Wheat-Germ Aspartate Transcarbamoylase

STEADY-STATE KINETICS AND STEREOCHEMISTRY OF THE BINDING SITE FOR L-ASPARTATE

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1. The steady-state kinetics of the bisubstrate reaction catalysed by aspartate transcarbamoylase purified from wheat (*Triticum vulgare*)-germ have been studied at 25°C, pH 8.5 and I 0.10–0.12. Initial-velocity and product-inhibition results are consistent with an ordered sequential mechanism in which carbamoyl phosphate is the first substrate to bind, followed by L-aspartate, and carbamoyl aspartate is the first product to leave, followed by P_i . The order of substrate addition is supported by dead-end inhibition studies using pyrophosphate and maleate as inhibitory analogues of the substrates. Product inhibition permitted a minimum value for the dissociation constant of L-aspartate from the ternary complex to be estimated. This minimum is of the same order as the dissociation constant (K_i) of succinate. 2. A range of dicarboxy analogues of L-aspartate were tested as possible inhibitors of the enzyme. These studies suggested that L-aspartate is bound with its carboxy groups in the eclipsed configuration, and that the stereochemical constraints around the binding site are very similar to those reported for the catalytic subunit of the enzyme from *Escherichia coli* [Davies, Vanaman & Stark (1970) *J. Biol. Chem.* **245**, 1175–1179].

Aspartate transcarbamoylase (EC 2.1.3.2) catalyses the bisubstrate reaction shown (eqn. 1), which is an early step in the biosynthesis of pyrimidine nucleotides. The reaction proceeds essentially to completion in the forward direction ($K_{eq.} = 1.8 \times 10^5$; Wedler & Gasser, 1974).

Changeux *et al.* (1968) noted that the enzyme from *Escherichia coli* bound succinate, an analogue of L-aspartate, more strongly in the presence of carbamoyl phosphate than in its absence, and suggested that the catalytic mechanism was ordered, carbamoyl phosphate binding before L-aspartate. Porter *et al.* (1969) carried out the first steady-state kinetic analysis of the catalytic subunit of the *E. coli* enzyme and suggested an ordered sequential mechanism with carbamoyl phosphate as the first substrate to bind and phosphate as the last product to leave. They also postulated several dead-end complexes involving substrates and products on the basis of non-linear product inhibition

and substrate inhibition. However, Heyde *et al.* (1973), in an independent kinetic study of the same enzyme, favoured a random-order mechanism. Subsequently Wedler & Gasser (1974) and Jacobson & Stark (1975) produced additional evidence in favour of an ordered mechanism. Other aspartate transcarbamoylases for which reports on the steady-state mechanism have appeared include the enzymes from mouse spleen (Hoogenraad, 1974) and mung bean (*Phaseolus aureus*) (Ong & Jackson, 1972; Savithri *et al.*, 1978) for which ordered mechanisms have been postulated, and from *Streptococcus faecalis* (Chang & Jones, 1974) for which a random mechanism has been postulated. The enzyme from baker's yeast was studied by Lue *et al.* (1976), but only an equivocal result, possibly favouring a random mechanism, was reported. In addition to studies on the steady-state mechanism, Porter *et al.* (1969) and Davies *et al.* (1970) used kinetic methods to explore the stereochemistry of the aspartate-binding site in the enzyme from *E. coli*.

In the present paper we report initial-velocity, product-inhibition, and dead-end-inhibition studies of the wheat-germ aspartate transcarbamoylase purified as described in the preceding paper (Grayson *et al.*, 1979) that strongly suggest that the mechanism

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is ordered. We also report a study of the effects of inhibitor analogues of L-aspartate, and conclude that there are strong similarities between the stereospecificities of the aspartate-binding sites in the enzymes from *E. coli* and wheat-germ.

Experimental

Materials

L-Aspartic acid, sodium succinate, sodium dihydrogen phosphate, sodium pyrophosphate and all buffer components were obtained from BDH, Poole, Dorset, U.K. Carbamoyl phosphate, carbamoyl-DL-aspartic acid, D- and L-malic acid, D-, L- and *meso*-tartaric acid, D-aspartic acid, L-glutamic acid and phthalic acid were obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Pyromellitic acid and succinamic acid were obtained from Ralph Emanuel, Wembley, Middx., U.K. The carbamoyl phosphate was estimated to contain not less than 95% acid-labile phosphate and less than 3% P_i by assaying the phosphate content before and after complete acid hydrolysis, by using the method of Baginski (1967).

