1. PEP binds to the R state with 10-fold higher affinity than to the T state, while Phe binds to the T state with 23-fold higher affinity. The R state appears to be responsible for almost all of the observed catalytic activity. Since both the catalytic rate and the affinity of the effectors are state dependent, this enzyme falls into the category of a mixed K- and V-type enzyme (Monod et al., 1965).

Although seven different constants are needed to fit the experimental data according to the proposed model, the assignment of the values of these constants is not arbitrary. In fact, several data sets provide almost direct access to some of the individual constants. A good estimate of K^R_S can be

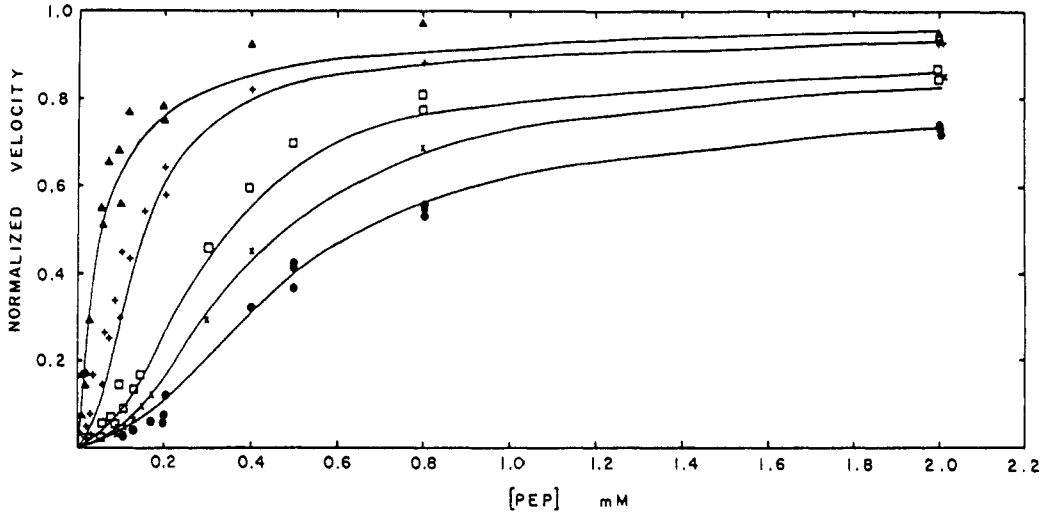

FIGURE 2: Comparison between calculated and experimental values for rates of the PK-catalyzed conversion of PEP to pyruvate at various Phe concentrations and 23 °C. Calculated values are represented by the smooth curves. Phe concentrations are (▲) 0, (+) 3, (□) 7, (×) 9, and (●) 12 mM.

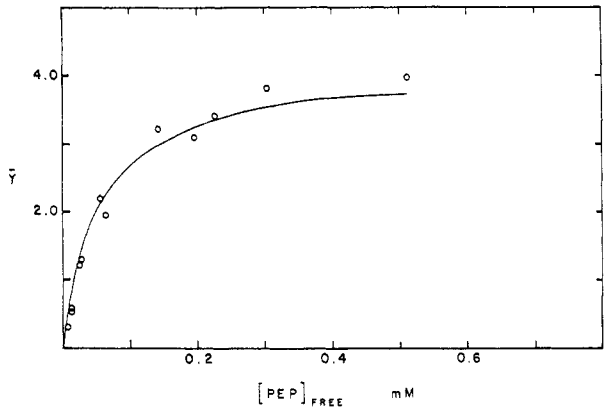

FIGURE 3: Comparison between calculated and experimental values for PEP binding to PK at 23 °C in the absence of ADP. Calculated values are represented by the smooth curve.

