sound), eqn. (11) of Topham (1990) is valid, since, although $[X]$ is not $\gg [E]_0$ during the whole course of the reaction, the condition (5) is fulfilled. Fig. 1(b) shows that if the condition (6) (and the remaining assumptions of Topham), but not condition (5), is satisfied, then eqn. (11) of Topham (1990) fails. The numerical integration to obtain the simulated curves in Fig. 1 was carried out by the use of the predictor-corrector algorithm of Adams-Moulton, starting with the fourth order Runge-Kutta method (Gerald, 1978). Before plotting eqn. (11) of Topham (1990), we corrected the mistake: $e^{ik't}$ instead of $e^{-ik't}$. For the acquisition of the points necessary to plot this equation, we elaborated a computer program using the BASIC language. This program is available to interested readers on request. It allows the choice of the number of terms in the infinite series in the kinetic equations. We progressively increased this number from 1 and, in all cases, the difference between the successive results vanished after less than 20 steps. For the plots of eqn. (11) of Topham (1990) in Fig. 1, we employed 20 terms. The algorithm for the numerical integration and the program elaborated by us were implemented and compiled in TurboBASIC 1.0 on an INVES PC-640A computer (IBM AT-compatible) with an Intel 80287 arithmetic coprocessor.

The unnecessary assumption (6) probably led Topham (1990) to propose a kinetic data analysis, which is based on the first instants of the reaction (at which this assumption is fulfilled because the modifier has not yet decayed appreciably), although his equations are valid in the entire time range. Since for short reaction times, the exponential terms cannot be neglected, that approach suffers from both mathematical and experimental difficulties. The assumption (6), which also has been used in earlier papers (Topham, 1987), has been questioned by Rakitzis (1987).

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Making sense of the kinetics of reactions of unstable modifiers with enzymes

The determination of kinetic parameters describing irreversible enzyme modification by unstable reagents may be achieved by analysis of reaction time-courses under conditions of modifier (X) excess over enzyme ($[X] \gg [E]_0$). Appropriate protocols have been devised for the discontinuous monitoring of residual enzyme activity during modification reactions conducted in the absence of substrate (Gomi & Fujioka, 1983; Topham, 1984, 1985, 1988; Topham & Dalziel, 1986) and for the continuous monitoring of enzyme-catalysed reactions in the presence of an unstable modifier (Topham, 1990). In order to derive all the integrated-rate equations described in these studies, it is necessary to assume that modifier decay is first-order. This enables the concentration

of modifier at any moment to be set equal to $[X]_0 e^{-k't}$ where k' is the (pseudo) first-order rate constant for modifier decomposition.

Varón *et al.* (1992) have challenged the validity of the time-course approach as it applies to the analysis of product (P) formation in the presence of an unstable inhibitor according to a direct-encounter mechanism (Schemes 1 of Topham, 1990 and Varón *et al.*, 1992). Their criticism centres on (a) the impropriety of the condition $[X] \gg [E]_0$ as a criterion by which inhibitor decay is adjudged to be first-order and (b) its replacement by the inequality

$$k' \gg (k_{\text{mod.}} + k'_{\text{mod.}})[E]_0$$

where $k_{\text{mod.}}$ and $k'_{\text{mod.}}$ are second-order rate constants for the reaction of the modifier with the free enzyme (E) and the enzyme-substrate (ES) complex, respectively. Varón *et al.* (1992) cite two extreme cases that purport to reveal inconsistencies between the results of their own numerical simulations and the integrated-rate equation for this mechanism [eqn. (11) of Topham (1990)], reproduced below after correction of the typographical error:

$[P] =$

$$v^*t + \frac{(v-v^*)}{k'} \exp\left(\frac{-A[X]_0}{k'}\right) \cdot \left(k't + \sum_{i=1}^{t-\infty} \left(\frac{A[X]_0}{k'}\right)^i \frac{[1-(e^{-k't})^i]}{i \cdot i!}\right) \quad (1)$$

where v^* and v are the usual Michaelis-Menten steady-state rate expressions for the modified and unmodified forms of the enzyme, respectively [see eqn. (10) of Topham (1990)] and A is an apparent second-order rate constant for modification:

$$A = \frac{k_{\text{mod.}}K_m + k'_{\text{mod.}}[S]}{K_m + [S]} \quad (2)$$

It is helpful to consider reactions of enzymes with unstable modifiers in terms of the relative flux (Q) occurring through reactions of the modifier with the various enzyme-containing species compared to that proceeding via the non-enzymic pathway for modifier decay. The flux ratio Q will decrease as the modification reaction proceeds and is at a maximum ($Q_{\text{max.}}$) in the limit $t \rightarrow 0$. For the mechanism under discussion:

$$Q = (k_{\text{mod.}}[E] + k'_{\text{mod.}}[ES])/k' \quad (3)$$

and

$$Q_{\text{max.}} = A[E]_0/k' \quad (4)$$

The example illustrated in Fig. 1(a) of Varón *et al.* (1992) concerns a highly unstable inhibitor ($t_{1/2} = 0.069$ s). The flux ratio $Q_{\text{max.}}$ for this reaction is $5.07 \times 10^{-5} \ll 1$. Since first-order kinetics with respect to inhibitor concentration clearly hold under these conditions, it is not surprising that eqn. (1) is also obeyed. However, by ensuring that $k' \gg (k_{\text{mod.}} + k'_{\text{mod.}})[E]_0$ while simultaneously permitting the deliberate violation of the condition $[X] \gg [E]_0$ over most of the observation period (~ 0.6 s), Varón *et al.* (1992) have been obliged to consider a system of little or no practical relevance. The inhibitor is so unstable that 99.995 % of it will decompose non-enzymically within one second! Furthermore its efficacy in the reaction mixture of Fig. 1(a) is minimal. It can be calculated [using eqn. 7 of Topham (1990)] that a mere 0.051 % of the total enzyme will have been modified (i.e. exists as E^* or ES^*) at the end of the observation period (taken as 0.6s). When more realistic experimental conditions apply, rates of enzyme inactivation and non-enzymic inhibitor decay can often be expected to be similar and $Q_{\text{max.}}$ approximates to unity [see Topham (1987) for experimental examples]. Ironically Varón *et al.* (1992) make this same point in their opening

sentence. When $Q_{\max} \approx 1$, $k' \approx (k_{\text{mod.}} + k'_{\text{mod.}})[E]_0$. The inequality proposed by Varón *et al.* (1992) (their eqn. 5) is therefore entirely inappropriate in precisely those circumstances that they rightly acknowledge to be most relevant.

First-order decay with respect to inhibitor concentration will strictly apply only when $Q < Q_{\max} \ll 1$ (Topham, 1987, 1988). However, the requirement for first-order kinetics can still be met experimentally when $Q \approx 1$ by ensuring that $[X]/[E]_0 \gg 1$ during the observation period. This has been demonstrated by computer simulation where it was found that deviations from first-order kinetics are trivial when $Q \approx 1$ provided that $[X]/[E]_0 \gg 1$ (Topham, 1987). There are of course cases where k' is not too large as to preclude experimentation and for which $Q < 1$ (or $Q \ll 1$). In these circumstances it could be argued that the restriction $[X]/[E]_0 \gg 1$ should be relaxed and replaced by either the inequality suggested by Varón *et al.* (1992) or, more consistently, by $k' \gg A[E]_0$. However, both the latter inequalities depend on the unknowns $k_{\text{mod.}}$ and $k'_{\text{mod.}}$. Since the flux ratio will not be known in advance, it is much safer from an experimental viewpoint to retain the requirement that $[X]/[E]_0 \gg 1$ (which only depends on an independent knowledge of k' and $[E]_0$) even when $Q < 1$ (or $Q \ll 1$).

The second example cited by Varón *et al.* (1992) involves what is essentially a stable inhibitor ($t_{1/2} = 19.25$ h). Non-enzymic decay over the time-course indicated on the abscissa of Fig. 1(b) of Varón *et al.* (1992) ($\sim 6.0 \times 10^{-4}$ %) is insignificant by comparison with acceptable limits of experimental error (1–5 %). Using the parameters given by Varón *et al.* (1992) in the legend to Fig. 1(b), $(A[X]_0/k')$ can be calculated to be 50.67 with $k' = 1.0 \times 10^{-5} \text{ s}^{-1}$. However, under these conditions the power series in eqn. (1):

$$\left(\sum_{i=1}^{t-\infty} \left(\frac{A[X]_0}{k'} \right)^i \frac{[1 - (e^{-k't})^i]}{i \cdot i!} \right)$$