Wheat (*Triticum vulgare*)-germ aspartate transcarbamoylase was purified to near-homogeneity as described in the preceding paper (Grayson *et al.*, 1979). The method used a small column of DEAE-cellulose in the final purification/concentration step, which also concentrated UMP present in the previous step. The final preparation therefore contained 2–3 mM-UMP as judged by A_{260} measurements. This preparation, even after removal of low-molecular-weight compounds by gel filtration on Sephadex G-25, gave kinetic results suggesting it still contained tightly-bound UMP (a potent inhibitor of the enzyme). To counter this effect it was necessary to pretreat the enzyme with carbamoyl phosphate before use. To the purified enzyme (0.2 ml) was added an equal volume of 15 mM-carbamoyl phosphate adjusted to pH 8.0. After 10 min this mixture was loaded on to a column (150 mm $\times$ 8 mm) of Sephadex G-25 equilibrated with the reaction buffer. Material in the void-volume peak was collected and further diluted as described below. These operations were carried out immediately before a set of kinetic experiments. Carbamoyl phosphate present in the pretreatment was removed during the gel filtration step, since the enzyme showed no activity when incubated in a reaction mixture containing L-aspartate only. The enzyme was diluted with the reaction buffer to give a value of V_{max} between 20 and 30 nmol/min. Dilution factors varied according to the specific activities of different preparations. For the most highly purified enzyme, such dilution gave 1.0–1.2 μ g of enzyme/ml of reaction mixture.

Initial-velocity measurements

All kinetic experiments were carried out at $25 \pm 0.1^\circ\text{C}$ and at pH 8.5 in 0.1 M-Tris adjusted with acetic acid. The reaction volume was 3.0 ml. The reaction was initiated by the addition of a substrate made up in the reaction buffer. After 5 or 10 min the reaction was terminated by the addition of 2 ml of the phenazone/ H_2SO_4 mixture, followed by 1 ml of the butanedione oxime/acetic acid mixture as described by Prescott & Jones (1969). Carbamoylaspartate was determined by the longer and more sensitive of the methods described by those authors. Rigid control of times and lighting conditions during the colour development ensured reproducible results. In studying product inhibition by carbamoylaspartate, the production of phosphate was assayed by the method of Baginski (1967) in which the colour development time was kept to 30 s to minimize the decomposition of carbamoyl phosphate in the acid conditions of the assay.

The production of carbamoylaspartate and of phosphate were linear with time up to 20% of the theoretical extent of the reaction. In most cases initial velocities were determined at single times corresponding to less than 10% conversion. Replicate determinations (five to ten values) were made at each combination of substrate concentrations shown in Fig. 1 (below). All other data points shown in Figs. 2–5 (below), and in obtaining the K_i values of Table 2 (below), are the means of duplicate determinations.

Preliminary double-reciprocal plots were made to check linearity. Experimental data were then fitted directly to the Michaelis-Menten equation by a computer program adapted from that of Cleland (1967). Each line was fitted separately and without reference to its intersection with other lines. Each replicate determination was entered separately; thus some of the lines in Fig. 1 were generated from as many as 30 value pairs (only the means of replicates are shown in Fig. 1). The program provided slopes and intercepts together with their s.e.m. values. In studying inhibition by aspartate analogues, preliminary double-reciprocal plots of the effects of the more potent inhibitors were drawn to confirm that inhibition was competitive with aspartate. Thereafter K_i values were determined from Dixon (1953) plots.

