Kinetics of protein-modification reactions

Determination of the fractional concentration of enzyme protein groups, or group reactivities, essential for catalytic function

Emmanuel T. RAKITZIS and Thalia B. MALLIOPOULOU

Department of Biological Chemistry, University of Athens Medical School, Athens 115 27, Greece

A mathematical treatment of modification-induced enzyme protein inactivation is presented, and it is shown that, at initial reaction conditions, the ratio of the first derivative of the equation describing enzyme activity loss to the first derivative of the equation describing protein groups modification is equal to the fractional concentration of enzyme protein reactive groups, or group reactivities, essential for catalytic function.

The number of enzyme protein reactive groups, i , per enzyme active site, essential for catalytic function, may be determined by plotting the $(1/i)$ th power of fractional enzyme activity versus the extent of protein groups modification (Tsou, 1962; Horiike & McCormick, 1979; Stevens & Colman, 1980; Horiike *et al.*, 1984; Rakitzis, 1984a,b). However, this procedure is not applicable in cases where there is interaction between sites during the modification process (Tsou, 1962; Rakitzis, 1978). Also, in cases where protein modification is described by a summation of exponential functions of reaction time, this procedure is only applicable to the fraction of groups exhibiting the slowest exponential function of reaction time (Tsou, 1962). The fractional concentration of enzyme protein groups, or, in the case where protein groups are not intrinsically identical, of protein group reactivities, essential for catalytic function may be determined by the use of an enzyme protein reactive groups concentration well in excess of modifying agent concentration (Rakitzis & Malliopolou, 1985). However, this method is accurate only in cases where the fractional concentration of group reactivities essential for catalytic function is close to unity. The number of protein reactive groups, per enzyme active site, essential for catalytic function may also be determined by a comparison of protein-modification, and of enzyme-inactivation, rate constants (Ray & Koshland, 1961; Rakitzis, 1977, 1978). However, in cases where the kinetic description of these processes consists of a summation of exponential functions of reaction time, a comparison of rate constants is not feasible without a thorough analysis of the enzyme protein modification, and inactivation, event into the microscopic reaction components constituting the overall process. Since an analysis of a protein-modification, and/or enzyme-inactivation, reaction is fraught with difficulties of many kinds (see Rakitzis, 1984a,c), a more direct approach to this end is desirable. In the present paper it is shown that, in cases where protein-modification, and/or enzyme-inactivation, reactions are described by summations of exponential functions of reaction time, the fractional concentration of enzyme protein group reactivities essential for catalytic function may be determined by a comparison of the first derivative values, at initial reaction conditions, of the enzyme-inactivation and of the protein-modification process. Horiike *et al.* (1984), by an extension of the probabilistic method of Tsou (1962), have shown that, in a plot of fractional

enzyme activity versus extent of protein groups modification, extrapolation of the plot on the extent of modification axis, indicates, under certain conditions, the stoichiometry of the modification-induced enzyme-inactivation reaction. Clearly, the extrapolated initial portion of the plot of fractional enzyme activity versus extent of protein modification is equal to the ratio, at initial reaction conditions, of the first derivative of the enzyme-inactivation reaction to the first derivative of the protein-modification reaction.

For a protein-modification reaction, a generalized reaction scheme is:

where E_{r+1} is the protein species produced by a reaction of either protein conformational isomerization, or one of covalent modification, from the species E_r , and also where k_r is the reaction rate constant. The first derivative, at initial reaction conditions, of the concentration of protein modified groups, $[E]_{\text{mod}}$, is:

$$[E]_{\text{mod}}'(0) = n \sum_j k_j [E]_{j(0)} \quad (2)$$

where n is the total number of protein reactive groups per enzyme protein molecule, $[E]_{j(0)}$ is the concentration of the j th class of protein conformational isomers at initial reaction conditions, and k_j is the modification rate constant for this class. Similarly, the first derivative, at initial reaction conditions, of the concentration of the catalytically inactive enzyme protein, $[E]_{\text{inact}}$, is given by the relationship:

$$[E]_{\text{inact}}'(0) = \sum_j i_j k_j [E]_{j(0)} \quad (3)$$

where i_j is the number of groups, per enzyme protein molecule, of each particular class j , essential for catalytic function. The ratio $[E]_{\text{inact}}'(0)/[E]_{\text{mod}}'(0)$ is equal to the fractional concentration of enzyme protein group reactivities essential for catalytic function. In cases where all protein reactive groups are intrinsically identical, the value for $[E]_{\text{inact}}'(0)/[E]_{\text{mod}}'(0)$ is equal to i/n , i.e., it is equal to the stoichiometry of the modification-induced enzyme-inactivation reaction. It should be noted that the procedure described above is also applicable in cases presenting with interaction between protein reactive groups during the modification event (protein-modifica-

tion co-operativity). This procedure is also applicable in cases where protein species are produced by reactions involving ligand binding to the protein, since, at constant ligand concentration, these reactions are kinetically indistinguishable from protein-isomerization reactions (Rakitzis, 1980).

