Volume 247, number 2, 361-366 FEB 07062 April 1989

Structural consequences of a one atom mutation on aspartate transcarbamylase from *E. coli*

J. Cherfils, R.M. Sweet†, S.A. Middleton°, E.R. Kantrowitz°, P. Tauc+ and P. Vachette*

*Laboratoire de Biologie Physicochimique, Bât. 430 and *LURE, Bât. 209d, Université Paris-Sud, 91405 Orsay, France, †Department of Biology, Center for Structural Biology, Brookhaven National Laboratory, Upton, LI 11973, °Department of Chemistry, Boston College, Chestnut Hill, MA 02167, USA* and *+Laboratoire d'Enzymologie, CNRS, 91190 Gif-sur-Yvette, France*

Received 8 February 1989

Tyr-240 of the catalytic chain of aspartate transcarbamylase from *E. coli* has been substituted by Phe using site-directed mutagenesis. The regulatory mechanisms of the mutant enzyme have been shown to be slightly less effective than the wild-type enzyme [1]. A study of the structural consequences of the mutation using solution X-ray scattering and computer simulations is reported here. No significant change from the wild-type enzyme is detectable in the quaternary structure. Simulations suggest that the only effect of the mutation is an increased mobility of the mutated side chain.

Aspartate transcarbamylase; Single amino acid mutation; X-ray solution scattering; Energy minimization; Computer graphics

1. INTRODUCTION

Aspartate transcarbamylase (ATCase) from *E. coli* catalyzes the first step of pyrimidine biosynthesis, the condensation of carbamyl phosphate and L-aspartate to *N*-carbamylaspartate. It shows homotropic cooperativity for aspartate, is feedback inhibited by CTP, and activated by ATP. This extensively studied allosteric enzyme is composed of two trimers of catalytic (c) chains carrying the active sites, and three dimers of regulatory (r) chains carrying the effector binding sites (see [2] and references therein).

Two functional and structural states have been well characterized by a variety of techniques: a less active T state, stabilized by CTP, and a more active R state stabilized by the substrates or their analogues, for instance PALA.

The crystal structures of unligated [3], CTP-ligated [4] and PALA-ligated [5] ATCases have been determined at high resolution. In the transition from T to R, the enzyme expands by 12 Å along the three-fold axis while subunits and domains undergo rotation movements. This quaternary rearrangement is accompanied by tertiary changes in the chains.

The loop at residues 230–245 of the c chains shows one of the largest changes. In particular, residue Tyr-240 in this loop is involved in two different types of interactions in the T and R forms. In T, its hydroxyl group gives an internal H-bond to the side chain of Asp-271 (fig.1a); this H-bond is broken in R as these two residues move 10 Å apart. In this form, the two phenyl rings of residues Tyr-240 from opposing catalytic chains are close enough to interact [6] (fig.1b).

In order to determine the role of the H-bond in the allosteric transition, site-directed mutagenesis was used to substitute a phenylalanine for tyrosine at position 240. This mutation removes a single ox-

Correspondence address: J. Cherfils, Laboratoire de Biologie Physicochimique, Bâtiment 430, Université Paris-Sud, 91405 Orsay, France

Abbreviations: ATCase, aspartate transcarbamylase (EC 2.1.3.2); Tyr-240→Phe, mutant enzyme; PALA, *N*-phosphonacetyl-L-aspartate; rms, root mean square