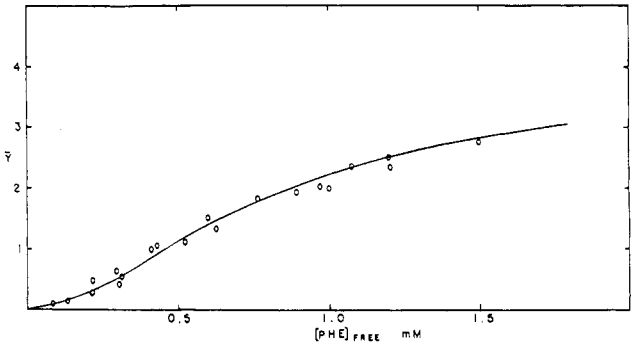

FIGURE 4: Comparison between calculated and experimental values for Phe binding to PK at 23 °C. The calculated values are represented by the smooth curve.

obtained directly from the equilibrium binding data in the absence of Phe. Examination of eq 2 shows that in the absence of Phe, all the terms involving inhibitor concentration can be discarded. Under the experimental conditions, PK is present predominantly in the R state as indicated by the value of 0.094 for L. Hence, the second term in both the denominator and the numerator of eq 2 will be small, and $\bar{Y}_S$ depends mainly on the PEP concentration and the equilibrium constant K^R_S. Similarly, the kinetic data in the absence of Phe should also provide a good, independent estimate of K^R_S for the same reason. Equation 1 shows that in the absence of Phe, V is determined mainly by substrate concentration, K^R_S, and k^R_2. The maximum enzyme activity under these experimental conditions should provide a reasonably good estimate of k^R_2. The experimental data provided by the preceding paper (Oberfelder et al., 1984) do not contain any means of estimating, K^R_I, K^T_I, K^T_S, k^T_2, and L independently. Since binding of Phe induces a conformational state change in PK, and since there is no way to fix PK in the R state in the presence of Phe, independent estimates of K^R_I are not possible. Similarly, PK cannot be obtained in the T state in the absence of Phe, so the parameters K^T_I, K^T_I, and k^T_2 cannot be estimated independently. Thus, these parameters can only be extracted from data collected under various conditions and by multiple approaches.

The observed behavior of the enzyme can be readily rationalized from the fitted parameters. According to the formalism of Monod et al. (1965), a state transition is indicated by a sigmoidal relationship in binding isotherms or in the plots of velocity vs. substrate concentration. Since the intrinsic allosteric equilibrium of PK at pH 7.5 and 23 °C favors the R state and since PEP binds preferentially to this state in the absence of Phe, one would expect hyperbolic shapes for plots of reaction velocity vs. PEP concentration and for the PEP binding isotherm. Such behavior can indeed be seen in the observed kinetic and binding data shown in Figures 2 and 3.

The effect of Phe upon the kinetic curves is also readily explained. The inhibitory effect of Phe results from its stabilization of the T state, which has a low affinity for PEP and a very low catalytic rate as well. The sigmoidal shape observed in the plots of velocity vs. PEP concentration results from the stabilization by PEP of the R state. In the presence of appreciable amounts of Phe, PK will be present in a mixture of E^T, $E^T{\cdot}I$, E^R, and $E^R{\cdot}I$ forms. The amounts of each species will be governed by their respective equilibrium constants and the concentration of Phe. At low concentrations, PEP will bind preferentially to the E^R or $E^R{\cdot}I$ species without perturbing the R–T equilibrium. As the PEP concentration is increased, it begins to shift the equilibrium distribution toward species in the R state. Now PEP binds 10-fold more strongly to the R state, and this state exhibits essentially all the catalytic activity of the enzyme. Thus, in the presence of Phe, high concentrations of PEP should cause sigmoidal shapes in V vs. substrate concentration plots.

The fitted constants also provide an explanation for the shapes of PEP and Phe binding isotherms at pH 7.5 and 23 °C. In the absence of Phe, the PEP binding isotherm should

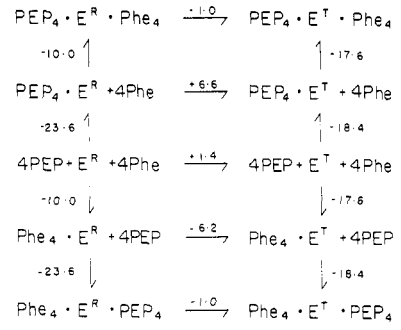

FIGURE 5: Free-energy changes for reactions of PK at 23 °C with substrate and inhibitor, as well as for the allosteric transitions among various liganded species. Ligand binding free energies are given as kilocalories per 4 mol of ligand while allosteric transition free energies are given as kilocalories per tetramer.

appear hyperbolic since PK is present principally in the R state as indicated by $L << 1$. On the other hand, at the low Phe concentrations, the inhibitor neither binds appreciably nor perturbs the R–T equilibrium. Higher Phe concentrations shift the equilibrium to favor the formation of the T state to which Phe binds with higher affinity. Additional Phe binding should then reflect the affinity of Phe for the T state, which is 23-fold higher than that for the R state. This leads to a sigmoidal relationship in the binding isotherm. The computer-fitted parameters, therefore, provide both a qualitative and a quantitative explanation for the observed allosteric behavior of PK at pH 7.5.