Results

Choice of reaction conditions

The pH profile of the activity of the enzyme shows two maxima, the higher at pH 10 and a second at pH 8.5 (Yon, 1972). The present study was carried out at pH 8.5, which was considered to be a more physiological value. Carbamoyl phosphate could not be used at concentrations much above 500 μ M,

Fig. 1. Initial-velocity studies

Double-reciprocal patterns are shown with (a) L-aspartate as variable substrate, and (b) carbamoyl phosphate as variable substrate. In (a), the fixed concentrations of carbamoyl phosphate were $8\mu\text{M}$ (■), $15\mu\text{M}$ (□), $30\mu\text{M}$ (●), and $100\mu\text{M}$ (○). In (b), the fixed concentrations of L-aspartate were 0.167mM (■), 0.25mM (□), 0.50mM (●) and 1.0mM (○). Insets are slope (▲) and intercept (△) replots against reciprocals of the changing fixed substrate concentration. The vertical bars show the calculated S.E.M. values.

Table 1. Kinetic constants at pH8.5 for the sequential reaction catalysed by wheat-germ aspartate transcarbamoylase

Kinetic constants are defined, and obtained from the replots of Fig. 1, as described by Cleland (1970). The subscripts *a* and *b* refer to carbamoyl phosphate and L-aspartate respectively, as judged by product-inhibition and dead-end-inhibition patterns (see the text). The kinetic constants are estimated to be correct to 20% or better, based on triplication of the whole experiment.

$$\begin{aligned} V_{\max} &= 26 \text{ nmol/min} \\ K_{ia} &= 27 \mu\text{M} \\ K_a &= 3.5 \mu\text{M} \\ K_b &= 120 \mu\text{M} \end{aligned}$$

Thus true saturation could not be achieved for either substrate in kinetic studies; in the following experiments the non-varied substrate was always at a non-saturating concentration.

Initial-velocity patterns

The terminology of Cleland (1963) is used in this and subsequent parts of the present paper. Since these experiments were conducted in the absence of UMP, all primary plots were hyperbolic and gave linear double-reciprocal plots [see Yon (1972) for the kinetic effects of UMP]. Fig. 1 shows the initial-velocity patterns obtained. When either substrate was varied the double-reciprocal plots gave an intersecting pattern; within the limits of error of the experiments the lines all intersected at a single point to the left of the vertical axis. The mechanism is therefore sequential, that is, both substrates must bind to the enzyme before either product leaves. Replots of slopes and intercepts from both patterns are shown (Fig. 1) and the kinetic constants derived from them by the method of Cleland (1970) are given in Table 1. The subscripts *a* and *b* are assigned to carbamoyl phosphate and L-aspartate respectively, as explained below.

Product-inhibition patterns

Figs. 2 and 3 show product-inhibition patterns for the four possible pairings of product inhibitor and variable substrate. The linear slope and intercept replots shown were fitted by eye. A competitive-inhibition pattern was given by phosphate when carbamoyl phosphate was the variable substrate. The inhibition was linear and the dissociation constant for phosphate was approx. 1.0mM from the horizontal intercept of the slope replot (Fig. 2a). Inhibition by phosphate with aspartate as variable substrate and by carbamoyl aspartate with phosphate as variable substrate (Figs. 2b and 3a) were both linear non-competitive, the points of intersection lying

at which value the non-enzymic carbamoylation of aspartate by isocyanate (a breakdown product of carbamoyl phosphate) becomes obtrusive. L-Aspartate could not be used at concentrations above 1mM owing to the onset of severe substrate inhibition.

Fig. 2. Product inhibition by phosphate

Double-reciprocal patterns are shown with (a) carbamoyl phosphate as variable substrate, L-aspartate fixed at 1.0 mM, and (b), L-aspartate as variable substrate, carbamoyl phosphate fixed at 100 μ M. In both cases the fixed concentrations of phosphate were 0 ($\bullet$), 2.5 mM ($\circ$), 5.0 mM ($\blacktriangle$), 10.0 mM ($\square$) and 25.0 mM ($\blacksquare$). Insets are slope ($\blacktriangle$) and intercept ($\triangle$) replots against phosphate concentration. The vertical bars show the calculated s.e.m. values.

well to the left of the vertical axis. The inhibition by carbamoyl aspartate with aspartate as variable substrate was also linear non-competitive, although the point of intersection was much nearer the vertical axis (Fig. 3b). All of these patterns are consistent with an ordered mechanism in which carbamoyl phosphate is the first substrate to bind and phosphate is the last product to leave (Cleland, 1963). This mechanism is represented in Scheme 1.

