New Relations for Activity Analysis of Associating Enzymes

Jean Claude THOMES, Jacques ARCHAMBAULT DE VENÇAY and Raymond JULIEN
Laboratoire de Biochimie, Unité d'Enseignement et de Recherche des Sciences Exactes et Naturelles,
 123 rue Albert Thomas, 87060-Limoges-Cedex, France

(Received 17 July 1979)

New relations for catalytic-activity analysis are proposed for simple association/dissociation equilibria between the mono- and oligo-meric forms of enzymes in the case where these equilibria evolve very slowly in comparison with the binding of the substrates. This analysis of activity versus total enzyme concentration leads rapidly to information, complementary to that given by physico-chemical methods, on specific activities, the degree of polymerization of the enzyme forms and on their dissociation constants.

Theoretical models for oligomeric enzymes undergoing association/dissociation have been advanced by many authors (Frieden, 1967; Nichol *et al.*, 1967; Klapper & Klotz, 1968; Levitzki & Schlesinger, 1974; Kurganov, 1974*a,b*; Colosimo *et al.*, 1976), who have shown the advantage of their cooperativity and regulatory properties in cell metabolism. The physical properties of such enzymes may be obtained by conventional physico-chemical means and by kinetic analysis, leading to complementary information and to the specific activities of the different enzymic forms. Kurganov (1968, 1973, 1974*a,b*, 1977) and Kurganov *et al.* (1976*a,b*), in particular, have worked out kinetic analyses of dissociating-enzyme models in order to determine their kinetic parameters and equilibrium constants.

For the eukaryotic aminoacyl-tRNA synthetases (EC 6.1.1) that we have studied, a significant simplification of this type of analysis occurs (Chazal *et al.*, 1977; Archambault de Vençay *et al.*, 1978; Carias *et al.*, 1978). If a sample of the dilute solution is introduced in the activity test medium, the evolution to equilibrium is 'frozen', allowing analysis of the properties of these enzymes in terms of dissociation/association. This 'freezing' phenomenon remains as yet unexplained. For given substrate concentrations and initial-reaction-rate conditions, this 'freeze' enables us to relate this rate, v , directly to the enzyme form concentrations ($[E_j]$) by means of apparent specific activities k_j^* , by:

$$v = \sum_j k_j^* [E_j]$$

where the subscript j designates all the different enzymic forms.

This basic relationship, which is always valid if the dissociation/association evolution is very slow com-

pared with the binding of the substrates to the enzyme (that is, there is no obvious shift during test activity time), is the starting point of this kinetic analysis. In the simplest cases the study of k_j^* as a function of the substrate concentrations leads to the Michaelis-Menten constants of the enzymic forms, according to:

$$k_j^* = k_j \bar{Y}$$

where $\bar{Y}$ is the saturation function.

Taking into account the reaction-rate derivative with respect to the total enzyme concentration, one can easily analyse the following two systems:

- (i) $\sum_i n_i E_i = C$
- (ii) $\sum_i n_i E_i = C_1; \quad rC_1 = C_2$
 (with only one active enzymic form)

where E_i is the i th monomer and C the oligomer, n_i and r being the number of E_i present in C or C_1 and the number of C_1 present in C_2 respectively. The relationships developed may be applied also to association between an enzyme and a simple protein or between different enzymes, in order to investigate subunit interactions and possible activity modulation in enzyme complexes.

Theoretical Considerations

Equilibrium between monomers and oligomers

The equilibrium between the monomers E_i and the oligomer C corresponds to eqn. (1) and (1*a*):

$$\sum_i n_i E_i = C \quad (1)$$

$$K_1 = \frac{\prod (E_i^{n_i})}{C} \quad (1a)$$

K_1 being the dissociation constant, and E_i and C being the concentrations of free E_i and complex C respectively. Π is the multiplication operator and E_{i0} the concentration of total E_i . We have, for all i :

$$E_{i0} = E_i + n_i C \quad (2)$$

Let us introduce C_0 , the total enzyme concentration expressed in relation to the oligomer structure. Thus:

$$n_i C_0 = E_{i0} \quad \text{and} \quad \frac{E_i}{n_i} = E = C_0 - C \quad (2a)$$

The reaction rate related to C_0 is expressed as:

$$v = \Sigma \alpha_i E_i + \beta C \quad (3)$$

where α_i and β are the specific activities for free E_i and the molecule C respectively.

