Regulatory kinetics of wheat-germ aspartate transcarbamoylase

Adaptation of the concerted model to account for complex kinetic effects of uridine 5'-monophosphate

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The kinetic effects of the end-product inhibitor UMP on aspartate transcarbamoylase (EC 2.1.3.2) purified to homogeneity from wheat germ were studied. In agreement with an earlier study of the relatively crude enzyme [Yon (1972) *Biochem. J.* **128**, 311–320], the half-saturating concentrations of UMP and of the first substrate, carbamoyl phosphate (but not of the second, L-aspartate), were found to be strongly interdependent. However, the kinetic behaviour of the pure enzyme differed from that of the crude enzyme in several important respects, namely: (a) the apparent affinity for UMP was lower with the pure enzyme; (b) sigmoidicity was absent from plots of initial rate versus carbamoyl phosphate concentration, each at a fixed UMP concentration; (c) sigmoidicity was greatly exaggerated in plots of initial rate versus UMP concentration, each at a fixed carbamoyl phosphate concentration, owing to the occurrence of a slight but definite maximum in each plot at low UMP concentration; (d) there was a relative increase in this maximum in the presence of *N*-phosphonacetyl-L-aspartate, an inhibitor competitive with carbamoyl phosphate. It is shown that a modified two-conformation concerted-transition model can be used to account for most of these features of the pure enzyme. The model treats carbamoyl phosphate and UMP as antagonistic allosteric ligands binding to alternative conformational states [Monod, Wyman & Changeux (1965) *J. Mol. Biol.* **12**, 88–118], carbamoyl phosphate binding non-exclusively (dissociation constants 20 μM and 85 μM respectively) and UMP binding exclusively (dissociation constant 2.5 μM). The model postulates further that the conformation with lower affinity for carbamoyl phosphate has the higher value of k_{cat} , and that it binds UMP in competition with carbamoyl phosphate. Parameters giving the best fit of experimental data to this model were found by a non-linear least-squares search procedure.

Control of the pyrimidine pathway is attributed in part to end-product inhibition of aspartate transcarbamoylase (L-aspartate:carbamoyl phosphate carbamoyltransferase, EC 2.1.3.2) in several organisms. A previous paper from this laboratory (Yon *et al.*, 1982) described the quaternary structure of wheat-germ aspartate transcarbamoylase as a 104 kDa trimer of similar or identical chains, and reviewed briefly the variety of quaternary structures reported for prokaryotic and eukaryotic forms of the enzyme. The enzyme from *Escherichia coli* is at present arguably the best-characterized allosteric enzyme in terms of its physical, catalytic and ligand-binding properties (reviewed by Kantrowitz *et al.*, 1980a,b). The striking re-

semblance in gross quaternary structure between the wheat-germ enzyme and the so-called 'catalytic subunit' (a 100 kDa trimer) of the *E. coli* enzyme naturally provokes speculation about possible evolutionary relatedness. Although the *E. coli* catalytic subunit does not show the strong co-operative behaviour of the holoenzyme, and in particular is not regulated by nucleotides at concentrations likely to be encountered *in vivo*, there have nevertheless been several recent reports of a non-functional, and possibly vestigial, nucleotide-binding site on the catalytic subunit (Thiry & Hervé, 1978; Monaco *et al.*, 1978; Issaly *et al.*, 1982). The fact that the wheat-germ enzyme has long been known to be regulated by UMP (Yon, 1970, 1972) there-

fore takes on new significance as a possible case in which function at this site has survived, a suggestion made by Issaly *et al.* (1982).

The effects of UMP on the kinetic behaviour of wheat-germ aspartate transcarbamoylase were examined by Yon (1972) with a crude enzyme extract. The enzyme was found to be strongly and specifically inhibited by UMP in what appeared to be a classic case of end-product inhibition. Kinetic coupling between the effects of UMP and the substrate carbamoyl phosphate was explained in terms of a concerted-transition model (Monod *et al.*, 1965) that predicted a trimeric structure for the enzyme, a prediction subsequently confirmed (Yon *et al.*, 1982). In view of the renewed interest in this regulation it was decided to re-examine the effects of UMP with the highly purified enzyme now available (Yon, 1981). The present paper describes features of the regulation not seen in the crude enzyme, and speculates on a possible mechanism to account for them.

