ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/19262360

# Quaternary structure changes in aspartate transcarbamylase studied by X-ray solution scattering. Signal transmission following effector binding

**ARTICLE** *in* JOURNAL OF MOLECULAR BIOLOGY · OCTOBER 1985

Impact Factor: 4.33 · DOI: 10.1016/0022-2836(85)90190-1 · Source: PubMed

CITATIONS
83

READS
5

**5 AUTHORS**, INCLUDING:

Guy Herve
Pierre and Marie Curie University - Paris 6
**122** PUBLICATIONS **1,676** CITATIONS
SEE PROFILE

Patrick Tauc
Ecole normale supérieure de Cachan
**75** PUBLICATIONS **1,774** CITATIONS
SEE PROFILE

Patrice Vachette
French National Centre for Scientific Rese…
**102** PUBLICATIONS **2,309** CITATIONS
SEE PROFILE

Available from: Patrick Tauc
Retrieved on: 23 January 2016

*J. Mol. Biol.* (1985) **185**, 189–199

# Quaternary Structure Changes in Aspartate Transcarbamylase Studied by X-ray Solution Scattering

## Signal Transmission Following Effector Binding

**Guy Hervé[1], Michael F. Moody[2]†, Patrick Tauc[1] Patrice Vachette[3] and Peter T. Jones[2]‡**

[1] *Institut d'Enzymologie C.N.R.S., 91190, Gif/Yvette, France*

[2] *European Molecular Biology Laboratory D-6900 Heidelberg, West Germany*

[3] *Laboratoire pour l'Utilisation du Rayonnement Electromagnetique Bâtiment 209c, Université Paris-Sud, 91405 Orsay, France*

*(Received 8 October 1984, and in revised form 24 March 1985)*

The result of binding the effectors ATP and CTP to aspartate transcarbamylase was studied by X-ray solution scattering. Binding of substrate analogues produces a substantial change in the solution scattering curve, allowing us to monitor the proportion of the different quaternary structure states present in solution. In the initial solution this ratio was made roughly unity by adding either carbamyl phosphate and succinate, or *N*-(phosphonacetyl)-L-aspartate (PALA). ATP or CTP were then added, and their effect on the proportion of the different quaternary structure states was followed. When using carbamyl phosphate and succinate (weakly bound), ATP or CTP had a clear effect, as observed previously by monitoring the sedimentation rate (Changeux *et al.*, 1968). However, when PALA (strongly bound) was used, the effect of CTP was very much smaller, and that of ATP was undetectable. This result supports the explanation by Tauc *et al.* (1982), that nucleotides act mostly through changing the affinity of the active sites for substrate, and only to a small extent by directly modifying the quaternary structure equilibrium in the case of CTP.

## 1. Introduction

Aspartate transcarbamylase of *Escherichia coli* ATCase§ catalyses the first reaction of the pyrimidine pathway, the carbamylation of the amino group of aspartate by carbamyl phosphate. ATCase activity is feedback-inhibited by the end product, CTP, and is activated by ATP, it is extensively used as a model system to study the molecular mechanisms of allosteric regulation (see the reviews by Gerhart, 1970; Jacobson & Stark, 1973; Kantrowitz *et al.*, 1980*a*,*b*). The enzyme consists of three regulatory subunits (each composed of two chains, and binding ATP or CTP), and of two catalytic subunits (each composed of three chains, and containing three active sites). The sequences of both chains have been established (Weber, 1968; Hoover *et al.*, 1983; Konigsberg & Henderson, 1983; Schachman *et al.*, 1984), and detailed structures are known for two forms of the enzyme without bound substrate-analogues (Honzatko *et al.*, 1982; Ke *et al.*, 1984). The binding of substrate-analogues causes a large quaternary structure change, originally seen by hydrodynamic techniques (Gehart & Schachman, 1968; Dubin &

† Present address: School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, England.

‡ Present address: MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Hills Road, Cambridge CB2 2QH, England.

§ Abbreviations used: ATCase, aspartate transcarbamylase (or carbamoyltransferase) from *E. coli* (EC 2.1.3.2); PALA, *N*-(phosphonacetyl)-L-aspartate; "C", X-ray solution scattering curve with no ligands or with CTP; "E", X-ray solution scattering curve with substrate-analogues at saturating concentrations.

0022-2836/85/170189-11 $03.00/0



© 1985 Academic Press Inc. (London) Ltd.