In such an ordered bisubstrate mechanism the dissociation constant for carbamoyl phosphate

Fig. 3. Product inhibition by carbamoylaspartate

Double-reciprocal patterns are shown with (a) carbamoyl phosphate as variable substrate, L-aspartate fixed at 1.0 mM and (b) L-aspartate as variable substrate, carbamoyl phosphate fixed at 100 μ M. In both cases the fixed concentrations of carbamoylaspartate were 0 ($\bullet$), 25 mM ($\circ$), 50 mM ($\blacksquare$) and 100 mM ($\square$). Insets are slope ($\blacktriangle$) and intercept ($\triangle$) replots against the concentrations of carbamoylaspartate. The vertical bars show the calculated s.e.m. values.

(k_{-1}/k_{+1}) from the enzyme-carbamoyl phosphate complex is given directly by the kinetic constant K_{ia} as 27 μ M (see Table 1). The horizontal co-ordinate of the intersection point in Fig. 3(b), in which carbamoylaspartate is the product inhibitor and aspartate the variable substrate, may be used to estimate a minimum value for the dissociation constant (k_{-2}/k_{+2}) of aspartate from the ternary complex. This horizontal co-ordinate, H , is given by the expression below for

Scheme 1. Mechanism of substrate binding to aspartate transcarbamoylase

Abbreviations used: E, enzyme active centre; CP, carbamoyl phosphate; Asp = L-aspartate; CA = N-carbamoyl-L-aspartate; P_i = inorganic phosphate.

the ordered bisubstrate mechanism; the denominator may be neglected at saturating concentrations of carbamoyl phosphate (Porter *et al.*, 1969; Segel, 1975):

$$H = \frac{\frac{k_{+2}}{k_{-2}} \left(1 + \frac{k_{+p}}{k_{-p}}\right)}{1 + \frac{K_{ia}}{[\text{carbamoyl phosphate}]}} \quad (2)$$

which may be rearranged to:

$$\frac{k_{-2}}{k_{+2}} = \frac{1 + \frac{k_{+p}}{k_{-p}}}{H \times D}$$

where D is the denominator of eqn. (2). Since k_{+p} and k_{-p} are both positive, k_{-2}/k_{+2} must be greater than $1/(H \times D)$. The denominator D has a value between 1.21 and 1.33 (allowing for the error in K_{ia} ; see Table 1). We estimate the value of H as being between 1 and 3 mm^{-1} ; the large margin of error is due to uncertainty in locating the intersection in Fig. 3(b). The minimum value of k_{-2}/k_{+2} is therefore estimated to lie between 0.25 and 0.83 mm . It is noteworthy that this is of the same order as the dissociation constant (K_i) of the strong inhibitor, succinate (0.21 mm ; see Table 2).

Dead-end inhibition patterns

Since the foregoing conclusions are only valid for the ordered sequential mechanism, it was important to seek further evidence of the proposed binding order. Cleland (1970) showed that the nature of the inhibition by structural analogues of the two substrates may be used to resolve sequential mechanisms and establish the binding order. We studied dead-end inhibition by maleate, an analogue of aspartate, and by pyrophosphate, an analogue of carbamoyl phosphate, to test the proposed mechanism.

Figs. 4 and 5 show the double-reciprocal patterns obtained from the four pairings of inhibitor and variable substrate. As expected, each inhibitor was competitive with respect to the substrate that it

Fig. 4. Dead-end inhibition by maleate

Double-reciprocal patterns are shown with (a) L-aspartate as variable substrate, carbamoyl phosphate fixed at $100 \mu\text{M}$, and (b) carbamoyl phosphate as variable substrate, L-aspartate fixed at 0.33 mm . The fixed concentrations of maleate were 0 (●), 0.10 mm (○), 0.20 mm (■), and 0.30 mm (□). The lines were fitted by eye.

structurally resembled (Figs. 4a and 5a). Pyrophosphate showed a non-competitive (intersecting) pattern when aspartate was the variable substrate (Fig. 5b). However, maleate showed an uncompetitive (parallel) pattern when carbamoyl phosphate was the variable substrate (Fig. 4b). The absence of a slope effect in the second case, but not in the first, establishes that maleate binds after carbamoyl phosphate