Experimental

Materials

Aspartate transcarbamoylase was purified from wheat germ as described by Yon (1981), with the exception that the two organic-solvent fractionation steps were combined. The enzyme was more than 96% pure by polyacrylamide-gel electrophoresis, and was stored at room temperature in 0.2M-Tris/acetic acid buffer, pH 7.5, containing 50% (v/v) glycerol. Under these conditions the enzyme is stable for several months; however, the present experiments were performed within 2 weeks of purification.

Substrates and buffers were obtained from sources listed previously (Grayson *et al.*, 1979). UMP (disodium salt) was from Sigma Chemical Co. *N*-Phosphonoacetyl-L-aspartate was synthesized by the procedure of Goodson *et al.* (1980) and shown to be approx. 90% pure by aspartate analysis (Locarte amino acid auto-analyser) before and after hydrolysis in 6M-HCl for 24 h at 105°C.

Methods

The enzyme concentration in the stock solution was determined by the method of Lowry *et al.* (1951). Initial reaction rates were obtained with the enzyme at 5 nM. Preliminary experiments at the highest and lowest substrate and UMP concentrations confirmed that product formation was linear with time for at least 20 min under the conditions described below. Thereafter the main body of the results were obtained by single-time-point (usually 10 min) assays, in duplicate. The reaction was in 0.1M-Tris adjusted to pH 8.5 with acetic acid. The temperature was $25 \pm 0.1^\circ\text{C}$. Substrate and inhibitor concentrations are given in Figure legends.

The reaction was normally initiated by the addition of carbamoyl phosphate. Other details were essentially as described by Grayson *et al.* (1979). Carbamoylaspartate was determined as described by Prescott & Jones (1969) with rigorously standardized heating and lighting conditions.

Data treatment

The set of values of K_{C1} , K_{C2} , K_U , L , k_1 and k_2 that gave the best fit of eqn. (1) (see the Results and theory section) to all the data points in Fig. 2 was obtained as follows. The value of K_{ia} for carbamoyl phosphate (Grayson *et al.*, 1979) and the half-saturating concentration for the protective effect of UMP against trypsin (Cole & Yon, 1984) were taken as first estimates of K_{C1} and K_U . The value of $V_{\max.}/([\text{enzyme}] \times 3)$ was taken as a first estimate of k_1 . By trial, values of the other parameters were found that gave a rough fit to the set of data points. The six values were then refined by systematically changing each by a small amount to diminish the sum of squares of differences between experimental and calculated velocities for the whole data-set. The sum-of-squares minimum was located by a steepest-descent search (Wong, 1975) until a change in any parameter value of 0.01% decreased the sum-of-squares by less than 0.01%.

The value of K_i for *N*-phosphonoacetyl-L-aspartate was similarly found by fitting the extended form of eqn. (1) given by eqns. (2) (see the Results and theory section) to the data points of Fig. 4(a) and assuming the values previously found for the other parameters.

Results and theory

Effects of UMP on the kinetics of crude and highly purified enzyme preparations

Pronounced differences were noted in the effect of UMP at fixed concentrations of substrates, depending on the state of purity of the enzyme. Fig. 1 shows UMP saturation curves and their corresponding Hill plots for a relatively crude enzyme preparation (immediately after the precipitation stage with industrial methylated spirit) and for the nearly homogeneous enzyme preparation. Although the degree of co-operativity is about the same (Hill coefficients are 2.54 for the crude enzyme and 2.65 for the pure enzyme), the half-saturating concentration of UMP differed by nearly 4-fold (70 μM for the crude and 270 μM for the pure enzyme, under the particular values of substrate concentration). Thus the purified enzyme appears to have a substantially lower affinity for UMP. An additional feature of the curve for the pure enzyme is the suggestion of a slight maximum at low concentrations of UMP. This has been confirmed in the experiments described below.

Fig. 1. Comparison of the effects of UMP on the initial reaction rate, v , catalysed by a relatively crude preparation (○) and by a highly purified preparation (●) of wheat-germ aspartate transcarbamoylase

The enzyme was purified to the alcohol-precipitation stage and to near-homogeneity respectively (Yon, 1981). In both cases the concentration of L-aspartate and of carbamoyl phosphate were 1.0 mM and 0.3 mM respectively, and the buffer was 0.1 M-Tris/acetic acid, pH 8.5. Inset: Hill plots of the same data. The slopes, h , are indicated.