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

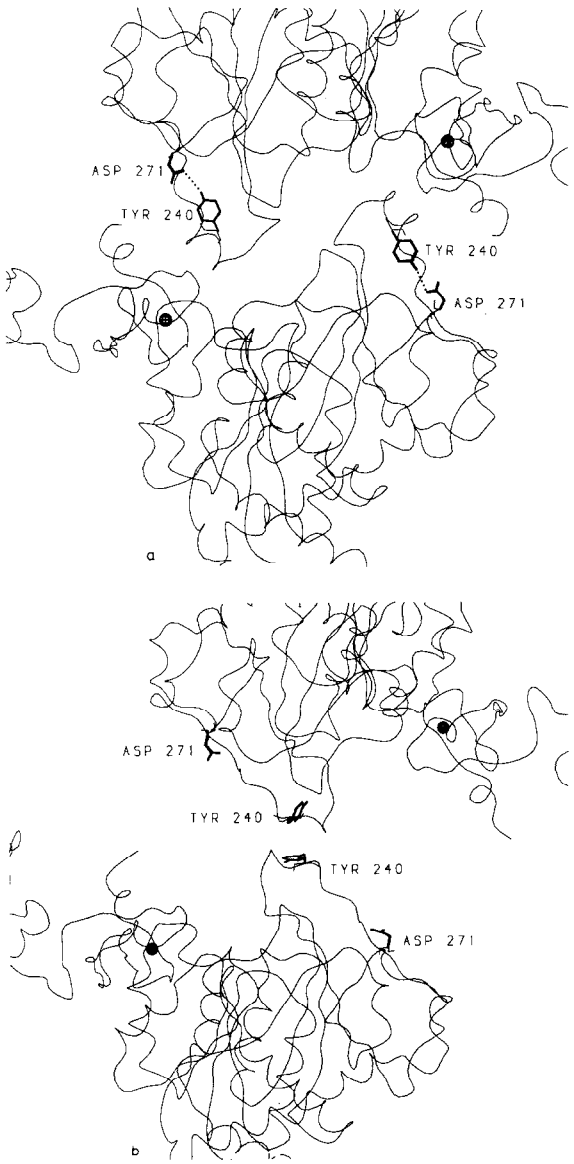

Fig.1. View of the c1–c4 interface between top and bottom halves of aspartate transcarbamylase (a) in the unligated T form [1] and (b) in PALA-ligated R form [3]. Spheres indicate the location of the structural Zn^{2+} of regulatory chains. In the T form, the 240's loop is in contact with both catalytic and regulatory chains of the opposite half of the molecule, whereas in the R form only the c1–c4 contact remains. The side chains of residues 240 and 271 are shown with heavy lines. H-bonds found in the T form are shown by dotted lines.

ygen atom. The mutant enzyme shows reduced cooperativity and increased affinity for aspartate, but no change in maximal specific activity; heterotropic interactions are only slightly altered [1].

We present here a study of the structural consequences of this mutation both at the global and at the local level. Solution X-ray scattering was used to investigate the quaternary structure of the mutant enzyme. The solution X-ray scattering curve of ATCase has been shown to undergo major changes upon addition of substrates, which reflect the transition from T to R [7,8]. It constitutes a sensitive and specific probe of the quaternary structure of the enzyme. Comparative modelling using computer graphics and energy minimization calculations is an appropriate approach to a structural study of the mutation at the residue level in both T and R forms [9]. Simulations of structural changes in the T form are compared to the crystal structure of the unligated mutant enzyme which has recently been determined [10].

2. MATERIALS AND METHODS

2.1. *Solution X-ray scattering*

The expression and purification of Tyr-240→Phe ATCase has been described in [1]. Samples were prepared from a stock solution in 50 mM Tris-borate buffer, pH 8.3, 0.1 mM EDTA and 0.1 mM dithiothreitol as in [8]. X-ray scattering curves were recorded on the small angle scattering instrument using synchrotron radiation at LURE-DCI, Orsay. The instrument is described in [11], the data acquisition system in [12]. Experimental procedures have been reported in [8].

2.2. *Molecular graphics and simulations*

Crystallographic coordinates of the unligated T-form [3], the CTP-ligated T-form [4] and PALA-ligated R-form [5] enzymes have been used in separate simulations as starting points for modelling the Tyr-240→Phe structure (see table 1). In these crystal structures, catalytic chains c1 and c4 and associated regulatory chains r1 and r4 are related by approximate two-fold symmetry. The structure of the mutant was obtained by removing the hydroxyl oxygen from residue Tyr-240.