The computer-fitted constants describing the interactions between PK and the ligands at pH 7.5 enable various free energies associated with the equilibrium to be determined. From these free energies, additional insights into the PK allosterism can be gained. The standard-state free-energy changes ($\Delta G°$) for the various reactions can be calculated as $\Delta G° = -RT \ln K$, where R is the gas constant, T is the absolute temperature, and K is the equilibrium constant for the reaction as written. Values of $\Delta G°$ associated with various steps in the two-state model are shown in Table I and Figure 5. These values indicate that the only T-state species for which the allosteric equilibrium favors is E–Phe. In the absence of allosteric effectors such as Phe, the allosteric equilibrium favors R-state tetramers, and PEP binding shifts this equilibrium even more strongly toward R-state molecules.

In the preceding paper (Oberfelder et al., 1984), the kinetic data were analyzed by using the linked-function approach of Wyman (1964); it was shown that a value of +1.2 kcal/mol of monomer can be assigned to ΔG_{XY}, the coupling energy between the two ligands, namely, Phe and PEP. It implies that the simultaneous binding of both ligands to the same PK subunit is unfavorable. Free-energy changes for PEP binding to the R and T state are −5.9 and −4.6 kcal/mol of PEP, respectively. The 1.3 kcal/mol of PEP difference in these values is similar to $\Delta G°_{XY}$ estimated from the kinetic data. Hence, this unfavorable coupling energy can be largely ascribed to the difference in $\Delta G°$ for PEP binding to the R and T states.

In conclusion, this study has analyzed results from various experimental approaches in terms of a simple two-state concerted model for the allosteric behavior of rabbit muscle PK. This two-state MWC model satisfactorily describes all experimental results. This situation contrasts with yeast PK whose kinetic behavior can only be described by an extended MWC model which includes the presence of a symmetrical hybrid state of R_2T_2 (Johannes & Hess, 1973). It would thus be of interest to determine the minimal models necessary to describe allosteric regulation for other isozymic forms of PK. Combining the solution properties of these PK isozymes with the respective structural information, it may be possible to elucidate the mechanisms of regulation of PK at the molecular level.

Appendix

The equilibria that exist for the binding of the substrate and the inhibitor are

$$E^R + S \overset{K^R_S}{\rightleftharpoons} E^R{\cdot}S \quad \text{(A1a)}$$

$$E^R + I \overset{K^R_I}{\rightleftharpoons} E^R{\cdot}S \quad \text{(A1b)}$$

$$E^R{\cdot}S + I \overset{K^R_I}{\rightleftharpoons} E^R{\cdot}S{\cdot}I \quad \text{(A1c)}$$

$$E^R{\cdot}I + S \overset{K^R_S}{\rightleftharpoons} E^R{\cdot}I{\cdot}S \quad \text{(A1d)}$$

and

$$E^T + S \overset{K^T_S}{\rightleftharpoons} E^T{\cdot}S \quad \text{(A2a)}$$

$$E^T + I \overset{K^T_I}{\rightleftharpoons} E^T{\cdot}I \quad \text{(A2b)}$$

$$E^T{\cdot}S + I \overset{K^T_I}{\rightleftharpoons} E^T{\cdot}S{\cdot}I \quad \text{(A2c)}$$

$$E^T{\cdot}I + S \overset{K^T_S}{\rightleftharpoons} E^T{\cdot}I{\cdot}S \quad \text{(A2d)}$$

