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QUATERNARY STRUCTURAL CHANGES IN ASPARTATE CARBAMOYLTRANSFERASE OF ESCHERICHIA COLI AT pH 8.3 and pH 5.8

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SUMMARY: Atomic coordinates obtained from the crystal structures of unliganded and liganded aspartate carbamoyltransferase at pH 5.8 yield calculated low angle scattering curves in substantial agreement with experimental curves obtained by Moody, Vachette and Foote at pH 8.3. Thus the major conformational changes produced upon binding of six molecules of ligand, N-phosphonacetyl-L-aspartate (PALA) are very similar at pH 8.3 where the enzyme shows activity and regulation, as at pH 5.8 where the enzyme is inactive.

INTRODUCTION: Aspartate carbamoyltransferase (Carbamoylphosphate: L-aspartate carbamoyltransferase, EC 2.1.3.2) initiates the pyrimidine pathway in Escherichia coli by forming carbamoyl-L-aspartate from carbamoylphosphate and L-aspartate. Substrates produce homotropic effects, cytidine triphosphate inhibits allosterically, and ATP stimulates at the same allosteric sites (1,2). The three-dimensional structure indicates two catalytic trimers c_3 and three regulatory dimers r_2 in a molecule $(c_3)_2(r_2)_3$ of symmetry D_3 (1-3). When the substrate analogue N-phosphonacetyl-L-aspartate (PALA) binds to each of the six catalytic chains, the structure elongates by 11-12 A along the molecular three-fold axis by separation of the two c_3 units (4). These c_3 units also undergo (4) a relative rotation by 8-9°, and each r_2 unit rotates (4) about its molecular two-fold axis by 14-15°. Earlier chemical evidence for very large conformational changes when substrates or their analogues bind has been summarized elsewhere (1,2,5), and earlier structural evidence for the elongation along the three-fold axis has been obtained from the unit cells of the X-ray diffraction studies (3,6) and from low angle scattering of X-rays (7).

The low angle X-ray scattering was carried out in solution at pH 8.3 where the activity and regulation are nearly maximal (8), while the X-ray

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diffraction studies (3,4) were made at pH 5.8 where neither activity nor regulation are appreciable. A study of the reactivity of the sulfhydryl ligands to the Zn^{+2} of the regulatory units with p-hydroxymercuri benzoate indicated that structural differences persist as the pH is lowered from 8.5 to 6.0 (5). However, this reaction is a local probe. In the present communication we use the atomic coordinates of the unliganded (T) structure and the PALA-liganded (R) structure to compute the low angle X-ray scattering at pH 5.8, and we compare our results with those obtained experimentally at pH 8.3 by Moody, Vachette and Foote (7).

MATERIALS AND METHODS: The intensity at each angle of scattering was calculated from the sum of absolute squares of the structure factors contributing near that angle (9). THese structure factors were computed on a VAX 11/780 Computer (Digital Equipment Company) by use of the fast Fourier transform (10) of the electron density of the complete protein molecule. No solvent was included. The map for the unligated enzyme had previously been refined to 2.8 A resolution, while that of the ligated enzyme is in an advanced stage of refinement (R = 0.36 to 3.0 A resolution) by the method of Konnert and Hendrickson (11). However, the unrefined structure (4) was used in the present calculations. The PALA molecule was omitted from the coordinates which were used to refine the electron density, but electron density is present in the map at its expected positions.

In these calculations an overall temperature factor B of 10 A^2 was assumed. In order to produce an even distribution of calculated intensity an orthorhombic unit cell of symmetry P1 and dimensions of 450 A x 500 A x 500 A was assumed. This large cell, containing one c_6r_6 molecule minimizes the effect of intermolecular vectors. The grid interval was 3A in each direction, and limits of 15 A to 200 A were taken for the Fourier transform. Squares of structure factors were sorted into about 45 bins of reciprocal radius, at an interval of about s = 0.0012 A^{-1}. Intensities were then calculated as the average of the squares of all structure-factors within a given sampling interval, ranging from 0.002 A^{-1} to 0.055 A^{-1}.

RESULTS: The dimensions of the unit cell are at least three times the molecular dimensions (1) (105 A x 105 A x 92 A) in each direction. Hence, only intramolecular interactions are included. In Figure 1a we show the calculated scattering (pH 5.8) as a function of $s = 2(\sin \psi/2)/\lambda$, where $\psi = 2\theta$ is the total scattering angle. In Figure 1b we show the experimental curves (7) at pH 8.3. Details of the comparisons are shown in Table 1. The small systematic shift of about 0.0005 A^{-1} to 0.0001 A^{-1} to higher s values of the two calculated curves relative to the experimental curves may be due to the omission from the calculated curves of incoherent scattering, or, more likely, of partially ordered solvent molecules near the surface of the protein.