Fig. 5. Dead-end inhibition by pyrophosphate

Double-reciprocal patterns are shown with (a) carbamoyl phosphate as variable substrate, L-aspartate fixed at 0.33 mM, and (b) L-aspartate as variable substrate, carbamoyl phosphate fixed at 100 μ M. The fixed concentrations of pyrophosphate were 0 ($\bullet$), 1.0 mM ($\circ$), 3.0 mM ($\blacksquare$) and 5.0 mM ($\square$). The lines were fitted by eye.

in the binding sequence, i.e. maleate binds to the enzyme-carbamoyl phosphate complex [see Cleland (1970) and Segel (1975) for a discussion of dead-end-inhibition patterns in ordered mechanisms]. This provides strong support for the order of substrate binding suggested by product-inhibition studies.

Steric constraints at the binding site for L-aspartate

With the assay of Prescott & Jones (1969), which gives a positive response for any *N*-carbamoyl compound, no products were found when the enzyme was incubated with 1 mM-carbamoyl phosphate and either D-aspartate or L-glutamate, even when the enzyme concentration was 100-fold greater than was used in kinetic studies with L-aspartate as second

Table 2. Inhibition by analogues of L-aspartate
 K_i values were obtained from the intersection of the two lines corresponding to 1.0 mM- and 0.25 mM-L-aspartate in a Dixon (1953) plot. Values greater than 500 mM could not be measured accurately.

Compound	K_i (mM)
D-Aspartate	> 500
Fumarate	> 500
Succinamate	> 500
Phthalate	> 500
Pyromellitate	> 500
L-Glutamate	160
D-Tartrate	150
L-Tartrate	140
D-Malate	7.6
L-Malate	6.7
meso-Tartrate	0.51
Succinate	0.21
Maleate	0.10

substrate. Thus D-aspartate and L-glutamate are not substrates.

The inhibition of the enzyme by a series of dicarboxylic analogues of L-aspartate was investigated (Table 2). Values of K_i were obtained from Dixon (1953) plots, and it was assumed that all dicarboxylic inhibitors bound to the enzyme-carbamoyl phosphate complex as has been shown (above) for maleate. These results are strikingly similar to those of Porter *et al.* (1969) and Davies *et al.* (1970) on the *E. coli* enzyme. Strong inhibition by maleate, a planar *cis*-dicarboxylic acid, and by succinate, which can rotate its carboxy groups into the *cis* (eclipsed) configuration, and the failure of fumarate (a *trans*-dicarboxylic acid) to inhibit even slightly, all suggest strongly that binding to the aspartate site requires two carboxy groups oriented in the *cis* configuration.

Stereochemical restrictions on substituents at the α - and β -carbon atoms of L-aspartate are best discussed by reference to Fig. 6 (adapted from Davies *et al.*, 1970), which is a schematic view of L-aspartate in its binding site with the *cis*-carboxy groups pointing away from the viewer. In this orientation the substituents at the α - and β -carbon atoms are situated in loci labelled *a*, *b*, *c* and *d*. Locus *a* is assumed to be near the bound carbamoyl phosphate molecule and normally accepts the amino group of L-aspartate. Loci *b*, *c* and *d* normally accept hydrogen atoms. Evidence for steric restrictions at some of these sites may be deduced from the inhibition studies (Table 2). Since locus *a* can accept an amino group, it may be assumed that the smaller hydroxy group will fit into this locus. This would account for the moderately strong inhibition by L-malate. D-Tartrate can only bind with its hydroxy groups in loci *a* and *c*; since it is a very weak inhibitor, one must conclude

that locus *c* is unable to accept a hydroxy group. Since D-malate is a moderate inhibitor, locus *b* or locus *d* must be able to accept a hydroxy group; that only one of these is accessible is shown by the extremely weak inhibition by L-tartrate. Thus only one of the locus pairs *a*-*b* or *a*-*d* can accept hydroxy groups. Of these alternatives, *meso*-tartrate would put its hydroxy groups into loci *a* and *b*; since this compound is an excellent inhibitor, one concludes finally that loci *a* and *b* will accommodate groups as bulky as hydroxy groups, whereas loci *c* and *d* will not. The failure of D-aspartate (which would put its amino group into locus *b* or locus *d*) to inhibit the enzyme reaction suggests that locus *b*, which can accept a hydroxy group, is unable to accept the larger amino group.