Two series of simulations have been performed. The first one used the BRUGEL package [13] running on a VAX-780, with all hydrogens included. Its potential energy function contains an explicit term for H-bonds. The other one used the CHARMM package running on a STAR-100 array computer [14] with only polar hydrogens explicitly represented.

Parallel pairs of energy minimizations were performed in vacuo for wild type and mutant structures for T and R forms of the enzyme. For the calculations, only atoms within a certain distance of Cα-240 of c chains were kept. As this residue is located near the c1–c4 and c1–r4 interfaces (fig.1a and b), a subset of the c1, c4, r1 and r4 chains was selected for the simulations (table 1). Individual segments produced by the truncation on the outer boundary were maintained by harmonic constraints.

All starting energies were positive except for the CTP-ligated structure. After 700 steps of energy minimization, the energy per residue for the optimized structures was about -3.5 kcal/residue using BRUGEL and -32 kcal/residue using CHARMM. The difference is mostly due to electrostatic interactions having much larger values in CHARMM. Coordinates for wild type and Tyr-240→Phe optimized structures were compared with the graphics software MANOSK implemented on an Evans and Sutherland PS300 [15]. Superimpositions of c1 and c4 chains were performed with the least square fitting program EZIFIT [16]. The root mean square displacements were about 0.4 Å using BRUGEL and 0.5–0.8 Å using CHARMM.

3. RESULTS

3.1. *X-ray scattering*

X-ray scattering spectra of both the wild-type and mutant enzymes were recorded in the absence of substrates as well as in the presence of a saturating amount of PALA. Fig.2 shows that the scattering curves of the wild type and the mutant are practically identical. Other patterns were recorded using several combinations of substrate analogues such as acetyl phosphate and aspartate or carbamyl phosphate and succinate: in each case, the curves were the same for both enzymes. Only in the following experiment did the mutant behave differently than the wild-type enzyme. A subsaturating amount of PALA was added so as to bring the curve about half-way between the T and the R patterns; the subsequent addition of ATP significantly shifted the curve of the Tyr-240→Phe ATCase towards the R pattern (fig.3a) while no detectable effect was observed in the case of the wild-type enzyme (fig.3b). With CTP, a shift towards the T curve was observed, as with the wild type (fig.3).

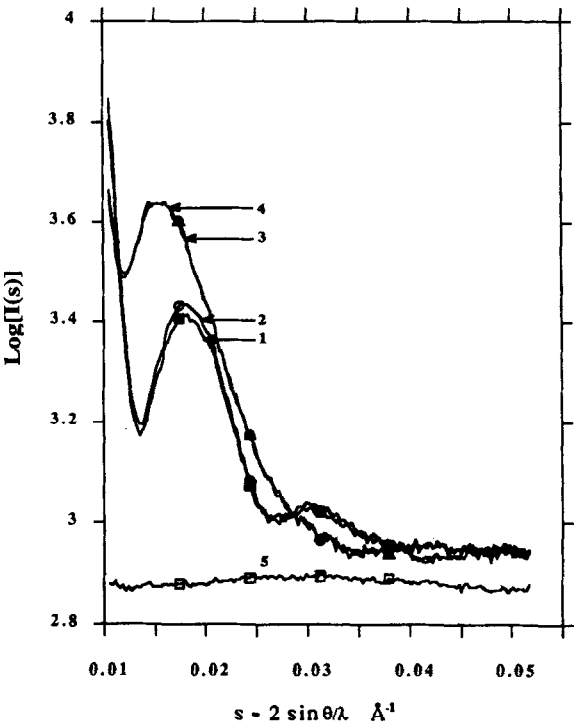

Fig.2. Solution X-ray scattering spectra of wild-type ATCase and Tyr-240-Phe ATCase. (1) Unligated wild-type ATCase; (2) unligated Tyr-240→Phe ATCase; (3) wild-type ATCase in the presence of 2 M excess PALA; (4) Tyr-240→Phe ATCase in the presence of 2 M execess PALA; (5) background scattering of buffer.