Kinetic interaction of UMP and carbamoyl phosphate

In a previous study in which a crude enzyme preparation was used (Yon, 1972), carbamoyl phosphate and UMP were found to interact in a classically heterotropic manner (Monod *et al.*, 1965), each showing strong kinetic co-operativity in the presence of a constant concentration of the other. The pure enzyme was found to differ from this pattern of behaviour. Fig. 2 shows the array of initial rates determined for nine UMP concentrations at each of eight carbamoyl phosphate concentrations. This result is typical of several experimental runs with the pure enzyme. Compared with the earlier study with the crude enzyme, the main differences are (a) little or no evidence of sigmoidicity (kinetic co-operativity) in the carbamoyl phosphate saturation curves (Fig. 2a) and (b) exaggeration of the sigmoidicity in the UMP saturation curves due to a slight but persistent maximum at low UMP concentrations.

Effect of UMP on kinetics with L-aspartate as variable-concentration substrate

Substrate inhibition is observed at high concentrations of L-aspartate, presumably owing to the formation of the non-productive aspartate-phosphate complex, L-aspartate being the second substrate in an ordered mechanism (Grayson *et al.*, 1979; see also the discussion on substrate inhibition in Cornish-Bowden, 1976). To avoid this complication, the effects of UMP on kinetic behaviour with L-aspartate as variable ligand were performed at aspartate concentrations well below saturation. The results are shown in Fig. 3. Linear double-reciprocal plots indicate hyperbolic kinetics in every case. The activating effect of low concentrations of UMP is again evident at 25 μ M-UMP, and is seen at all concentrations of L-aspartate in the range tested. The half-saturating concentration of L-aspartate (reciprocal of the horizontal intercept in Fig. 3) is relatively little affected by changing the UMP concentration, suggesting that the affinity of the enzyme for L-aspartate is not dependent on the UMP concentration.

Effect of UMP on inhibition by N-phosphonoacetyl-L-aspartate

N-Phosphonoacetyl-L-aspartate is a putative transition-state analogue of the reaction catalysed by aspartate transcarbamoylase (Collins & Stark, 1971) and is a potent inhibitor of the enzyme. The inhibition of wheat-germ aspartate transcarbamoylase is shown in Fig. 4(b). The inhibition constant K_i is approx. 5 nM; however, its accurate determination from Fig. 4(b) is precluded owing to the large error in measuring the small slope of the zero-inhibitor line.

Fig. 4(a) shows the combined effects of UMP and N-phosphonoacetyl-L-aspartate; the data are plotted with UMP as variable-concentration ligand for comparison with Fig. 2(b). Of particular note is the relative increase in the height of the maximum in the curve when N-phosphonoacetyl-L-aspartate is present at 0.1–1.0 μ M [see experimental points and broken curves in Fig. 4(a); the continuous lines are theoretical]. Thus in the absence of N-phosphonoacetyl-L-aspartate, 20 μ M-UMP activates the enzyme by only 6%, whereas in the presence of 0.3 μ M-N-phosphonoacetyl-L-aspartate the same concentration of UMP activates the enzyme by 70%.

A modified concerted-transition two-conformational model

The simplest exclusive-binding 'K-type' concerted model (Monod *et al.*, 1965), which accounted satisfactorily for the kinetics of the enzyme in its crude state (Yon, 1972), will not explain saturation curves having the features shown in

Fig. 2. Effects of UMP and carbamoyl phosphate on the initial reaction rate, v , catalysed by the pure preparation of wheat-germ aspartate transcarbamoylase

(a) Data plotted with carbamoyl phosphate as the variable-concentration ligand. The fixed UMP concentrations were (mM): 0 (●), 0.05 (○), 0.10 (▲), 0.20 (△), 0.30 (■), 0.40 (□), 0.50 (▼), 0.60 (▽) and 0.80 (◆). (b) The same data plotted with UMP as the variable-concentration ligand. The fixed concentrations of carbamoyl phosphate were (mM): 0.5 (●), 1.0 (○), 1.5 (▲), 2.0 (△), 3.0 (■), 4.0 (□), 6.0 (▼) and 8.0 (▽). For clarity the data at low UMP concentrations are shown, in the inset, on an enlarged scale. In both (a) and (b) the concentration of L-aspartate was 1.0 mM. The curves are theoretical, obtained by using the parameters given in Table 1 in eqn. (1) (see text).

Fig. 3. Effects of UMP and L-aspartate on the initial reaction rate, v , catalysed by the pure preparation of wheat-germ aspartate transcarbamoylase

Double-reciprocal plots with L-aspartate as the variable-concentration substrate are shown. The fixed UMP concentrations were (mM): 0 (●), 0.025 (○) and 0.20 (□). The concentration of carbamoyl phosphate was 0.5 mM.