does not even begin to converge by the 20th iteration when evaluated using fixed values of $t \leq 0.6$ s. For example, when $t = 0.1$ s, individual terms in the series increase from 5.07×10^{-5} at $i = 1$ through 3.07×10^4 at $i = 10$ to 5.11×10^9 at $i = 20$, an overall change of some 10^{14} -fold. The claim by Varón *et al.* (1992) that the difference between successive values (presumably of $[P]$ although this was not explicitly stated) had disappeared after less than 20 steps in all cases is meaningless because the power series has not simultaneously converged. As in the case of the well-known Maclaurin series expansion of e^x , the power series in eqn. (1) holds for all values of $(A[X]_0/k')$. Indeed, its convergence properties are much better. Thus the reason for the discrepancy between the two curves shown in Fig. 1(b) is simply that Varón *et al.* (1992) have used too low a cutoff for i for their extreme case. In curtailing the calculation at $i = 20$ (or less) the dominant term in eqn. (1) is v^*t . The truncated solution at $i = 20$ gives $[P] \sim v^*t = 8.33 \times 10^{-9} \text{ M}$ when $t = 0.1$ s. Readers wishing to check this value on the lower curve of Fig. 1(b) of Varón *et al.* (1992) should note that the scale depicted on the left-hand ordinate axis is in error by a factor of 10 (given $[E]_0 = 1.0 \times 10^{-8} \text{ M}$ according to the legend).

The reliability of eqn. (1) can be readily ascertained and the numerical simulation result reproduced with little loss in accuracy by setting $k' = 1.0 \times 10^{-4} \text{ s}^{-1}$ $[(A[X]_0/k') = 5.07]$, non-enzymic inhibitor decay ~ 0.006 % at 0.6s]. Convergence to three significant figures is now reached in the range $i = 11$ –15. For example, at $t = 0.1$ s, $[P] = 6.67 \times 10^{-8} \text{ M}$, in agreement with the simulation data. In fact the inhibitor is sufficiently stable over the observed time-course that the same result is found using the integrated-rate equation for a stable modifier. This may be derived from first principles or obtained by addition of eqns. (11)

and (18) of Tsou *et al.* (1985) (after setting $B = 0$ for a direct-encounter mechanism). To facilitate comparison with eqn. (1) the equation is given below in terms of the same symbols:

$$[P] = v^*t + \frac{(v - v^*)}{A[X]} \cdot (1 - e^{-A[X]t}) \quad (5)$$

It may be concluded that integrated-rate equations describing time-courses of enzyme modification by unstable modifiers (Gomi & Fujioka, 1983; Topham, 1984, 1985, 1988, 1990; Topham & Dalziel, 1986) are valid provided that experiments are conducted in such a way as to ensure that $[X] \gg [E]_0$ during the observed reaction period. Given this condition, which may be established independently, first-order modifier decay is **effectively** ensured irrespective of the prevailing flux ratio. Rigorous adherence to the argument that first-order decay is only possible when $Q \ll 1$ (Rakitzis, 1987; Varón *et al.*, 1992) overlooks two important practical points. First, there is a need to accommodate cases likely to be encountered experimentally, namely when $Q \approx 1$. Secondly, the flux ratio depends on unknown kinetic parameters describing the modification reaction and is therefore itself also unknown in real situations.

The apparent discrepancies illustrated in Fig. 1(b) of Varón *et al.* (1992) can be explained by the failure of their computer program to carry out rigorous tests for convergence. Although there are no theoretical limits on the magnitude of $(A[X]_0/k')$, the power series in eqn. (1) will not converge quickly when $(A[X]_0/k') > 5$. When this threshold is exceeded because $k' \rightarrow 0$, it is more sensible to use the analogous equation for a stable modifier.

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Low concentrations of adenine nucleotides enhance the receptor binding of inositol 1,4,5-trisphosphate

The Ca^{2+} -mobilizing second messenger inositol 1,4,5-trisphosphate (InsP_3) acts on specific binding sites, first described in hepatocytes and polymorphonuclear leukocytes [1], pituitary [2], and adrenocortical microsomes [3]. The receptor which was purified [4], cloned and sequenced from rodent cerebellum [5,6] is a homotetramer of M_r -313000 protein subunits, each of which contains two adenine nucleotide binding sites (Gly-Xaa-Gly-Xaa-Xaa-Gly) at amino acid residues 1773–1778 and 2016–2021. In non-neuronal tissues the deletion of a 120-nucleotide segment designated SII (at amino acid residues 1687–1732) [7] gives rise to the appearance of a third nucleotide binding site. The presence of ATP-binding sites has been confirmed by photoaffinity labelling