3.2. *Energy minimization*

Minimizations were performed on the wild-type and Tyr-240→Phe structures, and the results were evaluated by superimposing the minimized coordinates, so as to identify differences due to the mutation from movements generated by the optimization process. The results are summarized in table 1. Root mean square differences between the wild-type and mutant structures were between 0.15 and 0.30 Å.

As three calculations were performed for the T form (unligated and CTP-ligated T forms studied with CHARMM, unligated T structure studied with BRUGEL) on structures containing c1 and c4 chains, a total of six copies of c chains was available for analysis in this form. In the same way, the two simulations for the R form (BRUGEL and CHARMM) yield four copies of c chains.

For the T form, differences larger than 3σ were restricted to residue 240 and close neighbours. The minimization process strengthens (unligated T form) or maintains (CTP-ligated T form) the H-bond between Tyr-240 and Asp-271. In all copies of c chains except one, the side chains of residues Phe-240 and Asp-271 of the mutant T structures move from the wild-type positions (fig.4). This is accomplished by a rotation around the $C\alpha$–$C\beta$ bond. In three copies, these deviations increase by 1 Å or more the distance between the two side chains.

On the other hand, the minimization process does not lead to any significant differences between

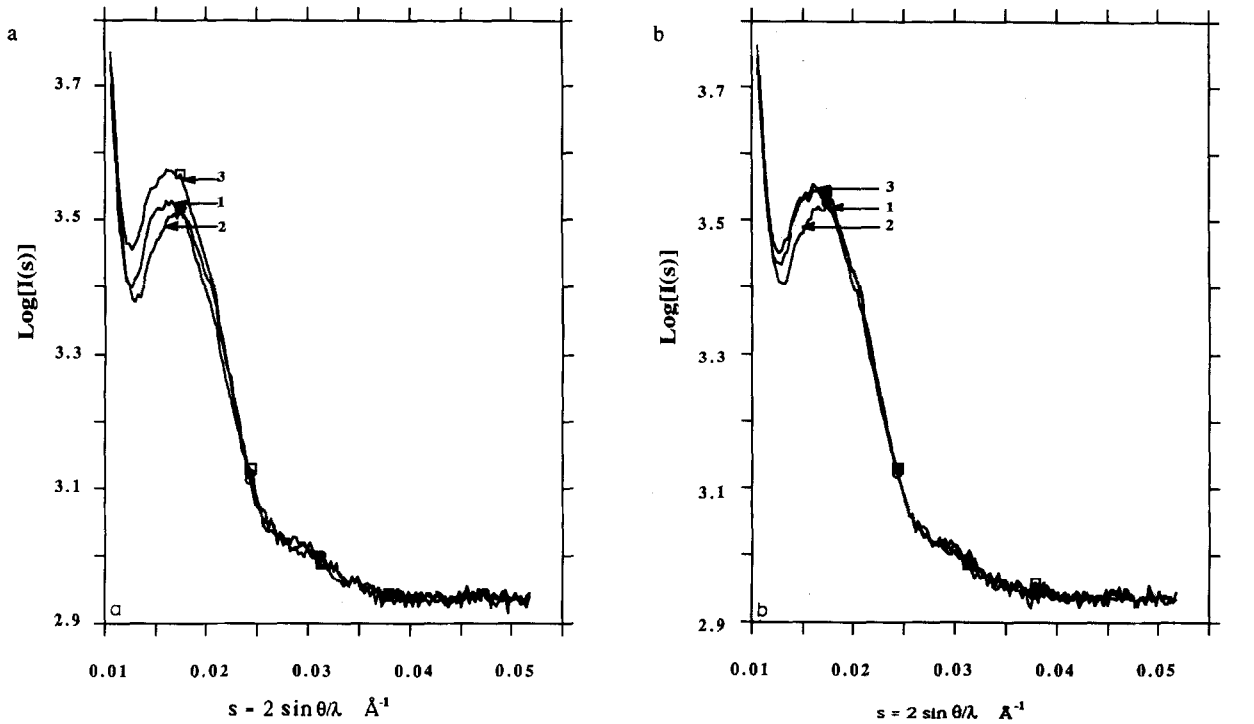

Fig.3. Effect of the nucleotide effectors on solution X-ray scattering curves of (a) Tyr-240→Phe ATCase and (b) wild-type ATCase in the presence of (1) 0.3 mol PALA/pmol active site; (2) 0.3 mol PALA + 10 mM CTP; (3) 0.3 mol PALA + 10 mM ATP.

Table 1

Results of energy minimizations

	T form			R form	
	Unligated		CTP ligated		
	BRUGEL	CHARMM	CHARMM	BRUGEL	CHARMM
Number of residues included in the simulation	300	414	414	162	414