Figs. 2 and 4(a). With a few modifications, however, a reasonable fit may be obtained; the continuous curves in Figs. 2 and 4(a) show the result of such an adaptation of the concerted model.

The model is shown in Fig. 5. It assumes a trimeric enzyme (Yon *et al.*, 1982) existing as a dynamic equilibrium between two conformational states designated 'circular' and 'hexagonal', the conformational transition being concerted. The dotted outline includes all equilibrating complexes in the presence of UMP (U) and carbamoyl phosphate (C); this model was used to fit the data of Fig. 2. The additional species outside of the dotted box appear in the presence of an inhibitor I competitive with carbamoyl phosphate in the 'circular' conformation. UMP is assumed to bind exclusively to the 'hexagonal' conformation with intrinsic (i.e. microscopical) association constant K_U . Carbamoyl phosphate is postulated to bind non-exclusively with intrinsic association constants K_{C1} and K_{C2} for the 'circular' and the 'hexagonal' states respectively, where $K_{C1} > K_{C2}$. To generate a maximum in the kinetic curves with variable UMP (Figs. 2b and 4a) one must make two additional postulates: (1) the catalytic constants of the two conformations are inversely related to their affinities for carbamoyl phosphate, i.e. $k_2 > k_1$, where

Fig. 4. Effects of (a) UMP and *N*-phosphonoacetyl-L-aspartate, and (b) *N*-phosphonoacetyl-L-aspartate alone, on the initial reaction rate, v , catalysed by the pure preparation of wheat-germ aspartate transcarbamoylase

(a) Data plotted with UMP as variable-concentration ligand. The fixed concentrations of *N*-phosphonoacetyl-L-aspartate were (μM): 0 (●), 0.1 (○), 0.3 (▲), 1.0 (△), 3.0 (■) and 10.0 (□). The concentrations of carbamoyl phosphate and L-aspartate were 0.7 mM and 1.0 mM respectively. The continuous curves are theoretical, obtained by using the parameters given in Table 1 in eqn. (1) and its extension (eqn. 2) (see the text). The broken lines emphasize the departure from theory at low concentrations of UMP and *N*-phosphonoacetyl-L-aspartate. (b) Double-reciprocal plots of initial rates, v , plotted versus carbamoyl phosphate concentration, in the absence (○) and in the presence (●, 0.1 μM) of *N*-phosphonoacetyl-L-aspartate. The concentration of L-aspartate was 1.0 mM.

k_1 and k_2 are the ' k_{cat} ' values of the 'circular' and 'hexagonal' conformations respectively; (2) UMP and carbamoyl phosphate bind competitively to the 'hexagonal' conformation. At low concentrations, UMP enhances the catalytic rate by creating more vacant active sites in the high- k_{cat} conformation, as a result of the concerted transition; at higher concentrations UMP inhibits these same sites by competition, thus producing the maximum in the rate curve.

According to this model the initial reaction rate v is dependent on the concentrations of UMP and carbamoyl phosphate as follows [cf. eqn. (6) of Smith (1977)]:

$$\frac{v}{[E_0]} = \frac{3k_1 \alpha (1+\alpha)^2 + 3k_2 L c \alpha (1+c\alpha+\beta)^2}{(1+\alpha)^3 + L(1+c\alpha)^3 + L\beta^3 + 3L\beta(1+c\alpha)(1+c\alpha+\beta)} \quad (1)$$

where $[E_0]$ is the molar enzyme concentration, $\alpha = K_{C1}[\text{carbamoyl phosphate}]$, $c = K_{C2}/K_{C1}$, $\beta = K_U[\text{UMP}]$ and L is the intrinsic allosteric constant (Monod *et al.*, 1965), i.e. the ratio of 'hexagonal' to 'circular' enzyme in the absence of ligands. In the presence of the inhibitor I, assumed to bind exclusively to the 'circular' conformation

with intrinsic association constant K_I in competition with carbamoyl phosphate, additional terms N and D are added to the numerator and denominator respectively of eqn. (1) as follows:

$$\left. \begin{aligned} N &= 3k_1 \alpha \gamma (2 + \gamma + 2\alpha) \\ D &= \gamma^3 + 3\gamma(1 + \alpha)(1 + \alpha + \gamma) \end{aligned} \right\} \quad (2)$$

where $\gamma = K_I[I]$.