The treatment of modification-induced enzyme-inactivation described above may be exemplified by its application to a simple model. A protein presenting with two modifiable groups per protein molecule is considered. For the sake of simplicity, it is assumed that modification of one of the two protein reactive groups is quickly followed by modification of the other group, so that the existence of monomodified protein species need not be taken into account (strong positive modification co-operativity) (Rakitzis, 1977, 1983). It is further assumed that the unmodified protein species exist in one of two conformational states, A_I and A_{II} , and also that each protein reactive group constitutes an enzyme active site. These changes may be written as follows:

where a is modified protein, k_1 and k_2 are the protein-isomerization rate constants, and k_a and k_b the modification rate constants. The dependence of the concentration of unreacted protein, $[E]$, where $[E] = [A_I] + [A_{II}]$, on reaction time is given by the equation (Rakitzis, 1980):

$$[E]' + (k_1 + k_2 + k_a + k_b)[E]' + (k_a k_2 + k_a k_b + k_b k_1)[E] = 0 \quad (5)$$

The solution of eqn. (5) is (Rakitzis, 1980):

$$([E]/[E]_0) = Ce^{m_1 t} + (1 - C)e^{m_2 t} \quad (6)$$

where:

$$C = \frac{m_2 + k_a[A]_{I(0)}/[E]_0 + k_b[A]_{II(0)}/[E]_0}{m_2 - m_1} \quad (7)$$

where m_1 and m_2 are the roots of the characteristic equation of eqn. (5), t is reaction time, and $[E]_0$, $[A]_{I(0)}$ and $[A]_{II(0)}$ are equal to $[E]$, $[A_I]$ and $[A_{II}]$, respectively, at $t = 0$. As will be seen, either by inspection of eqn. (4), or by differentiation of eqn. (6):

$$[E]_{\text{mod.}}'(0) = -[E]'(0) = k_a[A]_{I(0)} + k_b[A]_{II(0)} \quad (8)$$

Concerning modification-induced enzyme inactivation, in the reaction scheme of eqn. (4), two cases may be distinguished:

(a) modification of one particular protein group abolishes catalytic function for the active site associated with that group, or

(b) modification of one particular protein group abolishes catalytic function for both enzyme active sites of the protein molecule.

In case (a), $[E]_{\text{inact.}}'(0) = [E]_{\text{mod.}}'(0)$ while in case (b), $[E]_{\text{inact.}}'(0) = 2[E]_{\text{mod.}}'(0)$, where $[E]_{\text{mod.}}'(0)$ is as given in eqn. (8) (Rakitzis, 1978). In case (a), the value for the fractional concentration of group reactivities essential for catalytic function is 0.5, while in case (b), this value is equal to unity.

Examples of the determination of the fractional concentration of enzyme protein group reactivities essential for catalytic function, by the use of data from the literature, are given in Table 1. It will be seen that, in all cases given, the fractional concentration of group

Table 1. Determination of the fractional concentration of enzyme protein group reactivities essential for catalytic function

a is fractional enzyme activity, b is fractional concentration of unmodified protein groups, t is in h, n is the total number of modifiable groups per protein molecule, while the ratio $[E]_{\text{inact.}}'(0)/[E]_{\text{mod.}}'(0)$ is as defined in the text.

