Crystallographic analysis of the three-dimensional structure of baboon α -lactalbumin at low resolution

Homology with lysozyme

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The crystal structure of baboon α -lactalbumin has been determined at 6 Å and at 4.5 Å (0.6 nm and 0.45 nm) resolution by the method of isomorphous replacement. The principal derivative was prepared by reducing a disulphide bridge in the crystals and inserting a mercury atom. Detailed comparison of the electron-density maps with corresponding maps of hen egg-white lysozyme shows that they are closely similar, with correlation coefficients of 0.57 and 0.44 at 6 Å and 4.5 Å resolution respectively. This result, in accordance with earlier predictions based upon comparisons of amino-acid sequences, provides further evidence that class C lysozymes and α -lactalbumins are homologous proteins and it is in keeping with the hypothesis that the α -lactalbumins evolved from a lysozyme precursor.

INTRODUCTION

α -Lactalbumin (α -LAC) has been much studied as an important constituent of milk and for three particular reasons. First, as shown by Ebner *et al.* (1966), it is one of the two protein components of the lactose synthetase complex (UDP-galactose:D-glucose 4- β -D-galactosyl-transferase, EC 2.4.1.22). In this complex, α -LAC acts to modify the specificity of the enzyme galactosyltransferase (EC 2.4.1.38) so that the transferase (GT), which by itself catalyses galactosyl transfer to *N*-acetylglucosamine (GlcNAc) or to terminal GlcNAc residues of glycoproteins, specifies instead the synthesis of lactose by galactosyl transfer to acceptor glucose (Brew *et al.*, 1968). Second, α -LAC is a metalloprotein with binding sites for calcium (Hiraoka *et al.*, 1980) and zinc (Murakami & Berliner, 1983). The binding of these ions probably controls the conformation of α -LAC and its association with GT, which is also a metalloprotein (Musci & Berliner, 1985). Third, α -LAC is chemically very similar to hen egg-white lysozyme (Brew & Campbell, 1967) and comparisons of amino-acid sequences (e.g. Brew *et al.*, 1970; Findlay & Brew, 1972), gene sequences (Hall *et al.*, 1982) and the exon–intron organization of the genes (Qasba & Safaya, 1984) have established with some certainty that the two proteins are homologous, having evolved by divergence from a common ancestor. A plausible account of the evolution of α -LAC and the origins of lactation has been given by Hayssen & Blackburn (1985).

Deeper understanding of the activity and evolution of α -LAC depends upon knowledge of its three-dimensional structure and, for this reason, attempts to determine the

structure have been made for many years. When the chemical resemblance between hen egg-white lysozyme (HEWL) and bovine α -LAC was first established, efforts were made to derive the structure of the α -LAC by analogy from the known structure of HEWL (Blake *et al.*, 1965), first by model building alone (Browne *et al.*, 1969) and subsequently by model building combined with energy refinement (Warne *et al.*, 1974). These studies demonstrated that the amino-acid sequence of bovine α -LAC is consistent with a three-dimensional structure closely resembling HEWL. α -LACs from different mammalian species (Table 1) have similar sequences: 35 of the residues are invariant in those sequences that are known in detail and 15 of these are identical with the corresponding residues in HEWL, when structurally reasonable insertions and deletions are included to maximize the homology.

Direct X-ray crystallographic studies of the three-dimensional structure of α -LAC were begun many years ago but have been frustrated, first by the difficulty of finding amenable crystals to study (Aschaffenburg *et al.*, 1972*a,b*; Fenna, 1982*a*) and, subsequently, by the difficulty of preparing isomorphous heavy-atom derivatives for use in the multiple-isomorphous-replacement method of X-ray structure analysis. Baboon (*Papio cynocephalus*) α -LAC, which has an amino-acid sequence closely similar to that of human α -LAC shown in Table 1 (R. Greenberg, personal communication), was the first species found to give suitable crystals for X-ray analysis (Aschaffenburg *et al.*, 1979). Subsequently, conditions were found (Fenna, 1982*b*) under which human α -LAC gives crystals that are isomorphous with those from baboon. We report here the first results, from studies of

Abbreviations used: α -LAC, α -lactalbumin; HEWL, hen egg-white lysozyme; GT, galactosyltransferase; DTT, dithiothreitol.