Shifts between wild type and mutant enzyme (Å)					
rms shift (a)	0.15	0.18	0.18	0.15	0.30
Shift of Cζ 240 (b)					
c1 chains	0.92	0.41	1.66	0.18	0.53
c4 chains	0.51	0.91	1.02	0.34	0.55
Change of 240-271 distance (c)					
c1 chains	1.3	0.1	1.1	-	-
c4 chains	1.4	0.5	0.2	-	-
c1/c4 correlation coef. (d)	0.37	0.26	0.59	0.0	0.41

Shifts are calculated between wild-type and mutant superimposed coordinates. (a) Differences between wild-type and mutant r chains are negligible, therefore rms differences are computed for heavy atoms of c1 and c4 chains only. (b) Because the hydroxyl oxygen of Tyr-240 is not present in the Phe residue, the Cζ atom is chosen for comparisons between wild-type and mutant structures. This is also the atom where largest shifts occur. Only in the case of c1 chain of unligated T form optimized with CHARMM is the shift smaller than 3 times the rms shift. (c) Distances between Tyr-240/Phe-240 Cζ and the closest oxygen of Asp-271 side chain are compared. In the R form of the enzyme, residues 240 and 271 are not in contact (fig.1b). In the T form, the distance is larger in three copies of mutant catalytic chains and of comparable amplitude in the others. (d) The correlation coefficient is computed using the following formula:

$$r = \Sigma x_{i,1} \cdot x_{i,4} / (\Sigma_i [x_{i,1}]^2 \cdot \Sigma_i [x_{i,4}]^2)^{1/2}$$

$x_{i,1}$ is the vector connecting the ith atom of the c1 optimized wild-type structure to the same atom in the c1 optimized mutant structure. $x_{i,4}$ is the corresponding vector for the c4 chains after superimposition on the c1 chain

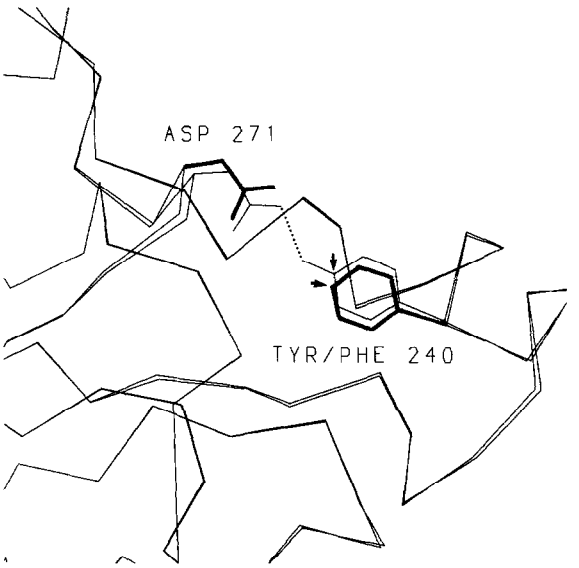

Fig.4. Superimposition of c1 chains of wild-type and Tyr-240→Phe ATCases in the unligated T form after energy minimization with BRUGEL. The 240 and 271 side chains of the mutant are shown with heavy lines. Arrows point to Cζ atoms of the rings. The H-bond in the wild-type structure is dotted.