Enzyme, reaction type, and reference	Kinetic description of inactivation and modification processes	$\frac{[E]_{\text{inact.}}'(0)}{[E]_{\text{mod.}}'(0)}$	
			$1/n$
Photo-oxidation of phosphoglucomutase methionine residues (Ray & Koshland, 1962)	$a = e^{-57.6t}$ $b = 0.43e^{-22.2t} + 0.57e^{-3.06t}$	0.43	0.08
Photo-oxidation of aspartic aminotransferase histidine residues (Martinez-Carrion <i>et al.</i> , 1967)	$a = e^{-2.72t}$ $b = 0.25e^{-2.77t} + 0.75e^{-0.27t}$	0.38	0.125
Alkylation of glutamate dehydrogenase SH residues (Rosen <i>et al.</i> , 1973)	$a = 0.73e^{-0.336t} + 0.27e^{-0.084t}$ $b = 0.70e^{-0.064t} + 0.30e^{-0.3t}$	0.35	0.17
Acylation of histidyl residues of pyridoxamine (pyridoxine) 5'-phosphate oxidase (Horiike <i>et al.</i> , 1979)	$a = e^{-55.2t}$ $b = 0.27e^{-52.2t} + 0.73e^{-5.88t}$	0.75	0.25
Trinitrophenylation of rhodanese amino groups (Malliopoloulou, 1984)	$a = e^{-37.7t}$ $b = 0.09e^{-32.6t} + 0.14e^{-5.94t} + 0.77e^{-0.724t}$	0.87	0.10

reactivities essential for catalytic function is several times higher than the $1/n$ value. It is clear, however, that, unless all protein groups are intrinsically identical, the fractional concentration of group reactivities essential for catalytic function is not a measure of the stoichiometry of the modification-induced enzyme-inactivation reaction, while it certainly is a measure of modifying agent effectiveness. An effective modifying agent should have a fractional concentration of group reactivities essential for catalytic function equal to unity, either because of modification limited to active-site residues only, or because of co-operative enzyme inactivation (Rakitzis, 1986).

It is concluded that, in cases of modification-induced enzyme inactivation, the determination of the fractional concentration of group reactivities essential for catalytic function is to be preferred to a *prima facie* interpretation of data on the assumption that multiexponential modification, and inactivation, processes are only due to the existence of groups of different reactivity towards the modifying agent. As will be seen from eqns. (2), (3) and (8), the first derivatives of these processes, at initial reaction conditions, are free of isomerization rate constants, and indeed are only dependent on rate constants pertaining to the modification of the protein species present at the start of the reaction [this may be contrasted with eqn. (6), where m_1 and m_2 are functions of the protein-modification and isomerization rate constants]. Clearly, unless a complete analysis of the modification, and inactivation, process is experimentally feasible, the use of first derivatives, at initial reaction conditions, is to be preferred when protein-modification,

and/or enzyme-inactivation, data are to be correlated with such parameters as modifying agent, ligand, or hydrogen ion concentration of the reaction medium.

REFERENCES

- Horiike, K. & McCormick, D. B. (1979) *J. Theor. Biol.* **79**, 403–414
Horiike, K., Tsuge, H. & McCormick, D. B. (1979) *J. Biol. Chem.* **254**, 6638–6643
Horiike, K., Tojo, H., Yamano, T. & Nozaki, M. (1984) *J. Biochem. (Tokyo)* **95**, 605–609
Malliopoulou, T. B. (1984) Habilitation Thesis, p. 37, University of Athens Medical School
Martinez-Carrion, M., Turano, C., Riva, F. & Fasella, P. (1967) *J. Biol. Chem.* **242**, 1462–1430
Rakitzis, E. T. (1977) *J. Theor. Biol.* **67**, 49–59
Rakitzis, E. T. (1978) *J. Theor. Biol.* **70**, 461–465
Rakitzis, E. T. (1980) *J. Math. Biol.* **10**, 79–87
Rakitzis, E. T. (1983) *Math. Biosci.* **66**, 93–96
Rakitzis, E. T. (1984a) *Biochem. J.* **217**, 341–351
Rakitzis, E. T. (1984b) *Biochem. J.* **223**, 259–262
Rakitzis, E. T. (1984c) *J. Mol. Catal.* **26**, 291–295
Rakitzis, E. T. (1986) *J. Mol. Catal.* **34**, 123–127
Rakitzis, E. T. & Malliopoulou, T. B. (1985) *Biochem. J.* **230**, 89–93
Ray, W. J. & Koshland, D. E., Jr. (1961) *J. Biol. Chem.* **236**, 1973–1979
Ray, W. J. & Koshland, D. E., JR. (1962) *J. Biol. Chem.* **237**, 2493–2505
Rosen, N. I., Bishop, L., Burnett, J. B., Bishop, M. & Colman, R. F. (1973) *J. Biol. Chem.* **248**, 7359–7369
Stevens, E. & Colman, R. F. (1980) *Bull. Math. Biol.* **73**, 185–197
Tsou, C.-L. (1962) *Sci. Sin.* **11**, 1535–1558

Received 31 December 1985/24 February 1986; accepted 2 April 1986