Since any enzyme form which contains substrate may contribute to the activity of the enzyme, eq A3 is a general

$$V = \{k^R_2([E^R{\cdot}S] + [E^R{\cdot}S{\cdot}I]) + k^T_2([E^T{\cdot}S] + [E^T{\cdot}S{\cdot}I])\}/\{4([E^R] + [E^T] + [E^R{\cdot}S] + [E^T{\cdot}S] + [E^R{\cdot}S{\cdot}I] + [E^T{\cdot}S{\cdot}I] + [E^R{\cdot}I] + [E^T{\cdot}I])\} \quad \text{(A3)}$$

equation describing the velocity. The numerator contains those species which may have catalytic activity, and the denominator contains all possible forms of the enzyme weighed by the number of active sites. In order to utilize this equation, the relative concentrations of the active species must be determined. Each active species must be weighed both for the number of substrate molecules bound and for the number of ways in which they can be arranged. $[E^R{\cdot}S]$ would be

$$[E^R{\cdot}S] = ([E^R][S])/K^R_S \quad \text{(A4)}$$

The concentration of a tetramer containing one substrate molecule bound would be

$$[E^R{\cdot}S] = \frac{[E^R][S]}{K^R_S} \times 1 \times 4 \quad \text{(A5)}$$

where 1 is the number of substrate molecules bound and 4 represents the number of possible configurations in the tetramer. If two substrate molecules are bound, then the following formulation describes the concentration:

$$S{\cdot}E^R + S \overset{K^R_S}{\rightleftharpoons} E^R{\cdot}S{\cdot}S \quad \text{(A6)}$$

$$[E^R{\cdot}S{\cdot}S] = \frac{[E^R][S][S]}{K^R_S K^R_S} \quad \text{(A7)}$$

Incorporating the fact that 2 mol of substrate is bound and there are six possible configurations for two substrate molecules on a tetramer, the concentration of the complex is

$$[E^R{\cdot}S{\cdot}S] = \frac{[E^R][S]^2}{K^{R_S{}^2}} \times 2 \times 6 \quad \text{(A8)}$$

Similar treatment results in the following values for the concentration of the protein with either three or four molecules of substrate bound:

$$[E^R{\cdot}S{\cdot}S{\cdot}S] = \frac{[E^R][S]^3}{K^R{}_S{}^3} \times 3 \times 4 \quad \text{(A9)}$$

$$[E^R{\cdot}S{\cdot}S{\cdot}S{\cdot}S] = \frac{[E^R][S]^4}{K^R{}_S{}^4} \times 4 \times 1 \quad \text{(A10)}$$

The total contribution of all of the $E^R{\cdot}S$ forms can be reduced to the following form by factoring:

$$[E^R{\cdot}S] = 4[E^R]\frac{[S]}{K^R{}_S}\left(1 + \frac{[S]}{K^R{}_S}\right)^3 \quad \text{(A11)}$$

A similar sequence was employed in determining the value for the term for the concentration of the $E^R{\cdot}S{\cdot}I$ species. The configuration of both ligands must be taken into account in the weighing of this term, as shown in eq A12:

$$[E^R{\cdot}S{\cdot}I] = \frac{4[E^R][S]}{K^R{}_S}\left(1 + \frac{[S]}{K^R{}_S}\right)^3\left(\frac{4[I]}{K^R{}_I} + \frac{6[I]^2}{K^R{}_I{}^2} + \frac{4[I]^3}{K^R{}_I{}^3} + \frac{[I]^4}{K^R{}_I{}^4}\right) \quad \text{(A12)}$$

Adding eq A11 and A12 together results in eq A13:

$$[E^R{\cdot}S] + [E^R{\cdot}S{\cdot}I] = \frac{4[E^R][S]}{K^R{}_S}\left(1 + \frac{[S]}{K^R{}_S}\right)^3\left(1 + \frac{[I]}{K^R{}_I}\right)^4 \quad \text{(A13)}$$

Similar manipulations of the T-state enzyme produce eq A14:

$$[E^T{\cdot}S] + [E^T{\cdot}S{\cdot}I] = \frac{4[E^T][S]}{K^T{}_S}\left(1 + \frac{[S]}{K^T{}_S}\right)^3\left(1 + \frac{[I]}{K^T{}_I}\right)^4 \quad \text{(A14)}$$

Defining the intrinsic allosteric equilibrium constant as $L = [E^T]/[E^R]$, we can describe the concentration of the T-state species as

$$[E^T{\cdot}S] + [E^T{\cdot}S{\cdot}I] = \frac{4[E^R][S][L]}{K^T{}_S}\left(1 + \frac{[S]}{K^T{}_S}\right)^3\left(1 + \frac{[I]}{K^T{}_I}\right)^4 \quad \text{(A15)}$$