the wild-type and mutant R structures. In both structures, the center to center distance between the phenyl rings of Tyr/Phe-240 at the c1–c4 interface remains essentially constant (6.5 Å), while the dihedral angle between the two planes is identical in the wild-type and mutant minimized structures.

A statistical analysis of the results was made possible due to the redundant information provided by the c chains from the top and bottom halves of the molecule, which are crystallographically independent. A correlation coefficient was calculated to evaluate the systematic nature of differences between mutant and wild-type structures. All r values are presented in table 1. The correlation is highly significant except in the case of the R form using BRUGEL, for which all differences are smaller than 0.2 Å. This supports the validity of our simulations.

4. DISCUSSION

X-ray scattering spectra show that the quaternary structures obtained in the absence of substrate (T) and in the presence of saturating PALA (R) are identical for both the wild-type and Tyr-240→Phe enzymes. Thus, in spite of the absence of the stabilizing H-bond between Tyr-240 and Asp-271, the enzyme is able to adopt the T structure. No other quaternary structure is detectable in the absence of substrate. The unusually large reactivity of the sulfhydryl groups of the mutant toward *p*-hydroxymercuribenzoate (7-fold higher than that of the wild-type enzyme) does not therefore indicate a conformation distinctly different from the unligated conformation of the wild-type enzyme [1]. More generally the reactivity of the enzyme towards *p*-hydroxymercuribenzoate appears to be an unreliable probe of the quaternary structure of mutant versions of the enzyme. The reduced stability of the T state in the case of the mutant might be enough to account for the increased reactivity of the sulfhydryl groups, even in the absence of any structural change. The effect of ATP at subsaturating concentrations of PALA is another, more direct manifestation of the destabilization of the T structure following the loss of the Tyr-240–Asp-271 H-bond.

The absence of significant changes in the quaternary structure of the enzyme, both in the unligated and in the PALA-saturated form, legitimates the use of the coordinates of the wild-type enzyme for energy minimization calculations, which remain close to the starting structure. Movements of different amplitude and directions are observed when different algorithms are applied to the same set of coordinates (table 1). In spite of these software-dependent differences, all simulations suggest that differences between wild type and mutant structures are restricted to residues 240 and 271 in the T form. In this form, the distance between the side chain of Phe-240 and that of Asp-271 increases by over 1 Å relative to the wild type during refinement with BRUGEL. With CHARMM, the distance either increases or remains the same, depending on starting coordinates (table 1). This suggests that the native conformation is physically permitted in spite of the loss of the H-bond. Alternative conformations separated by low energy barriers might be accessible to the two side chains in the mutant enzyme. The mutation thus leads to an increased local mobility.

In the R form, no difference is significantly larger than the rms difference in any simulation, even at the mutated side chain. The hydroxyl group of the tyrosine residue points towards the solvent

while the phenyl ring, partially buried at the c1–c4 interface [17], interacts with its counterpart. The conserved conformation of the mutated side chain suggests that the detection of the hydroxyl group does not affect close non-bonded contacts. Besides, the interaction between the two phenyl rings remains essentially unaltered.

The unligated mutant enzyme has been studied by X-ray crystallography [10]. No change is seen between the wild-type and mutant quaternary structures. The scattering experiments reported here complement the study in the crystal as they show that the quaternary structure is also conserved in solution. The only detectable difference between the two crystal structures is a rotation of the Phe-240 side chain in half of the c chains. In the other half this residue has not moved. Gouaux et al. propose that this might reflect either a structural asymmetry of the structures in the T form, or an effect of crystal constraints. Our simulations favor the second hypothesis, since side chains 240 and 271 of the mutant exhibit a greater rotational mobility, and could therefore adopt an alternative conformation in response to constraints from crystal packing.