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Table 1. Amino-acid sequences of various lysozymes and α -lactalbumins

Lysozymes (LYS): B, baboon (Hermann *et al.*, 1973); h, human (Canfield *et al.*, 1971; Jolles & Jolles, 1972); C, chicken (Canfield, 1963). α -Lactalbumins (α -L): h, human (Hall *et al.*, 1982); g, guinea pig (Hall *et al.*, 1982); b, cow (Shewale *et al.*, 1984). Disulphide bridges are 6-127, 30-115, 64-80 and 76-94.

	LYS	α -L		LYS	α -L	
	BhC	hgb		BhC	hgb	
1	KKK	KKE	1	66	DDD SSN	63
2	IVV	QQQ	2	67	GGG SSD	64
3	FFF	FLL	3	68	KKR QTQ	65
4	EEG	TTT	4	69	TTT VTD	66
5	RRR	KKK	5	70	PPP PVP	67
6	CCC	CCC	6	71	GGG QQH	68
7	EEE	EAE	7	72	AAS SSS	69
8	LLL	LLV	8	73	VVR RRS	70
9	AAA	SSF	9	74	NNN NNN	71
10	RRA	QHR	10	75	AAL III	72
11	TTA	LEE	11	76	CCC CCC	73
12	LLM	LLL	12	77	HHN DDN	74
13	KKK	KNK	13	78	ILI III	75
14	RRR	---		79	SSP SSS	76
15	LLH	---		80	CCC CCC	77
16	GGG	DDD	14	81	NSS DDD	78
17	LML	ILL	15	82	AAA KKK	79
18	DDD	DAK	16	83	LLL FLF	80
19	GGN	GGG	17	84	LLL LLL	81
20	YYY	YYY	18	85	QQS DDD	82
21	RRR	GRG	19	86	DDS DDD	83
22	GGG	GDG	20	87	NND DDD	84
23	IIV	IIV	21	88	III ILL	85
24	SSS	ATS	22	89	TAT TTT	86
25	LLL	LLL	23	90	DDA DDD	87
26	AAG	PPP	24	91	AAS DDD	88
27	NNN	EEE	25	92	VVV III	89
28	WWW	LWW	26	93	AAN MMM	90
29	VMV	ILV	27	94	CCC CCC	91
30	CCC	CCC	28	95	AAA AVV	92
31	LLA	TIT	29	96	KKK KKK	93
32	AAA	MIT	30	97	RRK KKK	94
33	KKK	FFF	31	98	VVI III	95
34	WWF	HHH	32	99	VVV LLL	96
35	EEE	TIT	33	100	SRS ---	
36	SSS	SSS	34	101	DDD DDD	97
37	DGN	GGG	35	102	PPG IIK	98
38	YYF	YYY	36	103	QQD KKV	99
39	NNN	DDD	37	104	GGG GGG	100
40	TTT	TTT	38	105	IIM III	101
41	QRQ	QQE	39	106	RRN DDN	102
42	AAA	AAA	40	107	AAA YYY	103
43	TTT	III	41	108	WWW WWW	104
44	NNN	VVV	42	109	VVV LLL	105
45	YVR	EKQ	43	110	AAA AAA	106
46	NNN	NNN	44	111	WWW HHH	107
47	PAT	---		112	RRR KKK	108
	GG-	---		113	NNN APA	109
48	DDD	NSN	45	114	HRR LLL	110
49	QRG	EDD	46	115	CCC CCC	111
50	SSS	SHS	47	116	QQK TSS	112
51	TTT	TKT	48	117	NNG EDE	113
52	DDD	EEE	49	118	RRT KKK	114
53	YYY	YYY	50	119	DDD LLL	115
54	GGG	GGG	51	120	VVV EED	116
55	III	LLL	52	121	SRQ QQQ	117
56	FFL	FFF	53	122	QQA ---	
57	QQQ	QQQ	54	123	YYW WWW	118
58	III	III	55	124	VVI LYL	119
59	NNN	SNN	56	125	QQR ---	
60	SSS	NDN	57	126	GGG ---	
61	HRR	KKK	58	127	CCC CCC	120
62	YYW	LDI	59		--- EEE	121
63	WWW	WFW	60	128	GGR KAK	122
64	CCC	CCC	61	129	VVL LQL	123
65	NNN	KEK	62			

the baboon α -LAC crystals, which show that the three-dimensional structures of α -LAC and HEWL are closely similar.

