BIOCHEMICAL JOURNAL LETTERS

Kinetics of protein modification and enzyme inactivation reactions: interpretation of reaction order

In cases of protein modification, or of modification-induced enzyme inactivation (irreversible enzyme inhibition), reaction order, with respect to modifying agent concentration, is mainly determined by the application of a simplified form of the Hill (1913) equation:

$$k_{app.} = k[M]^h \quad (1)$$

where $k_{app.}$ is the experimentally determined first-order rate constant of the protein modification, or enzyme inactivation, reaction, $[M]$ is modifying agent concentration, h is the Hill coefficient, and k is a proportionality constant (Levy *et al.*, 1963). Eqn. (1) has been applied in a large number of cases of modification-induced enzyme inactivation, and in most of these cases reaction order, with respect to modifying agent concentration, has been misinterpreted in one of three different ways.

(a) Reaction order has been assumed to be identical with the number, per enzyme active site, of groups essential for enzyme catalytic activity (e.g., by Reinsch & Dunlap, 1980; Kaminski & Jeżewska, 1982; Ceccarelli & Vallejos, 1983). However, this number may only be determined by a comparison of enzyme inactivation and of protein modification rate constants (Rakitzis, 1978, 1980), or by a juxtaposition of the $(1/i)$ th power of fractional enzyme activity (where i is the number, per enzyme active site, of groups essential for activity), with the extent of protein modification (Tsou, 1962; Horiike & McCormick, 1979; Stevens & Colman, 1980; Horiike *et al.*, 1984; Rakitzis, 1984a,b).

(b) Reaction order has been assumed to be identical with the total number of groups, per enzyme active site, modification of which occurs concomitantly with enzyme inactivation (e.g., by Armstrong *et al.*, 1976; Marcus *et al.*, 1976; Andreo & Vallejos, 1977; Rippa *et al.*, 1978; Cheung & Fonda, 1979; Franks *et al.*, 1980; Kremer *et al.*, 1980; Sönderling & Mäkinen, 1983). However, this number is identical with the total number of modifiable groups, per enzyme active site, provided all of these groups react at the same rate with the modifying agent (Tsou, 1962). Levy *et al.* (1963) have indicated that the total number of modifiable groups, per enzyme active site, must at least be equal to, but not higher than, the value for h in eqn. (1). The total number of modifiable groups, per enzyme protein molecule, or per enzyme active site, may be determined by the use of a reaction time long enough to ensure complete protein groups modification, or by one of the interpolation methods developed for cases where the final reading of the reaction is unknown (Guggenheim, 1926; Roseveare, 1931; Sturtevant, 1937;

Sokolnikoff & Sokolnikoff, 1941; Kezdy *et al.*, 1958; Swinbourne, 1960; Faddejew & Faddejewa, 1964; Frampton *et al.*, 1973; Glick *et al.*, 1978; Lachmann *et al.*, 1980a,b; Schwartz, 1981; McKinnon *et al.*, 1984). Alternatively, the total number of modifiable groups may be determined graphically, as described by Tsou (1962) (however, see Rakitzis, 1980, 1984b).

(c) Reaction order has been assumed to be identical with the number of modifying agent molecules, per enzyme active site, utilization of which is necessary to produce enzyme inactivation (e.g., by Bookjans & Böger, 1978; Griffith, 1979; Han *et al.*, 1980; Kebbij *et al.*, 1980; Shoshan & Selman, 1980; Chang & Huang, 1981; Meyer, 1981; Nishimura *et al.*, 1981; Jabalquinto *et al.*, 1983; Manohar & Rao, 1984). However, the relation between the fraction of enzyme activity remaining, a , and the number of modifying agent molecules utilized, per enzyme active-site, i.e., the relative concentration of protein groups modified, p , is:

$$a = (1 - p/n)^i \quad (2)$$

where n is the total number of reactive groups, per enzyme active site, and i is as defined above (Tsou, 1962; Rakitzis, 1980, 1984b). The assumption is made that all of the n groups react at the same rate with the modifying agent. If protein modification groups are of two kinds, with different reactivities towards the modifying agent, then eqn. (3) applies:

$$a = (1 - p_1/n_1)^i (1 - p_2/n_2)^j \quad (3)$$

where p_1 and p_2 are the relative concentrations of groups modified, and also where i and j are the numbers, per enzyme active site, of groups essential for activity, for the classes of groups containing n_1 and n_2 groups, in each instance (Tsou, 1962). It is accordingly seen that the number of modifying agent molecules, per enzyme active site, utilization of which is necessary to produce enzyme inactivation, is not a function of modifying agent concentration, and consequently, this number is entirely independent of the experimentally determined reaction order. In this connection, it should be noted that eqns. (2) and (3) apply only in cases where modification of protein reactive groups is not interdependent, i.e., in the absence of protein modification co-operativity. Where modification of protein reactive groups is interdependent, the stoichiometry of modification-induced enzyme inactivation may actually be a function of modifying agent concentration (Tsou, 1962; Rakitzis, 1978, 1980).

The erroneous interpretation of reaction order, in cases of modification-induced enzyme inactivation referred to above, mainly is [cases (b) and (c)] a result of confusing reaction order with reaction stoichiometry. Protein-

modification, and enzyme-inactivation, reactions are of the general form:

where M is modifying agent, E_i is the partially modified, or unmodified, protein species, or active enzyme species, with a modification rate constant of k_i , and $E_{\text{mod.}}$ is modified protein. In the case where all protein reactive groups are intrinsically identical, E_i is the stoichiometric protein species with i mol of unmodified groups per mol of protein, while in the case where protein groups are intrinsically nonidentical, E_i is the i th class of differently reacting groups. Protein-modification, and enzyme-inactivation, reactions, provided that modifying agent concentration is in large excess over protein concentration, are described by linear differential equations with constant coefficients, with unmodified protein groups concentration, or residual enzyme activity, as the dependent variable, and reaction time as the independent variable. The characteristic equations of these differential equations have solutions which are identical with the reaction rate constants (Rakitzis, 1977, 1983; Lachmann *et al.*, 1980a,b). The solution of these differential equations is given by a summation of exponential functions of reaction time. Reduction of these summation of exponentials equations to one single exponential equation is possible under certain conditions (Rakitzis, 1983). In these cases, the single exponential of the protein-modification, or enzyme-inactivation, equation describes the modification of one single protein species. It may therefore be concluded that the experimentally determined reaction order, in accordance with eqn. (1), is actually a reflection of the dependence on modifying agent concentration, of the single protein species, modification of which is the rate-limiting step in the overall reaction.

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