Taking into account the activity A defined by $A = v/C_0$, the concentration of the molecular species may be expressed as:

$$C = C_0 \frac{A - \bar{\alpha}}{\beta - \bar{\alpha}}; \quad E = C_0 \frac{\beta - A}{\beta - \bar{\alpha}}$$

with

$$\bar{\alpha} = \Sigma n_i \alpha_i \quad (4)$$

Given $\bar{n} = \Sigma n_i$ and $\Pi \equiv \Pi(n_i^n)$, the activity, A , is expressed as a function of C_0 according to relationship (1a):

$$A - \bar{\alpha} = \frac{\Pi}{(\beta - \bar{\alpha})^{\bar{n}-1}} \frac{C_0^{\bar{n}-1}}{K_1} (\beta - A)^{\bar{n}} \quad (5)$$

Introducing δ , the reaction-rate derivative with respect to C_0 , according to the relationship:

$$C_0 \frac{dA}{dC_0} = \delta - A$$

one may write the following equation:

$$\delta x_1 + A x_2 + x_3 = A \delta \quad (6)$$

where x_1 , x_2 and x_3 appear as constants that represent the following enzyme parameter combinations:

$$x_1 = \frac{\bar{\alpha}\bar{n} - \beta}{\bar{n} - 1}; \quad x_2 = \frac{\beta\bar{n} - \bar{\alpha}}{\bar{n} - 1}; \quad x_3 = -\bar{\alpha}\beta \quad (7)$$

From three points taken on the representative curve of A versus C_0 , that is, with three couples (A, δ) , one may write three first-order equations whose unknowns are x_1 , x_2 , x_3 , with:

$$x_1 + x_2 = \bar{\alpha} + \beta \quad (8)$$

The $\bar{\alpha}$ and β values are then the solutions of a second-order equation. The shape of the curve of A versus C_0 allows us to choose which root is valid for $\bar{\alpha}$ or β , because $\bar{\alpha}$ and β are the limits of A if C_0 falls to zero and reaches a high value respectively. The

number of subunits composing the oligomers may be deduced from the $\bar{\alpha}$ and β values and x_1 or x_2 .

A quick way for determining K_1/Π is obtained by using the total concentration, C_0 , which corresponds to a particular value of the activity A , for example, to the activity A^* , defined by:

$$A^* = \frac{\bar{\alpha} + \beta}{2} \quad (9)$$

is related C_0^* , and thus:

$$\frac{K_1}{\Pi} = \frac{C_0^{*\bar{n}-1}}{2^{\bar{n}-1}} \quad (9a)$$

It is clear that no further information is available from the present study, but if now the composition of the oligomer is known, that is, if $\Pi(n_i^n)$ is known, then the constant K_1 is available. If there are isomers of different enzymic forms, the dissociation constants are overall constants including isomerization equilibrium constants. This is also valid for specific activities. Specific information on these isomers cannot be obtained.

In order to check the accuracy of the results, linear plots may be drawn, corresponding to the following equations:

$$A = \beta - \left(\frac{K_1}{\Pi} \right)^{\frac{1}{\bar{n}}} (\beta - \bar{\alpha})^{\frac{\bar{n}-1}{\bar{n}}} \left[\frac{A - \bar{\alpha}}{C_0^{\bar{n}-1}} \right]^{\frac{1}{\bar{n}}} \quad (10)$$

$$A = \bar{\alpha} + \frac{\Pi}{K_1} (\beta - \bar{\alpha})^{1-\bar{n}} [C_0^{\bar{n}-1} (\beta - A)^{\bar{n}}] \quad (11)$$

$$\ln |C_0(A - \bar{\alpha})| = \ln \left| \frac{\Pi}{K_1} (\beta - \bar{\alpha})^{1-\bar{n}} \right| + \bar{n} \ln |C_0(\beta - A)| \quad (12)$$

Numerical analysis shows that, although numerical differentiation is sensitive to small variations in experimental data, accurate values can be obtained (Fig. 1a and Table 1).