EXPERIMENTAL

Crystallization of α -LAC

The preparation and crystallization of baboon α -LAC have been described by Aschaffenburg *et al.* (1979). The crystals, from 45% saturated $(\text{NH}_4)_2\text{SO}_4$ and phosphate buffer (pH 6.8), are diamond-shaped plates in the orthorhombic spheroidal class with unit-cell dimensions $a = 35.5 \text{ \AA}$, $b = 69.1 \text{ \AA}$, $c = 46.1 \text{ \AA}$. (Note: distances in this paper are given in $\text{\AA}$; $1 \text{ \AA} = 0.1 \text{ nm}$.) The space group is P2_12_12 and there is one molecule of α -LAC per asymmetric unit.

Heavy-atom derivatives

K_2PtBr_4 . An extensive search for heavy-atom derivatives was conducted by soaking crystals of baboon α -LAC in heavy-atom solutions in 0.1 M-phosphate buffer, 50% saturated with $(\text{NH}_4)_2\text{SO}_4$. Resultant cell-dimension and intensity changes were monitored by means of $hk0$ and $0kl$ precession photographs. The outcome was disappointing: only K_2PtBr_4 gave promising results although $\text{K}_2\text{Au}(\text{CN})_2$ in one experiment gave small intensity changes and K_2HgI_4 was apparently incorporated, giving well-ordered but non-isomorphous crystals.

K_2PtCl_4 was the first Pt salt to be tried because it has been used successfully in many analyses, even in the presence of $(\text{NH}_4)_2\text{SO}_4$ which leads to the formation of ionic species with reduced reactivity with protein ligands (Petsko *et al.*, 1978). Even at low concentrations of chloroplatinite, however, the baboon α -LAC crystals were rapidly etched and cracked and conditions were not found that led to a stable derivative. K_2PtBr_4 was tried in the expectation that it would be less reactive with both ammonium ions and protein. Although the crystals prepared by soaking in a 1 mM solution for 24 h were more fragile than native crystals they were stable enough for data collection and gave promising intensity changes. These crystals were difficult to handle and to reproduce but they were the only ones prepared by the soaking method to promise a useful derivative.

Mercury. When the normal soaking method gave such disappointing results a different approach was tried, prompted by the observation of Schechter *et al.* (1973) that a mercury atom can be inserted in the disulphide bridge linking residues 6 and 120 in bovine α -LAC, and by the finding of Ely *et al.* (1973), in their studies of a Bence-Jones protein, that such a reaction can be accomplished in the crystalline state. Baboon α -LAC crystals were soaked in deaerated mother liquor containing 2 mM-DTT for 24 h under an atmosphere of N_2 . The crystals were then transferred to DTT-free mother liquor for 2 min before the addition of mercury(II) acetate to a concentration of 20 mM and they were kept in this medium in the dark and under N_2 for 48 h. Finally, they were washed and back-soaked in mother liquor for 3 h before being mounted in the usual way in capillary tubes for X-ray examination. Precession photographs of the $h0l$ and $0kl$ zones showed that the unit-cell dimensions were essentially unaffected by this treatment but that the intensities of many reflections were

Table 2. Heavy-atom parameters

X , Y and Z are fractional atomic co-ordinates; B is the temperature factor ($\text{e} \cdot \text{\AA}^{-2}$), and

$$R = \frac{\sum |F_{\text{PH}} - F_{\text{P}}| - |F_{\text{H}}|}{\sum |F_{\text{PH}} - F_{\text{P}}|}$$

where F_{PH} and F_{P} are the derivative and native-protein structure amplitudes and F_{H} is the calculated heavy-atom contribution.

Derivative	Occupancy	X	Y	Z	B	R
6 $\text{\AA}$ resolution						
Hg	0.88	0.836	0.927	0.885	15	0.072
Pt	0.38	0.066	0.337	0.465	15	0.055
2.7 $\text{\AA}$ resolution						
Hg	0.75	0.839	0.937	0.881	11	0.102
Pt	0.34	0.067	0.336	0.456	19	0.091

significantly changed. Experiment showed that these intensity changes increased with time of soaking in DTT, whereas soaking crystals in mercury acetate solution without prior treatment with DTT gave no intensity changes. This derivative provided the key to the structure analysis.