Discussion

Steady-state kinetics

Kinetic studies suggesting ordered sequential mechanisms have been reported for the enzymes

from mung bean (Ong & Jackson, 1972; Savithri *et al.*, 1978) and mouse spleen (Hoogenraad, 1974). Although controversy has surrounded the kinetic mechanism of the catalytic subunit of the *E. coli* enzyme (reviewed by Jacobson & Stark, 1973) the present weight of evidence would appear to favour an ordered rather than a random mechanism (Wedler & Gasser, 1974; Jacobson & Stark, 1975). The present study suggests that the wheat-germ enzyme also utilizes an ordered mechanism, on the evidence of product-inhibition and dead-end-inhibition kinetic patterns. Dead-end inhibition by substrate analogues has been used in several studies on aspartate transcarbamoylase. Savithri *et al.* (1978) by using acetyl phosphate and succinate as the substrate analogues, observed a similar set of inhibition patterns as reported in Figs. 4 and 5 above. In a contrasting study of the enzyme from *Streptococcus faecalis*, Chang & Jones (1974), by using pyrophosphate and maleate as substrate analogues, noted a definite slope effect in plots of maleate (as constant inhibitor) against carbamoyl phosphate as variable substrate. On the basis of this and other evidence they proposed a

Fig. 6. Stereochemistry of the L-aspartate-binding site

L-Aspartate is shown schematically *in situ* with its carboxy groups eclipsed and pointing into a cleft away from the viewer. The loci *a*, *b*, *c* and *d* are represented by dotted outlines. Dicarboxylic analogues of L-aspartate would tend to place substituents into these loci according to the table below. Note that only compounds that place nothing larger than a hydrogen atom into loci *c* and *d* bind effectively.

Locus	...	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
(a) Substrate or effective inhibitors					
L-Aspartate		-NH ₃ ⁺	-H	-H	-H
Maleate		<-H	<-H	<-H	<-H
Succinate		-H	-H	-H	-H
<i>meso</i> -Tartrate		-OH	-OH	-H	-H
L-Malate		-OH	-H	-H	-H
D-Malate		-H	-OH	-H	-H
(b) Ineffective or very weak inhibitors					
L-Tartrate		-H	-OH	-H	-OH
D-Tartrate		-OH	-H	-OH	-H
D-Aspartate either (i)		-H	-NH ₃ ⁺	-H	-H
or (ii)		-H	-H	-H	-NH ₃ ⁺
(c) Presumed ineffective due to 'wrong-way-round' binding					
L-Aspartate		-H	-H	-NH ₃ ⁺	-H
L-Malate		-H	-OH	-OH	-H
D-Malate		-H	-H	-H	-OH

random sequential mechanism for the enzyme from *S. faecalis*.

It is worthwhile to compare the kinetic properties of the wheat-germ enzyme with that from *E. coli*, which has been extensively studied. The dissociation constant for carbamoyl phosphate (K_{1a}) is about the same for both enzymes. However, there is a very large difference in affinity for L-aspartate, the Michaelis constant, K_b , being more than 100 times smaller for the wheat-germ enzyme (0.12 mM compared with 20 mM). Porter *et al.* (1969) estimated that the minimum value for the dissociation constant of L-aspartate from the ternary complex was 0.2 M, i.e. aspartate was bound very poorly to the enzyme-carbamoyl phosphate complex. In comparison, succinate was bound with much greater affinity ($K_i = 3$ mM). On the basis of this and other evidence a compression mechanism was postulated in which the amino group of aspartate and the carbamoyl group of carbamoyl phosphate were forced together, favouring the transfer of the carbamoyl group (Collins & Stark, 1969). In our study of the wheat-germ enzyme a similar comparison of the minimum value of the dissociation constant for aspartate (k_{-2}/k_{+2}) with the value of K_i for succinate was less conclusive, suggesting that the affinity for aspartate was at most as great as, and possibly less than, the affinity for succinate. However, any difference in the affinities of aspartate and succinate is much less marked than in the *E. coli* enzyme.