TABLE 1. Features of the scattering curves of Figure 1.

Expl.		Min 1	Max 1	Shoulder	Min 1	Max 2
Native:	s^a	0.0135	0.0185	0.03	0.0425	0.049
	I^b	0.59	1.6	0.21	0.054	0.08
Ligated:	s(i)	0.0125	0.0162	----	------	0.048
	I	1.8	2.7	----	------	0.11
Calc.						
Native:	$s^{(A-1)}$	0.0144	0.0199	0.030	0.045	0.050
	I	0.39	1.6	0.23	0.033	0.035
Ligated:	$s^{(A-1)}$	0.0130	0.0171	-----	------	0.049
	I	1.13	2.7	-----	------	0.049

a, $s = 2\lambda^{-1} \sin \psi/2 = 2\lambda^{-1} \sin\theta$
b, I = relative intensity

The general agreement between the curves of Figure 1a and those of Figure 1b indicates that the gross quaternary structural changes, particularly the increase in separation of the two c_3 units upon ligation of the enzyme with PALA, are similar at pH 5.8 and pH 8.3. Particular attention is called to the major feature of the experimental curves, the shift on ligation of the first maximum from 0.0185 A^{-1} to 0.0162 A^{-1}, and to the lesser feature, the shoulder at 0.03 A^{-1} which disappears in the ligated form at pH 8.3. These features are very similar in the calculated curves at pH 5.8 (Table 1 and Figure 1a).

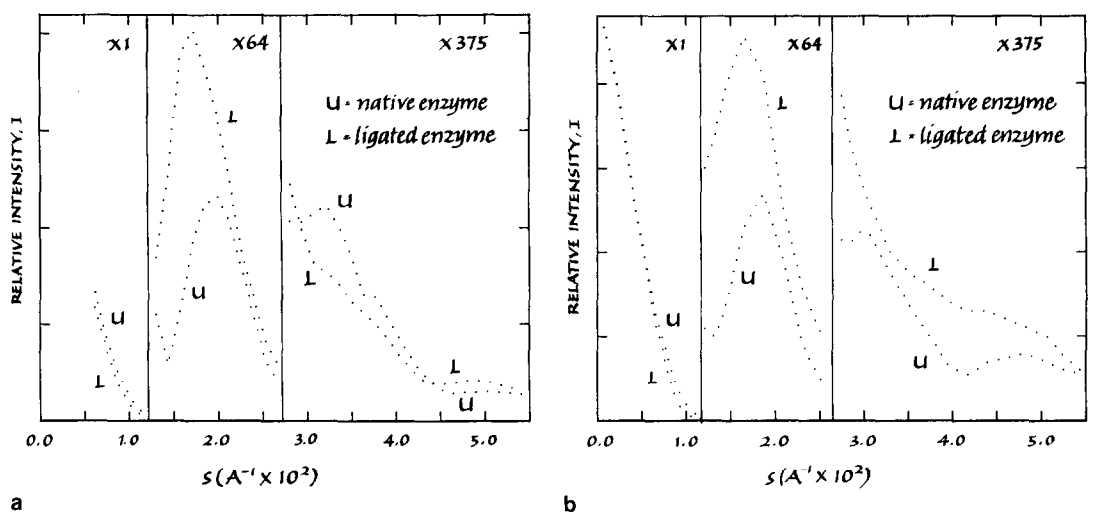

Figure 1. (a) Calculated solution scattering curves, pH 5.8.
(b) Experimental solution scattering curves, pH 8.3.

Further study may resolve the small differences, such as the systematic shift attributed above to solvent, and the relative intensities of ligated and unligated curves in the region from 0.03 A^{-1} to 0.055 A^{-1} in s (Figures 1a and 1b). Systematic studies of the effects of temperature factors, molecular occupancy, omission of partial electron density, and variation of unit cell dimensions change the relative positions of the calculated curves very little. Although we anticipate changes in tertiary structure, we believe it more likely that there is some ambiguity in the relative backgrounds of the two experimental curves in the region of magnification by a factor of 375. However, these small differences do not modify our major conclusion of the previous paragraph.

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