Calculations have made it clear that the highest specific activity ($\bar{\alpha}$ or β) is obtained with the greatest precision. Moreover, there is no doubt regarding the $\bar{n}$ value.

It also appears in Table 2 that the sum $(x_1 + x_2)$ is obtained with good precision, leading to an accurate K_1 value if Π is known. The more accurate specific-activity value may now be used to draw linear plots by using eqns. (10) or (11) and then the other specific activity is obtained with a higher accuracy. This last value may be used to determine the first specific-activity value with an ever better precision (Figs. 1b and 1c, and Table 2). A final check is possible by drawing the plot corresponding to eqn. (12); a straight line must be obtained whose slope is $\bar{n}$, as can be seen in Fig. 1(d).

Fig. 1. Analysis of a theoretical equilibrium $\Sigma n_i E_i = C$ (a) Curve for $A = f(C_0)$, corresponding to eqn. (5) with $\bar{n} = 4$, $K_1 = 1$, $\bar{\alpha} = 40$, $\beta = 8$ (arbitrary units); (b) determination of β according to eqn. (10) and by using the $\bar{\alpha}$ and $\bar{n}$ values obtained applying eqn. (6) (see Table 1); (c) determination of $\bar{\alpha}$ according to eqn. (11), by using the new β -value; (d) check of the parameter values according to eqn. (12) and with the $\bar{\alpha}$ and β values obtained in (b) and (c) (see Table 1).

Table 1. Analysis of Fig. 1(a) according to eqn. (6)

In order to determine average values, five triplets of points of the curve shown in Fig. 1(a) are considered. Because it is difficult to determine δ on a curve of $v = f(C_0)$, δ is calculated from the dA/dC_0 values, which are more easily obtained.

C_0	A	$\Delta = \frac{dA}{dC_0}$	$\delta = C_0 \Delta + A$	$A\delta$	Results
0.100	35.50	-78.47	27.65	981.6	$\bar{\alpha} = 41.19$
0.300	24.40	-31.59	14.92	364.0	$\beta = 6.100$
0.500	20.00	-16.00	12.00	240.0	$\bar{n} = 3.24$
0.200	28.60	-55.21	17.56	502.2	$\bar{\alpha} = 42.22$
0.400	21.90	-22.89	12.74	279.1	$\beta = 6.690$
0.600	18.70	-12.75	11.05	206.6	$\bar{n} = 3.65$
0.124	33.60	-76.80	24.08	809.0	$\bar{\alpha} = 39.51$
0.230	27.20	-47.37	16.31	443.5	$\beta = 6.850$
0.910	16.00	-6.909	9.713	155.4	$\bar{n} = 4.05$
0.150	31.60	-66.66	21.60	682.6	$\bar{\alpha} = 38.34$
0.250	26.20	-39.26	16.38	429.1	$\beta = 10.14$
0.350	23.10	-24.56	14.50	335.0	$\bar{n} = 4.70$
0.140	32.30	-71.79	22.25	718.7	$\bar{\alpha} = 41.00$
0.320	23.90	-28.57	14.76	352.8	$\beta = 8.305$
0.700	17.60	-8.759	11.47	201.9	$\bar{n} = 3.71$

Table 2. Theoretical and calculated parameter values corresponding to the curve of Fig. 1(a)

	$\bar{\alpha}$	β	$\bar{n}$	$x_1 + x_2$	K_1
Theoretical values	40	8	4	48	1
Average values (from Table 1)	40.45	7.617	3.870	48.07	1.000*
From Fig. 1(b) and $\bar{\alpha} = 40.45$	—	8.0 ± 0.1	—	—	0.95 ± 0.06
From Fig. 1(c) and $\beta = 7.617$	40.00 ± 0.25	—	—	—	1.00 ± 0.05

value obtained from the enzyme concentration C_0^ , which corresponds to $A^* = \frac{x_1 + x_2}{2}$ on the curve of Fig. 1(a), the monomers being assumed to be identical.