The denominator was derived in a similar manner. Each term in the demoninator was weighed only for a number of possible configurations, since the number of active sites has already been accounted for in eq A3. Substitution for the concentration terms in the numerator and demoninator of eq A3 results in eq A16:

$$V = \{(k^R{}_2[S]/K^R{}_S)(1 + [S]/K^R{}_S)^3(1 + [I]/K^R{}_I)^4 + (k^T{}_2[S]L/K^T{}_S)(1 + [S]/K^T{}_S)^3(1 + [I]/K^T{}_I)^4\}/\{(1 + [S]/K^R{}_S)^4(1 + [I]/K^R{}_I)^4 + L(1 + [I]/K^T{}_S)^4(1 + [I]/K^T{}_I)^4\} \quad \text{(A16)}$$

The derivation of the equations describing substrate and inhibitor binding was performed in a manner similar to that employed for the velocity equation. The general form of the equation is

$$\bar{Y}_S = \{[E^R{\cdot}S] + [E^R{\cdot}S{\cdot}I] + [E^T{\cdot}S] + [E^T{\cdot}S{\cdot}I]\}/\{[E^R] + [E^T] + [E^R{\cdot}S] + [E^T{\cdot}S] + [E^R{\cdot}S{\cdot}I] + [E^T{\cdot}S{\cdot}I] + [E^R{\cdot}I] + [E^T{\cdot}I]\} \quad \text{(A17)}$$

The numerator is the same as that employed in the velocity equation except that the rate constants are not employed in this equation. In order to determine the number of moles of substrate bound per tetramer, the denominator was weighed only for the number of moles of tetramer and not for the number of binding sites (eq A18). The amount of bound inhibitor is described by eq A19.

$$\bar{Y}_S = \{(4[S]/K^R{}_S)(1 + [S]/K^R{}_S)^3(1 + [I]/K^R{}_I)^4 + (4[S]/K^T{}_S)L(1 + [S]/K^T{}_S)^3(1 + [I]/K^T{}_I)^4\}/\{(1 + [S]/K^R{}_S)^4(1 + [I]K^R{}_I)^4 + L(1 + [S]/K^T{}_S)^4(1 + [I]/K^T{}_I)^4\} \quad \text{(A18)}$$

$$\bar{Y}_I = \{(4[I]/K^R{}_I)(1 + [S]/K^R{}_S)^4(1 + [I]/K^R{}_I)^3 + (4[I]L/K^T{}_I)(1 + [S]/K^T{}_S)^4(1 + [I]/K^T{}_I)^3\}/\{(1 + [S]/K^R{}_S)^4(1 + [I]/K^R{}_I)^4 + L(1 + [S]/K^T{}_S)^4(1 + [I]/K^T{}_I)^4\} \quad \text{(A19)}$$

Similarly, the equations describing the fraction of the protein molecules in each state have also been derived, as shown in eq A20 and A21.

$$\bar{R} = \{(1 + [S]/K^R{}_S)^4(1 + [I]/K^R{}_I)^4\}/\{(1 + [S]/K^R{}_S)^4(1 + [I]/K^R{}_I)^4 + L(1 + [S]/K^T{}_S)^4(1 + [I]/K^T{}_I)^4\} \quad \text{(A20)}$$

$$\bar{T} = \{L(1 + [S]/K^T{}_S)^4(1 + [I]/K^T{}_I)^4\}/\{(1 + [S]/K^R{}_S)^4(1 + [I]/K^R{}_I)^4 + L(1 + [S]/K^T{}_S)^4(1 + [I]/K^T{}_I)^4\} \quad \text{(A21)}$$

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References

Barisas, B. G., & Gill, S. J. (1979) *Biophys. Chem. 9*, 235–244.

Johannes, K. J., & Hess, B. (1973) *J. Mol. Biol. 76*, 181–205.

Marquardt, D. W. (1963) *J. Soc. Ind. Appl. Math. 11*, 431.

Monod, J., Wyman, J., & Changeux, J. P. (1965) *J. Mol. Biol. 12*, 88–118.

Oberfelder, R. W., Lee, L. L.-Y., & Lee, J. C. (1984) *Biochemistry* (preceding paper in this issue).

Wyman, J. (1964) *Adv. Protein Chem. 19*, 223–286.