The continuous lines in Fig. 2 and 4(a) are the least-squares fits of the experimental data to eqn. (1) and its extension, eqn. (2), respectively. The fitting was done by the procedure described in the Experimental section (under 'Data treatment'),

which yielded the parameter values in Table 1. The theoretical fit was less satisfactory to the data of Fig. 4(a), particularly at low concentrations of UMP and at high concentrations of *N*-phosphonoacetyl-L-aspartate, when systematic deviations clearly occurred. This fit was not significantly improved by assuming that I bound non-exclusively.

Fig. 5. Diagrammatic representation of the multiple equilibria in a trimeric concerted-transition model used to account for complex kinetic effects

Circles and hexagons represent subunits in alternative conformational states. C, U and I represent bound carbamoyl phosphate, UMP and *N*-phosphonoacetyl-L-aspartate respectively. For each ligand-binding equilibrium step, the stoichiometric association constant is shown as the product of a statistical factor and an intrinsic (microscopical) association constant (Klotz, 1946). For circles $k_{cat} = k_1$, and for hexagons $k_{cat} = k_2$; it is postulated the $k_2 > k_1$. The complexes within the dotted box are postulated to exist in the presence of carbamoyl phosphate and UMP, and lead to eqn. (1), to which the data of Fig. 2 were fitted. Complexes outside this box occur when *N*-phosphonoacetyl-L-aspartate is present additionally, and lead to an extended equation (eqn. 2) to which the data of Fig. 4(a) were fitted.

Table 1. Kinetic parameters generated by a non-linear least-squares fitting procedure

The experimental data of Figs. 2 and 4(a) were fitted to the model shown in Fig. 5 by the procedure described in the Experimental section (under 'Data treatment'). Equilibrium constants are given as intrinsic (microscopical) association constants; the corresponding dissociation constants are given in parentheses. k_1 and k_2 are turnover numbers.

K_{C1}	$= 5.04 \times 10^4 \text{ M}^{-1}$ (19.9 μM)
K_{C2}	$= 1.18 \times 10^4 \text{ M}^{-1}$ (85 μM)
K_U	$= 4.08 \times 10^5 \text{ M}^{-1}$ (2.45 μM)
L	$= 7.78$
k_1	$= 80 \text{ min}^{-1}$
k_2	$= 236 \text{ min}^{-1}$
K_1^*	$= 1.10 \times 10^8 \text{ M}^{-1}$ (9.0 nM)

* I = *N*-phosphonoacetyl-L-aspartate.

Discussion

Comparison of the results in the present paper and in an earlier kinetic study (Yon, 1972) indicate several differences in regulatory behaviour of the enzyme between the two studies. The data published in the 1972 study were obtained at pH 10; however, these early experiments had also been done at pH 8.5 with essentially the same results, except for a lower specific activity at pH 8.5 (R. J. Yon, unpublished work). The differences appear to be more the result of the different states of purity of the enzyme, a conclusion supported by Fig. 1, in which the pH was constant. The difference is also not due to low-molecular-mass modifiers in the crude enzyme preparation, which was extensively dialysed before use. It will be of interest to know which behavioural state most closely portrays the enzyme *in vivo*, a question we cannot answer at the present time.

Except for one point of detail, the earlier set of kinetic results were readily explicable in terms of the simplest concerted model (Monod *et al.*, 1965), with UMP and carbamoyl phosphate as antagonistic allosteric ligands. Analysis in terms of this model predicted a trimeric structure, which was subsequently confirmed (Yon *et al.*, 1982). To explain the present results, in particular the maximum in the kinetic curves with UMP as variable-concentration ligand, the concerted model was modified in several respects. As shown in Fig. 2, the adapted model gives a reasonable fit to initial rates obtained over a range of carbamoyl phosphate and UMP concentrations, when the parameter values in Table 1 are used. It can also generate, though with a poorer fit, the enhancement of the maximum in the UMP saturation curve in the presence of *N*-phosphonoacetyl-L-aspartate (Fig. 4a). The analysis suggests that, in the

absence of ligands, the enzyme is predominantly in the conformation with low affinity for carbamoyl phosphate (i.e. L is considerably greater than 1). A consequence of this should be that the binding of carbamoyl phosphate in the absence of UMP is co-operative; however, this co-operativity would not be seen in kinetic curves due to the postulated inverse relation of k_{cat} and carbamoyl phosphate affinity of the two conformations. In a study of the carbamoyl phosphate saturation curve for labilization of the enzyme towards proteinase, evidence of a co-operative conformational change concomitant with the binding of this ligand has been deduced (Cole & Yon, 1984). The present analysis also provides the first suggestion that the modifier UMP may in fact be a competitive inhibitor of the enzyme, a conclusion not readily apparent from its kinetic behaviour.