The one atom mutation studied here results in an enzyme endowed with the same functional properties – binding, catalysis, cooperativity, allosteric regulation – as the wild-type enzyme, but with slightly less effective regulatory mechanisms. The effect of ATP is the only detectable difference in terms of quaternary structure. Our energy minimization calculations suggest an increased local mobility of two side chains in the T form rather than a different conformation. The mutant R form is predicted to be identical to the wild type. These results are consistent with the view that the only effect of the mutation is a destabilization of the T structure directly following the loss of an H-bond, with no significant structural change for the unligated enzyme. This is an excellent illustration of the subtle balance of interactions which determine the behaviour of the enzyme as they control the equilibrium between the two extreme quaternary T and R structures [18].

Acknowledgements: We thank W.N. Lipscomb and J.E. Gouaux for providing the X-ray coordinates of the wild-type structures and J. Janin and J.E. Gouaux for critical reading of the manuscript. The efficient support from the technical staff from LURE-DCI and from the computing center of LURE is gratefully acknowledged. E.R.K. is supported by USPHS Research Grants GM26237 and DK1249. Work by R.M.S. was supported by USPHS grant GM35716 and by a visiting professorship from Université Paris-Sud, Orsay.

REFERENCES

[1] Middleton, S.A. and Kantrowitz, E.R. (1986) Proc. Nat. Acad. Sci. USA 83, 5866–5870.
[2] Kantrowitz, E.R. and Lipscomb, W.N. (1988) Science 241, 669–674.
[3] Ke, H.M., Hontzako, R.B. and Lipscomb, W.N. (1984) Proc. Natl. Acad. Sci. USA 81, 4037–4040.
[4] Kim, K.H., Pan, Z., Honzatko, R.B., Ke, H.M. and Lipscomb, W.N. (1987) J. Mol. Biol. 196, 853–875.
[5] Krause, K.L., Volz, K.W. and Lipscomb, W.N. (1987) J. Mol. Biol. 193, 527–553.
[6] Burley, S.K. and Petsko, G.A. (1985) Science 229, 23–28.
[7] Moody, M.F., Vachette, P. and Foote, A.M. (1979) J. Mol. Biol. 133, 517–532.
[8] Hervé, G., Moody, M.F., Tauc, P., Vachette, P. and Jones, P.T. (1985) J. Mol. Biol. 185, 189–199.
[9] Levitt, M. (1982) Annu. Rev. Biophys. Bioeng. 11, 251–271.
[10] Gouaux, J.E., Lipscomb, W.N., Middleton, S.A. and Kantrowitz, E.R. (1989) Biochemistry, in press.
[11] Depautex, C., Desvignes, C., Leboucher, P., Lemonnier, M., Dagneaux, D., Benoit, J.P. and Vachette, P. (1987) LURE Annual Report.
[12] Bordas, J., Koch, M.H.J., Clout, P.N., Dorrington, E., Boulin, C. and Gabriel, A. (1980) J. Phys., E: Sci. Instrum. 13, 938–944.
[13] Delhaise, P., Bardiaux, M., and Wodak, S. (1984) J. Mol. Graphics 2, 103–106.
[14] Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, J.S., Swaminathan, S. and Karplus, M. (1983) J. Comp. Chem. 2, 187–217.
[15] Cherfils, J., Vaney, M.C., Morize, I., Surcouf, E., Colloch, N. and Mornon, J.P. (1988) J. Mol. Graphics 6, 155–160.
[16] McLachlan, A.D. (1982) Acta Cryst. A, 6, 871–873.
[17] Cherfils, J. (1987) Thesis, Université Paris-Sud, Orsay, France.
[18] Schachman, H.K. (1988) J. Biol. Chem. 263, 18583–18586.