BIOCHEMICAL LETTERS

Effect of ligands on chemical modification of proteins

Chemical modification of proteins is useful for identifying the essential residues involved in the biological activities of the proteins. Analytical methods for determining the type and number of the essential residues in the absence of a ligand are available (the papers cited by Dixon *et al.*, 1979; Horiike & McCormick, 1979, 1980; Horiike *et al.*, 1984; Rakitzis, 1984). To investigate whether the susceptible residues are at the active site or not, it is usually informative to study the effects of specific ligands, e.g. the substrate or an analogue, on inactivation and modification of the protein. Recently, in this journal, Rakitzis (1984) presented a review on protein modification. We feel it is timely to report briefly the points that should be considered in interpretation of the ligand effect, since most results have been explained without regard for the quantitative methods of analysis here reported, and it would seem that many biochemists are not familiar with the analytical methods for the ligand effect.

We considered most of the cases encountered (Horiike & McCormick, 1980). The basic model is briefly as follows. A protein has i essential amino acid residues of a given type, which are modifiable by a chemical reagent. In the absence of ligand A, the fraction of i residues remaining at a given time, t , of the modifying reaction is represented by X . In the presence of a ligand, only the fraction of j residues remaining of i essential residues at t is altered from X to X_A . In the absence and the presence of a ligand, the number of residues modified per protein molecule at t is denoted by m_0 and m_A , respectively. Then the difference between m_0 and m_A at t is:

$$\Delta m = m_0 - m_A = j(X_A - X) \quad (1)$$

When $X_A > X$, the ligand has a protective effect. When $X_A < X$, the ligand accelerates the modification of the j residues.

Suppose that the modification of any one of the i essential residues results in a complete loss of activity. In the absence and the presence of a ligand, the fraction of activity remaining at t is denoted by a_0 and a_A , respectively. Then the following equation is obtained (Horiike &

McCormick, 1980):

$$\begin{aligned} \Delta m &= j(a_A^{1/j} \cdot a_0^{(1/i)-(1/j)} - a_0^{1/i}) \\ &= j \cdot a_0^{1/i} [(a_A/a_0)^{1/j} - 1] \end{aligned} \quad (2)$$

where $\Delta m \leq j \leq i$. Note that the value of Δm (or X_A) will depend on the reaction time, t , (or rate constant) and the concentration of free ligand, $[A]$, (or dissociation constant, K_A). Δm will have a stationary value (including an extremal value) dependent on $[A]$ at a certain reaction time. If a ligand protects the protein against modification, increasing $[A]$ results in a higher maximum of Δm , but $\Delta m \leq j$.

To determine the number of j residues, many investigators have examined the effects of specific ligands on inactivation and modification of proteins [e.g. Akeroyd *et al.*, 1981; Ramakrishna & Benjamin, 1981; Carrillo *et al.*, 1981; Tso & Zalkin, 1981; Cromartie, 1981; Brooker & Slayman, 1983; Hennecke & Plapp, 1983; as well as the papers cited by Horiike & McCormick (1980) and Rakitzis (1984)]. In the absence and the presence of a ligand, the differences in both the number of residues modified (m) and the fractional remaining activity (a), i.e. the values of Δm and Δa ($= a_A - a_0$), have been commonly determined at arbitrarily chosen times of the modifying reaction and at arbitrarily chosen concentrations of the ligand. Also it seems to be common practice to interpret such data without regard for the quantitative methods of analysis. As mentioned above, since the value of Δm is a function of t and $[A]$, we cannot directly determine the value of j from an arbitrary value of Δm . Conversely are not always true. Brooker & Slayman (1983) plotted Δm against Δa , and presumed that the plot is linear and its slope corresponds to the number of moles of modifier required to inhibit the activity. As can be seen easily from eqn. (2), this is not necessarily so except in the case where $i = j = 1$. Seid & Sakmar (1981) reported a theoretical method for the case where modification of nonessential residues results in enzyme activity changes. Their $\alpha(t)_{\text{obs}}$ and $g(t)$ correspond to a_0 and a_∞ in eqns. (28)–(31) of Horiike & McCormick (1980) when $i = j$ and $X_A = 1$, these equations being more general.

Although there are some assumptions and limitations, the ligand effect should be considered on a firm theoretical basis.

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