Equilibrium between three enzymic forms

Let us now consider the following set of reaction equations:

$$\Sigma n_i E_i = C_1; \quad rC_1 = C_2 \quad (13)$$

To eqn. (1a) one must add:

$$K_2 = \frac{C_1^r}{C_2} \quad (14)$$

If the oligomers are dissolved and diluted, the subunit concentrations are in stoichiometric ratios and the enzyme conservation law is obeyed; for all i , the following relationship occurs:

$$rC_{20} = E + C_1 + rC_2 \quad (15)$$

where C_{20} is the total enzyme concentration expressed in relation to the C_2 structure.

Eqn. (3) for reaction rate must be completed as:

$$v = \bar{\alpha}E + \beta C_1 + \gamma C_2 \quad (16)$$

where γ is the specific activity of the enzymic form C_2 .

In order to express the concentrations E , C_1 and C_2 as functions of measurable quantities, let us introduce the reaction-rate derivative, δ , with respect to C_{20} . Taking into account relationships (15) and (16), one may write:

$$E(\delta - r\bar{\alpha}) + C_1\bar{n}(\delta - r\beta) + C_2r^2\bar{n}(\delta - \gamma) = 0 \quad (17)$$

Because of the multilinear form of eqn. (16), there is no general solution, but if it is possible to obtain experimental data for the ranges of high and low enzyme concentrations, where E and C_2 are negligible respectively, the treatment of the previous section may be applied to these two ranges. Then the approximate values obtained may be adjusted by using the procedure developed by Archambault de Vençay *et al.* (1978); the calculations are, however, long.

If only one enzymic form is active, the problem simplifies and linear plots may be drawn that permit one to determine the dissociation constants K_1 and K_2 and the specific activity. These plots are only linear for the values of $\bar{n}$ and r , chosen by the experi-

menter if these are unknown, which characterize the enzyme.

One may write the following equations:

(a) only $\bar{\alpha} \neq 0$. The activity A is a decreasing function of C_0 (Fig. 2a)

$$v^{\bar{n}r-1} = \frac{K_1^r K_2}{\Pi^r} \frac{\bar{\alpha}^{\bar{n}r-1}}{\bar{n}r(r-1)} \left[(\bar{n}-1) + \bar{\alpha}r \left(\frac{1}{\delta} - \frac{\bar{n}}{A} \right) \right] \quad (18) \text{ (see Fig. 2c)}$$

$$v^{\bar{n}-1} = \frac{K_1}{\Pi} \frac{\bar{\alpha}^{\bar{n}-1}}{\bar{n}(r-1)} \left[(1-\bar{n}r) + \bar{\alpha}r \left(\frac{\bar{n}r}{A} - \frac{1}{\delta} \right) \right] \quad (18a) \text{ (see Fig. 2b)}$$

(b) Only $\beta \neq 0$. In this case, the plot of A versus C_{20} exhibits a maximum (Fig. 3a)

$$v^{r-1} = \frac{K_2}{r} \frac{\beta^{r-1}}{(\bar{n}r-1)} \left[(1-\bar{n}) + \beta r \left(\frac{\bar{n}}{\delta} - \frac{1}{A} \right) \right] \quad (19) \text{ (see Fig. 3b)}$$

$$v^{\frac{1-r}{\bar{n}}} = \left(\frac{\Pi}{K_1} \right)^{\frac{1}{\bar{n}}} \left(\frac{\bar{n}\beta^{\frac{1-r}{\bar{n}}}}{\bar{n}r-1} \right) \left[(1-r) + \beta r \left(\frac{r}{A} - \frac{1}{\delta} \right) \right] \quad (19a) \text{ (see Fig. 3c)}$$