X-ray data collection and processing

All of the X-ray measurements were made on a Hilger and Watts Y290 diffractometer, in the symmetrical A setting, with a scintillation counter and an Elliot Automation sealed X-ray tube generating Cu radiation and operated at 40 kV and 30 mA. The α -LAC crystals were mounted for rotation about the c -axis and measurements were made by the ordinate-analysis procedure of Watson *et al.* (1970). Data were collected from two native crystals, one Hg derivative crystal and two Pt derivative crystals (PT1 and PT2). Absorption corrections were applied by the method of North *et al.* (1968). Irradiation-damage corrections were based upon repeated measurement of three reference reflections: they were small and varied linearly with exposure time.

Heavy-atom parameters

Analysis of the derivatives was conducted in two stages, first to 6 $\text{\AA}$ and then to higher resolution. At 6 $\text{\AA}$ resolution both the Hg and Pt derivatives gave interpretable difference-Patterson maps indicating single sites of substitution. The Hg parameters derived from the Patterson map together with the scale factor and occupancy were improved by centric refinement and used to calculate preliminary phases (figure-of-merit 0.74). These phases were used to calculate a difference-electron-density map of the Pt derivative which confirmed the Pt position and revealed no significant minor sites. Subsequent F_{HLE} refinement (Blundell & Johnson, 1976) of the Hg derivative and phased refinement of both derivatives gave the heavy-atom parameters shown in Table 2. Temperature factors were not refined. Phases calculated with these parameters by the standard method (Blow & Crick, 1959; North, 1965) had a mean figure of merit at 0.87.

In an assessment of the derivatives at 2.7 $\text{\AA}$ resolution the data from PT2 were first kept separate and the parameters for Hg, PT1 and PT2 were refined independently by the F_{HLE} method. The results indicated that

PT2 was less isomorphous with the native than PT1 and that it had a lower occupancy. Nevertheless, in the next stage the Pt data sets were combined, with the omission of data in the range 4.4–2.7 $\text{\AA}$ from PT2, and parameters refined by the F_{HLE} method were used in a final phased refinement. The resultant heavy-atom parameters were closely similar to those obtained at 6 $\text{\AA}$ resolution though refinement of isotropic temperature factors gave values of 11 and 19 $\text{\AA}^2$ for Hg and Pt respectively and the apparent occupancies were reduced to 0.75 and 0.34. The phases calculated to 2.7 $\text{\AA}$ resolution had an overall mean figure-of-merit of 0.55.

Analysis of these high-resolution results showed clearly, however, that the phases beyond about 4.5 $\text{\AA}$ resolution were unsatisfactory, with mean figure-of-merit less than 0.4. The Pt contribution to phasing is negligible and for the Hg derivative both the isomorphous and anomalous contributions to F_{H} are less than the associated errors throughout this range. Overall the Pt derivative suffered from a low level of substitution and poor isomorphism and crystal PT2 was inadequate. Given the way in which it was prepared, the Hg derivative is inevitably non-isomorphous with the native crystals and it was encouraging to discover that it gave good phase information, at least over the low-resolution range.

RESULTS

Electron-density maps of baboon α -LAC were calculated at 6 $\text{\AA}$, 4.5 $\text{\AA}$ and, despite the poor quality of the high-angle phases, at 2.7 $\text{\AA}$ resolution. At 6 $\text{\AA}$ resolution the electron density corresponding to a single α -LAC molecule was clearly distinguishable from solvent and included strong rod-like features of the kind associated with α -helices. A solid model made of balsa wood and representing the electron density above about $0.5\text{e} \cdot \text{\AA}^{-3}$ was constructed from this electron-density map for comparison with a corresponding model of HEWL. Inspection of the two models (Fig. 1) showed immediately that the electron-density maps of the two proteins are closely similar. Examination of the maps and models also showed that the site of mercury substitution is in the region corresponding to the disulphide bridge I–VIII, between residues 6 and 127 in HEWL or 6 and 120 in α -LAC (Table 1). The site of platinum binding appears,

Fig. 1. Stereo photographs of solid models of the electron density greater than about $0.5e \cdot \text{\AA}^{-3}$ at 6 Å resolution (a) in hen egg-white lysozyme and (b) in baboon α -lactalbumin

View is from opposite side of molecule to active-site cleft. Numbers indicate the approximate locations of amino-acid residues: the effect of the deletion in α -LAC corresponding to residues 14 and 15 in HEWL is evident.

not unexpectedly, to be near residue 93 (HEWL) which corresponds to methionine-90 in human and probably in baboon α -LAC.