On the basis of kinetic evidence alone one cannot rule out other explanations for the regulatory kinetics of this enzyme, although a conformational model must be favoured in the light of independent evidence of conformational changes concomitant with the binding of UMP and carbamoyl phosphate (Cole & Yon, 1984). One such possibility is an induced-fit model (Koshland *et al.*, 1966) in which the binding of UMP to one subunit constrains adjacent subunits to take up a high- k_{cat} conformation. Since the model requires more parameters than the concerted model, it was not investigated. The literature also contains other models that explain maxima in curves of initial rate plotted versus inhibitor concentration. In the simplest concerted-transition model, inhibition by a structural analogue (competitive inhibitor) of a co-operatively binding substrate has long been known to produce such a maximum (Monod *et al.*, 1965; Smith *et al.*, 1975; Smith, 1977), a classic example being inhibition by succinate (or maleate) of aspartate transcarbamoylase from *E. coli* (Gerhart & Pardee, 1963; Collins & Stark, 1971). The model proposed by Mazat *et al.* (1977) considers two (generally different) binding sites per subunit for the same ligand, one of which may be occupied by a structurally related ligand. Although the present adaptation of the concerted model shares certain features with each of these models, neither of them was considered entirely appropriate for the

present case, in which the kinetic maximum was seen with UMP as variable-concentration ligand, but not when other inhibitors, known to be competitive with carbamoyl phosphate, are used, such as *N*-phosphonoacetyl-*L*-aspartate (the present study), phosphate or pyrophosphate (Grayson *et al.*, 1979).

References

- Cole, S. C. J. & Yon, R. J. (1984) *Biochem. J.* **221**, 289–296
- Collins, K. D. & Stark, G. R. (1971) *J. Biol. Chem.* **246**, 6599–6605
- Cornish-Bowden, A. (1976) *Principles of Enzyme Kinetics*, pp. 87–90, Butterworth, London
- Gerhart, J. C. & Pardee, A. B. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 491–496
- Goodson, J. J., Wharton, C. J. & Wrigglesworth, R. (1980) *J. Chem. Soc. Perkin Trans. 1* 2721–2727
- Grayson, J. E., Yon, R. J. & Butterworth, P. J. (1979) *Biochem. J.* **183**, 247–254
- Issaly, I., Poiret, M., Tauc, P., Thiry, L. & Hervé, G. (1982) *Biochemistry* **21**, 1612–1623
- Kantrowitz, E. R., Pastra-Landis, S. C. & Lipscomb, W. N. (1980a) *Trends Biochem. Sci.* **5**, 124–128
- Kantrowitz, E. R., Pastra-Landis, S. C. & Lipscomb, W. N. (1980b) *Trends Biochem. Sci.* **5**, 150–153
- Klotz, I. M. (1946) *Arch. Biochem.* **9**, 109–117
- Koshland, D. E., Nemethy, G. & Filmer, D. (1966) *Biochemistry* **5**, 365–385
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mazat, J.-P., Langla, J. & Mazat, F. (1977) *J. Theor. Biol.* **68**, 365–383
- Monaco, H. L., Crawford, J. L. & Lipscomb, W. N. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5276–5280
- Monod, J., Wyman, J. & Changeux, J.-P. (1965) *J. Mol. Biol.* **12**, 88–118
- Prescott, L. M. & Jones, M. E. (1969) *Anal. Biochem.* **32**, 408–419
- Smith, G. D. (1977) *J. Theor. Biol.* **69**, 275–285
- Smith, G. D., Kuchel, P. K. & Roberts, D. V. (1975) *Biochim. Biophys. Acta* **377**, 197–202
- Thiry, L. & Hervé, G. (1978) *J. Mol. Biol.* **125**, 515–534
- Wong, J. T. (1975) *Kinetics of Enzyme Mechanisms*, pp. 230–233, Academic Press, London and New York
- Yon, R. J. (1970) *Biochem. J.* **121**, 18p–19p
- Yon, R. J. (1972) *Biochem. J.* **128**, 311–320
- Yon, R. J. (1981) *Anal. Biochem.* **113**, 219–228
- Yon, R. J., Grayson, J. E., Chawda, A. & Butterworth, P. J. (1982) *Biochem. J.* **203**, 413–417