(c) Only $\gamma \neq 0$. A is an increasing function of C_{20} (see Fig. 4a)

$$v^{\frac{1-r}{r}} = \frac{r\gamma^{\frac{1-r}{r}}}{(\bar{n}-1)K_2^{\frac{1}{r}}} \left[(1-\bar{n}r) + \gamma \left(\frac{\bar{n}r}{\delta} - \frac{1}{A} \right) \right] \quad (20) \text{ (see Fig. 4b)}$$

$$v^{\frac{1-\bar{n}r}{\bar{n}r}} = \left(\frac{\Pi}{K_1} \right)^{\frac{1}{\bar{n}}} \frac{\bar{n}r\gamma^{\frac{1-\bar{n}r}{\bar{n}r}}}{K_2^{\frac{1}{\bar{n}}}(\bar{n}-1)} \left[(r-1) + \gamma \left(\frac{1}{A} - \frac{r}{\delta} \right) \right] \quad (20a) \text{ (see Fig. 4c)}$$

In all cases, the calculations make it clear that the specific-activity value is obtained with good pre-

Fig. 2. Activity analysis for a three-enzymic-form model with $\bar{\alpha} \neq 0$

(a) Curve of activity versus enzyme concentration for the model corresponding to: $\bar{n} = r = 2$, $\bar{\alpha} = 200$, $K_1 = 20$, $K_2 = 100$ (arbitrary units); (b) determination of $\bar{\alpha}$ and K_1 according to eqn. (18a); (c) determination of $\bar{\alpha}$ and $K_1'K_2$ according to eqn. (18). The values obtained are summarized in Table 3.

Fig. 3. Three-enzymic-form model with $\beta \neq 0$
 (a) Curve of activity versus enzyme concentration for the model corresponding to: $\bar{n} = r = 2$, $\beta = 100$, $K_1 = 20$, $K_2 = 100$; (b) determination of β and K_2 according to eqn. (19); (c) determination of β and K_1 according to eqn. (19a). The values obtained are summarized in Table 4.

Fig. 4. Three-enzymic form model with $\gamma \neq 0$
 (a) Curve of activity versus enzyme concentration for the model corresponding to: $\bar{n} = r = 2$, $\gamma = 100$, $K_1 = K_2 = 44.72$; (b) determination of γ and K_2 according to eqn. (20); (c) determination of γ and $K_1 K_2^{1/\bar{n}r}$ according to eqn. (20a). Table 5 summarizes the results.

cision (Figs. 2b, 2c, 3b, 3c, 4b and 4c, and Tables 3, 4 and 5). The equilibrium constants may be calculated with more precision by using eqn. (15) entirely expressed in terms of either E , C_1 or C_2 , according to the active enzyme form. One obtains then an equation that contains K_1 and K_2 . With two

Fig. 5. Effect of erroneous $\bar{n}$ and r values
 This effect is only shown for case (b) (see Fig. 2), but it has been demonstrated that, for the two other cases, erroneous $\bar{n}$ and r values also give curves that largely differ from the expected straight lines. (a) and (a') refer to eqn. (19); (a): $\bullet$, $\bar{n} = 2$, $r = 2$; $\blacksquare$, $\bar{n} = 3$, $r = 2$; (a'): $\bullet$, $\bar{n} = 2$, $r = 3$; $\blacksquare$, $\bar{n} = 3$, $r = 3$; (b) and (b') refer to eqn. (19a); (b) $\bullet$, $\bar{n} = 2$, $r = 2$; $\blacksquare$, $\bar{n} = 2$, $r = 3$; (b') $\bullet$, $\bar{n} = 3$, $r = 2$; $\blacksquare$, $\bar{n} = 3$, $r = 3$.

Table 3. Theoretical and calculated values corresponding to Fig. 2
 $n = r = 2$; $\bar{\alpha} \neq 0$. The subunits are identical.