Detailed comparison of the two structures requires analysis at higher resolution but, as expected from the analysis of the phases, the electron-density map of baboon α -LAC at 2.7 Å resolution was of poor quality and did not provide a satisfactory basis for the immediate determination of the structure in detail. But it was not totally uninterpretable. The general course of the main polypeptide chain could be followed throughout most of the molecule and, in particular, helical density, albeit poorly defined, was evident in the regions corresponding to residues 24–36, 80–85 and 88–95 in HEWL and fairly good electron density was also found for the disulphide bridges 30–115, 64–80 and 76–94 (HEWL) but not for 6–127, presumably because of the disturbance to this part of the structure brought about by the incorporation of mercury in the major derivative.

Atomic co-ordinates for parts of the α -LAC molecule were derived from a partial interpretation of the 2.7 Å map and these were used to provide the starting point for

a more systematic comparison of α -LAC and HEWL at lower resolution. The objective was to compare the electron-density maps of the two structures by superimposing them in such a way as to minimize the differences between them within the molecular boundaries.

Corresponding points in the two structures, x and x' , may be related by the equation:

$$x' = Cx + d \quad (1)$$

where C represents a rotation matrix and d a vector translation. The rotation matrix and translation that gave the best fit between the two electron densities were derived by the least-squares procedure of Cox (1967), starting with values determined from comparison of the partial model of α -LAC and the known structure of HEWL (Imoto *et al.*, 1972). The progress of the refinement was monitored by calculation of the residual:

$$R = \Sigma | \rho_{\text{HEWL}} - \rho_{\alpha\text{-LAC}} | / \Sigma | \rho_{\text{HEWL}} + \rho_{\alpha\text{-LAC}} | \quad (2)$$

and the correlation coefficient:

$$C = \Sigma(\rho_{\text{HEWL}} \times \rho_{\alpha\text{-LAC}}) / (\Sigma \rho_{\text{HEWL}}^2 \times \Sigma \rho_{\alpha\text{-LAC}}^2)^{1/2} \quad (3)$$

Fig. 2. Electron density in baboon α -LAC and HEWL at 6 Å resolution with superimposed C^α positions from HEWL

(a) Lower half of molecules, with $z = 0$ –19.1 Å in HEWL unit cell (Imoto *et al.*, 1972); (b) upper half of molecules, $z = 18.8$ –37.9 Å. Contours at equal and arbitrary intervals from about $0.5e \cdot \text{\AA}^{-3}$.

Fig. 3. Electron density in baboon α -LAC and HEWL at 4.5 Å resolution with C α positions from HEWL superimposed (a) Lower half of molecules; (b) upper half. W indicates the side-chain density for residue tryptophan-108.

where ρ_{HEWL} and $\rho_{\alpha\text{-LAC}}$ are corresponding electron-density values in the maps of HEWL and α -LAC.

Use of these measures is meaningful only if the electron densities are on the same scale and this was achieved by making $\Sigma\rho_{\text{HEWL}} = \Sigma\rho_{\alpha\text{-LAC}}$ within the molecular boundaries.

The electron-density maps of baboon α -LAC and HEWL (Blake *et al.*, 1965) were compared in this way at 6 Å and at 4.5 Å resolution. At 6 Å resolution approximately 4000 grid points were used to define each of the electron densities and the initial rotation and translation, derived from the partial model, gave a residual of 0.54 and a correlation coefficient 0.43. Seven cycles of refinement showed that the two electron densities are best related by:

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} \begin{bmatrix} -0.979 & 0.109 & 0.169 \\ -0.201 & -0.500 & -0.842 \\ -0.007 & -0.859 & 0.512 \end{bmatrix} + \begin{bmatrix} 28.27 \\ 43.99 \\ 19.78 \end{bmatrix} = \begin{bmatrix} X' \\ Y' \\ Z' \end{bmatrix} \quad (4)$$

with $R = 0.48$ and $C = 0.57$

The two electron-density maps, with superimposed HEWL co-ordinates, are shown in the same orientation in Fig. 2. The common orientation is the familiar one of HEWL so that the map of α -LAC has been rotated by the inverse of the matrix defined in eqn. (4).