Stereochemistry of the L-aspartate-binding site

Studies on the effectiveness of a range of structural analogues of L-aspartate as inhibitors of the enzyme suggest that this substrate is bound to the enzyme with its two carboxy groups in or near the eclipsed configuration. Further, the four other substituents on the α - and β -carbon atoms define loci, termed *a*, *b*, *c* and *d*, on the surface of the enzyme, that offer various degrees of hindrance to these substituents; in general, loci *a* and *b* are more accessible than loci *c* and *d* (see Fig. 6). Porter *et al.* (1969) and Davies *et al.* (1970) came to the same conclusions about the aspartate-binding site of the catalytic subunit of the *E. coli* enzyme. Moreover, they confirmed the steric restrictions at loci *c* and *d* by showing that, of the two diastereoisomeric β -hydroxy-L-aspartic acids, only the *erythro* diastereoisomer (with amino and hydroxy groups in loci *a* and *b* respectively) showed substrate activity. There are pronounced differences between the wheat-germ and *E. coli* catalytic subunit enzymes

in polypeptide chain length, quaternary structure and regulatory mechanism (Jacobson & Stark, 1973; Grayson & Yon, 1978). Notwithstanding these differences, the active-site regions of the two enzymes show striking similarities in the topography of the aspartate-binding pocket, in the order of binding and release of substrates and products, and possibly in the catalytic mechanism. These similarities have either been conserved since the divergence of plants and prokaryotes, or they represent a case of independent convergent evolution.

References

- Baginski, E. S. (1967) *Clin. Chim. Acta* **15**, 155–158
- Chang, T.-Y. & Jones, M. E. (1974) *Biochemistry* **13**, 638–645
- Changeux, J.-P., Gerhart, J. C. & Schachman, H. K. (1968) *Biochemistry* **7**, 531–538
- Cleland, W. W. (1963) *Biochim. Biophys. Acta* **67**, 104–137
- Cleland, W. W. (1967) *Adv. Enzymol. Relat. Areas Mol. Biol.* **29**, 1–32
- Cleland, W. W. (1970) *Enzymes 3rd Ed.* **2**, 1–65
- Collins, K. D. & Stark, G. R. (1969) *J. Biol. Chem.* **244**, 1869–1877
- Davies, G. E., Vanaman, T. C. & Stark, R. G. (1970) *J. Biol. Chem.* **245**, 1175–1179
- Dixon, M. (1953) *Biochem. J.* **55**, 170–171
- Grayson, J. E. & Yon, R. J. (1978) *Biochem. Soc. Trans.* **6**, 197–200
- Grayson, J. E., Yon, R. J. & Butterworth, P. J. (1979) *Biochem. J.* **181**, 239–245
- Heyde, E., Nagabhushanam, A. & Morrision, J. F. (1973) *Biochemistry* **12**, 4718–4726
- Hoogenraad, N. J. (1974) *Arch. Biochem. Biophys.* **161**, 76–82
- Jacobson, G. R. & Stark, G. R. (1973) *Enzymes 3rd Ed.* **9**, 226–309
- Jacobson, G. R. & Stark, G. R. (1975) *J. Biol. Chem.* **250**, 6852–6860
- Lue, P. F., Aitken, D. M. & Kaplan, J. G. (1976) *Biochimie* **58**, 19–25
- Ong, B. L. & Jackson, J. F. (1972) *Biochem. J.* **129**, 571–581
- Porter, R. W., Modebe, M. O. & Stark, G. R. (1969) *J. Biol. Chem.* **244**, 1846–1954
- Prescott, L. M. & Jones, M. E. (1969) *Anal. Biochem.* **32**, 408–419
- Savithri, H. S., Vaidyanathan, C. S. & Rao, N. A. (1978) *Proc. Indian Acad. Sci.* **87B**, 81–94
- Segel, I. H. (1975) *Enzyme Kinetics*, pp. 579 and 774–778 Wiley-Interscience, New York
- Wedder, F. C. & Gasser, F. J. (1974) *Arch. Biochem. Biophys.* **163**, 57–68
- Yon, R. J. (1972) *Biochem. J.* **128**, 311–320