	$\bar{\alpha}$	K_1	K_2
Theoretical values	200	20	100
From Fig. 2(b)	203 ± 2	19.7 ± 0.3	—
From Fig. 2(c)	199 ± 4	—	$102 \pm 2^*$
Values calculated according to eqn. (21a) with $\bar{\alpha} = 201$	—	19.8 ± 0.3	99.5 ± 0.8

* K_2 is calculated taking into account the K_1 value from Fig. 2(b).

Table 4. Theoretical and calculated values corresponding to Fig. 3
 $\bar{n} = r = 2$; $\beta \neq 0$.

	β	K_1	K_2
Theoretical values	100	20	100
From Fig. 3(b)	102 ± 2	—	96 ± 3
From Fig. 3(c)	100 ± 2	20.2 ± 0.7	—
From eqns. (15), (14) and (1a); $\beta = 101$	—	20.4 ± 0.2	96.0 ± 0.1

Table 5. Theoretical and calculated values corresponding to Fig. 4
 $\bar{n} = r = 2$; $\gamma \neq 0$.

	γ	K_1	K_2
Theoretical values	100	44.72	44.72
From Fig. 4(b)	100 ± 1	—	48 ± 1
From Fig. 4(c)	100 ± 1	24.5 ± 0.6	—
From eqns. (15), (14) and (1a); $\gamma = 100$	—	43 ± 5	45 ± 2

Fig. 6. Kinetic analysis of the methionyl-tRNA synthetase
(a) Experimental curve of activity versus enzyme concentration; (b) determination of $\bar{\alpha}$ according to eqn. (11) and the β -value determined by using eqn. (6) (see Table 6); (c) determination of β according to eqn. (10). The results are summarized in Table 6.

Table 6. Analysis of the activity of methionyl-tRNA synthetase

	$\bar{\alpha}(\text{min}^{-1})$	$\beta(\text{min}^{-1})$	$\bar{n}$	$K_1(\text{nM})$
Literature values (see the text)	—	—	2	13
From eqn. (6)	0.03 ± 0.03	19.5 ± 0.5	1.95	$13.5 \pm 0.5^*$
From Figs. 6(b) and 6(c)	0 ± 1	19.3 ± 0.1	—	13.3 ± 0.4

* Value calculated by using the curve of Fig. 6(a), assuming the subunits are identical.

Table 7. Analysis of the activity of phenylalanyl-tRNA synthetase

The results are consistent with $\bar{n} = r = 2$; the corresponding erroneous curves are not shown.

	$\beta(\text{min}^{-1})$	$K_1(\text{nM})$	$K_2(\text{nM})$
Literature values (see the text)	570	17	70
From Fig. 7(b)	559 ± 10	—	72 ± 4
From Fig. 7(c)	573 ± 10	15 ± 2	—

points of the experimental curve of A versus $f(C_{20})$, it is now possible to determine these constants. The constant values are more accurate if the powers of the enzyme-form concentration is close to zero.

For example, if at least one of the monomers is active (case a), the following equation:

$$E^{\bar{n}} \frac{\Pi}{K_1} + E^{\bar{n}r} \frac{r}{K_2} \left(\frac{\Pi}{K_1} \right)^r = rC_{20} - E \quad (21)$$

may be written as:

$$E^{\bar{n}-1} \frac{\Pi}{K_1} + E^{\bar{n}r-1} \frac{r}{K_2} \left(\frac{\Pi}{K_1} \right)^r = \frac{r\bar{\alpha}}{A} - 1 \quad (21a)$$

This procedure provides precise results as shown on Table 3, 4 and 5 corresponding to the curves of $A = f(C_{20})$ in Figs. 2(a), 3(a) and 4(a).

If $\bar{n}$ and r are not known by physicochemical means, arbitrary values are used in order to obtain the previous linear plots. Figs. 5(a), 5(a'), 5(b) and 5(b'), show, for example, the effect of erroneous values in the case where $\beta \neq 0$. It is now possible to check the accuracy of all the enzyme parameters obtained by comparing the calculated curve of $A = f(C_{20})$ with the experimental one.