At 4.5 Å resolution, comparison of the two electron densities was conducted at finer intervals over about 6000 grid points and nine cycles of refinement gave the relationship:

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} \begin{bmatrix} -0.981 & 0.130 & 0.140 \\ -0.186 & -0.471 & -0.861 \\ -0.046 & -0.872 & 0.487 \end{bmatrix} + \begin{bmatrix} 28.17 \\ 43.57 \\ 21.22 \end{bmatrix} = \begin{bmatrix} X' \\ Y' \\ Z' \end{bmatrix} \quad (5)$$

with $R = 0.54$ and $C = 0.44$. The two electron-density maps are shown in Fig. 3, again in the HEWL orientation with superimposed lysozyme co-ordinates. Inspection shows that they are closely similar.

DISCUSSION

Detailed comparison of the structures of α -LAC and lysozyme must await completion of the structure analysis at high resolution that is now in progress, but some features of interest are already clear. In particular, there are good indications of the relationship between α -LAC and HEWL in regions of the molecule that were difficult to predict with confidence by model building and energy refinement (Browne *et al.*, 1969; Warne *et al.*, 1974). In the following discussion the numbers of residues in HEWL are quoted; corresponding numbers in α -LAC can be derived from Table 1.

In HEWL, residues 5–16 form an α -helix with residue 6 participating in a disulphide bridge with residue 127. The mercury of the principal heavy-atom derivative is incorporated in this bridge so that the electron density in the vicinity might be expected to be affected by the local lack of isomorphism between the native and derivative structures. Nevertheless, the electron densities at both 6 Å (Figs. 1 and 2) and 4.5 Å resolution (Fig. 3) clearly show the presence of an α -helix in α -LAC in this region of the molecule. Residues 14 and 15 are deleted in α -LAC and the predicted structures accommodated this by a shortening of the helix and small adjustments of the conformation on either side of the deleted residues. The observed electron densities are fully consistent with this prediction, as appears particularly clearly in Fig. 1.

Residues 24–36 form an α -helix in HEWL and the electron densities indicate that the corresponding residues form a very similar helix in α -LAC. This resolves a difficulty that was noted by Browne *et al.* (1969) and discussed further by Warne *et al.* (1974). Maximum correspondence between the amino-acid sequences of bovine α -LAC and HEWL is achieved by deleting residue 33 and inserting an extra residue after 35 in α -LAC. This observation, together with the fact that the sequence for residues 31–35 is not helix-forming in α -LAC, suggested that this part of the α -LAC structure might be non-helical and differ radically from HEWL. Conservatively, however, the helical conformation was retained in the predicted structures and the electron densities now observed suggest strongly that this judgement was correct.

Residue 47, which is the most exposed to solvent in HEWL and forms part of an irregular β -turn, is deleted in α -LAC. In the predicted structures this was accommodated by retaining the adjacent anti-parallel β -pleated sheet and forming a tighter β -turn with three peptide groups rather than four. The present maps, which show residue 47 in HEWL lying outside the α -LAC densities, suggest that this conservative approach was correct.

Residue 100 is deleted in α -LAC and again the observed electron densities suggest that this can be accommodated by small adjustments of the HEWL conformation, in accordance with the predictions. The electron densities for the following residues to 110 are weaker in α -LAC than in HEWL, but it is interesting to note that density is present for the side chain of Trp-108 at 4.5 Å resolution (Fig. 3b), indicating that this conserved indole group occupies essentially the same position in the two structures. This residue is a part of the so-called hydrophobic box in HEWL which n.m.r. studies (Koga & Berliner, 1985) have shown to be conserved in α -LAC.

In the model-building and energy-refinement studies, the greatest difficulty was experienced in predicting the structure of the C-terminus of α -LAC: neither Browne *et al.* (1969) nor Warne *et al.* (1974) were able to suggest a unique conformation for this part of the molecule. The electron densities for residues 115 to the end are weaker and less continuous for α -LAC than for HEWL at both 6 Å and 4.5 Å resolution and, apart from suggesting a more extended conformation, they do not reveal the structure. It seems unlikely, however, that the short helix formed by residues 119–124 in HEWL is retained in α -LAC.

Overall, the electron density distributions in baboon α -LAC, at 6 Å and at 4.5 Å resolution, strongly support the main conclusions of Browne *et al.* (1969) and Warne *et al.* (1974) that α -LAC and HEWL have closely similar three-dimensional structures and that the differences between their amino-acid sequences are accommodated with minimal disturbance of the lysozyme conformation.

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