mathematical nature of the common-factor conditions given by Whitehead (1976, 1979).

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APPENDIX

As mentioned in the first paragraph, as well as eqn. (1) there is another independent condition for reduction of degree. It can be written (among several equivalent expressions):

$$\begin{vmatrix} a_{0(12)} & a_{0(13)} & a_{0(34)} \\ a_{1(12)} & a_{1(13)} & a_{1(34)} \\ a_{2(12)} & a_{2(13)} & a_{2(34)} \end{vmatrix} = 0 \quad (5)$$

where, if E, SE, SES, and ES are labelled 1, 2, 3, 4 respectively the a 's have the meaning explained in Whitehead (1979) and can be calculated directly from the mechanism to be:

$$a_{0(12)} = 0$$

$$a_{1(12)} = k_{+3}(k'_{-1}k_q + k_{-4}k_p) - k_{+1}k_p(k'_{-3} + k_{-4})$$

$$a_{2(12)} = k_qk_{+4}(k_{+3} - k_{+1}) + k_{+1}k_{+3}(k_q - k_p)$$

$$a_{0(13)} = k_{-2}(k'_{-1}k_q + k_{-4}k_p)$$

$$a_{1(13)} = k_q[k_{-2}(k_{+1} + k_{+4}) + k'_{-1}(k_{+2} + k_{+3})] + k_p[k_{-4}(k_{+2} + k_{+3}) - k_{+1}(k_{-2} + k'_{-3})]$$

$$a_{2(13)} = k_qk_{+4}(k_{+2} + k_{+3}) + k_{+1}k_{+3}(k_q - k_p)$$

$$a_{0(34)} = k_{-2}(k'_{-3}k_p - k'_{-1}k_q)$$

$$a_{1(34)} = k_p k'_{-3}(k_{+1} + k_{+2}) - k_q k'_{-1}(k_{+2} + k_{+3}) + k_{+1}k_{+2}(k_p - k_q)$$

$$a_{2(34)} = k_{+3}(k_{+1} + k_{+2})(k_p - k_q)$$

and $k'_{-1} = (k_{-1} + k_p)$, $k'_{-3} = (k_{-3} + k_q)$.

A factor k_{-2} can be extracted from this determinant. The determinant of eqn. (5) and $(k_{-1}k_q - k_{-3}k_p)$ [subject to condition (2)] are both

factors—the only physically significant ones—of the Sylvester eliminant R_0 (considered by Bardsley *et al.*, 1980) whose vanishing is the general common-factor condition.

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Kinetics of irreversible enzyme inhibition by an unstable inhibitor

Makoff & Malcolm (1981) have studied the modification of aldolase by methyl acetimidate. Methyl acetimidate is a water-unstable compound with a half-life of 30 min at pH 7.6, and Makoff & Malcolm (1981) have used eqn. (1) to plot enzyme protein modification data:

$$\ln \left(\frac{L}{L-L'} \right) = \frac{k}{k'} [M_0] \quad (1)$$

where L is the initial number of lysine residues in the enzyme preparation, L' is the number of lysine residues modified, k is the second-order rate constant for lysine modification, k' is the first-order rate constant for methyl acetimidate hydrolysis, and $[M_0]$ is the initial concentration of methyl acetimidate. The assumption is made that the modifying agent has been incubated with the enzyme solution for a long enough period for complete disappearance of the inhibitor to take place. Use of relationships like eqn. (1) in studies of enzyme inactivation by unstable inhibitors was initiated by Purdie & Heggie (1969) and extended by Ashani *et al.* (1972) and by Rakitzis (1974, 1978). Makoff & Malcolm (1980, 1981) appear to have overlooked the work of these authors.

Makoff & Malcolm (1981) have found that eqn. (1) is applicable at low methyl acetimidate concentrations, while at higher concentrations of this compound there is some deviation from linearity when $\ln[L/(L-L')]$ is plotted against $[M_0]$. Makoff & Malcolm (1981) attribute this deviation from linearity to a change in pH at high methyl acetimidate concentrations. However, departure from linearity when $\log$ [residual enzyme] is plotted against [hydrolysed inhibitor] may be due to a rate saturation effect, i.e. a non-linear dependence of the fraction of the enzyme protein reversibly complexed with inhibitor at any one moment on inhibitor concentration at that moment during the course of the reaction. When this happens eqn. (1) is no longer applicable, and more complicated expressions have to be used (Purdie & Heggie, 1969; Rakitzis, 1974). Data compatible with a rate-saturation effect in cases of enzyme inactivation by unstable inhibitors have been obtained for the inactivation of cathepsin D by dithiophosgene and by 2,2-dichloro-1,3-dithia-cyclobutanone (Rakitzis & Malliopoulou, 1976), and of the inactivation of serine proteinases by

p-amidinophenylmethanesulphonyl fluoride (Laura *et al.*, 1980). Data compatible with eqn. (1) have been obtained for the inactivation of acetylcholinesterase by a variety of water-unstable organophosphorus compounds (Ashani *et al.*, 1972; Maglothin & Wilson, 1974; Maglothin *et al.*, 1975; Barnett & Rosenberry, 1978) and for the inactivation of acid proteinases by water-unstable diazomethane derivatives (Rakitzis, 1974; Johnson & Poisner, 1980). A linear dependence of log[residual enzyme activity] (or enzyme protein) on [hydrolysed inhibitor] has also been obtained in a study of the inactivation of cathepsin D activity of stomach mucosa and of gastric adenocarcinoma by diazoacetyl-DL-norleucine methyl ester (Dionyssiou-Asteriou & Rakitzis, 1978), as well as in a study of the modification of lysine residues of RNA polymerase by methyl acetimidate (Makoff & Malcolm, 1980). However, in these cases the plots of log [residual enzyme] against [hydrolysed inhibitor] did not meet the log [residual enzyme] axis at the origin of the graph. This effect may be due to the presence of two or more isoenzymes, with different reactivities towards the modifying agent in the enzyme preparation, or it may be due to a time-dependent interconversion step of different species of the enzyme protein (Rakitzis, 1978). A study of the kinetics of biphasic protein modification reactions, when the modifying agent is stable in solution, has been presented (Rakitzis, 1980).

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