Results and Discussion

In order to check the usefulness of the preceding relationships, they were applied to our already published results on aminoacyl-tRNA synthetases. In

Fig. 7. Activity analysis of the phenylalanyl-tRNA synthetase

(a) Experimental curve of activity versus enzyme concentration; (b) determination of β and K_2 according to eqn. (19); (c) determination of β and K_1 according to eqn. (19a). Table 7 summarizes the results.

both cases reported here, the substrate concentrations are saturating and the production of aminoacyl-tRNA is linear with time under the test conditions.

It is reasonable to consider that the equilibrium state of the sample of the dilute solution introduced in the test medium is 'frozen', because this simple hypothesis permits a consistent explanation of the experimental data.

The activities and reaction rates are related to the total enzyme concentrations in the dilution medium (preincubation medium). δ is calculated by using the derivative of activity with respect to C_0 (or C_{20}).

(a) Methionyl-tRNA synthetase (EC 6.1.1.10) from wheat-germ

The experimental data for this dimer enzyme were published by Chazal *et al.* (1977). Fig. 6(a) shows the curve for $A = f(C_0)$. Figs. 6(b) and 6(c) and Table 6 provide the parameter values obtained by using eqn. (6), and eqns. (10), (11) and (12). These values, obtained without preliminary hypothesis on structure and activity, are in good agreement with those already obtained. We conclude that eqn. (6) easily gives accurate values.

(b) Phenylalanyl-tRNA synthetase (EC 6.1.1.20) from human placenta

Eqns. (19) and (19a) were applied to experimental data obtained by Archambault de Vençay *et al.* (1978). Figs. 7(b) and 7(c) and Table 7 show that for $\bar{n} = 2$ and $r = 2$, these equations provide linear

plots that easily lead to enzyme parameters in good agreement with those published previously. It is clear that this procedure provides the parameter values more quickly, avoiding the necessity of proceeding by trial and error.

This work was supported by grants from the Centre National de la Recherche Scientifique and the Délégation Générale à la Recherche Scientifique et Technique.

References

- Archambault de Vençay, J., Thomes, J. C. & Julien, R. (1978) *FEBS Lett.* **89**, 98–102
- Carias, J. R., Mouricout, M., Quintard, B., Thomes, J. C. & Julien, R. (1978) *Eur. J. Biochem.* **87**, 583–590
- Chazal, Ph., Thomes, J. C. & Julien, R. (1977) *Eur. J. Biochem.* **73**, 607–615
- Colosimo, A., Brunori, M. & Wyman, J. (1976) *J. Mol. Biol.* **100**, 47–57
- Frieden, C. (1967) *J. Biol. Chem.* **242**, 4045–4052
- Klapper, M. H. & Klotz, I. M. (1968) *J. Am. Chem. Soc.* **7**, 223–231
- Kurganov, B. I. (1968) *Mol. Biol.* **2**, 430–447
- Kurganov, B. I. (1973) *Mol. Biol.* **7**, 429–447
- Kurganov, B. I. (1974a) *Mol. Biol.* **8**, 525–535
- Kurganov, B. I. (1974b) *Symp. Biol. Hung.* **18**, 31–45
- Kurganov, B. I. (1977) *J. Theor. Biol.* **68**, 521–543
- Kurganov, B. I., Dorozhko, A. I., Kagan, Z. S. & Yakovlev, V. A. (1976a) *J. Theor. Biol.* **60**, 271–286
- Kurganov, B. I., Dorozhko, A. I., Kagan, Z. S. & Yakovlev, V. A. (1976b) *J. Theor. Biol.* **60**, 287–299
- Levitzki, A. & Schlessinger, J. (1974) *Biochemistry* **13**, 5214–5219
- Nichol, L. W., Jackson, W. J. H. & Winzor, D. J. (1967) *Biochemistry* **6**, 2449–2456