Cannell, 1975), and recently investigated by X-ray solution scattering (Moody *et al.*, 1979). The extensive alterations in the scattering curve showed that the main structural change is an increased separation of the catalytic trimers, a conclusion that has been reached also by X-ray crystallography (Ladner *et al.*, 1982).

Although solution scattering is far inferior to crystallography as a source of further structural details, it has the advantage that it can give information about the proportions of different forms present in solutions. This is important because many allosteric proteins show a change in quaternary structure when their activity state changes. In the case of the most thoroughly studied allosteric protein, haemoglobin, the quaternary structure change is tightly coupled to all the allosteric functions (see the reviews by Perutz & Fermi, 1981; Dickerson & Geis, 1983). It will be interesting to see the extent to which other allosteric proteins conform to this model. ATCase is a particularly favourable experimental system for studying the role of quaternary structure change, since the alteration in scattering curve is exceptionally large. The curve of the unligated enzyme will be referred to as the "C" curve (of the contracted molecule); substrate-analogues cause this to change to what we shall refer to as the "E" curve (of the expanded molecule).

In earlier studies (Gerhart & Schachman, 1968; Howlett & Schachman, 1977; Yang & Schachman, 1980; Wang *et al.*, 1981; Johnson & Schachman, 1983), the test for quaternary structure was sedimentation rate, which decreases by about 3·6% after binding substrate-analogues. The availability of intense synchrotron radiation sources makes X-ray solution scattering a technique superior in both accuracy and information content. We shall present, in a series of articles, the results of using this new technique to investigate the role of quaternary structure changes in the allosteric behaviour of ATCase.

In this first paper, the mechanism of effector action is examined. Binding of an allosteric effector is energetically coupled to some other functions of the enzyme, of which enzyme activity and quaternary structure are of particular interest. It has long been assumed (Changeux & Rubin, 1968; Howlett *et al.*, 1977) that the allosteric effectors ATP or CTP act by modifying the equilibrium between the two forms of ATCase that differ both in affinity for substrate and in quaternary structure, and that all three allosteric features are tightly coupled together. However, Thiry & Hervé (1978) and Tauc *et al.* (1982) found that the allosteric consequences of ATP or CTP binding proceed by a mechanism quite different from that of homotropic co-operativity. Their interpretation was that nucleotide effectors have no direct effect on the equilibrium between the different quaternary structural states; instead, bound nucleotides affect the affinity of the active sites for substrates. This could change the number of sites binding substrate, and it is the active site occupancy which would determine the quaternary structure. However, these results were obtained from steady-state kinetic experiments, so it was interesting to test these conclusions using a more direct measure of the quaternary structure.

This has been done using X-ray scattering to monitor the proportion of the two quaternary structure states present in solution, and determining how this is changed by the binding of effectors. However, in the absence of substrates or substrate-analogues, the quaternary structure equilibrium lies so heavily on the side of the "C" form that the effects of ATP or CTP are undetectable. The proportions of "C" and "E" forms must first be brought near equality by adding substrate-analogues. This was achieved with either carbamyl phosphate and succinate (weakly bound), or else *N*-(phosphonacetyl)-L-aspartate (strongly bound). At these subsaturating concentrations there is more succinate free in solution than bound to the active sites ($K_D = 4 \times 10^{-3}$ M). PALA, on the other hand, has such a very high affinity ($K_D = 10^{-8}$ M) that it is almost stoichiometrically bound to the active site, leaving almost no PALA free in solution under the conditions of our experiments. ATP or CTP was then added, and its effect on the proportion of the two quaternary structure states was followed. If the effectors act directly on the quaternary structure equilibrium involved in the homotropic co-operative interactions between the catalytic sites, the precise substrate-analogue used should be unimportant. But if the explanation given by Thiry & Hervé (1978) and Tauc *et al.* (1982) is correct, the binding constant of the substrate-analogue should determine how far the quaternary structure equilibrium can change after effector binding. Indeed, this explanation predicts that there would not be any influence of the effectors ATP and CTP on this structural equilibrium in the absence of unbound substrates or substrate-analogues. Our results support the second alternative.

## 2. Materials and Methods

### (a) *Chemicals*

Dilithium carbamyl phosphate was purchased from Serva and further purified by precipitation from 50% (v/v) ethanol (Gerhart & Pardee, 1962). The carbamyl phosphate solution was freshly prepared just before each experiment to avoid hydrolysis (Allen & Jones, 1964). EDTA was from Merck; dithioerythritol, aspartic acid, *N*-carbamyl-DL-aspartic acid and phenyl methane sulphonyl fluoride for Sigma; and CTP (disodium salt) from Pharma Waldhof. ATP was from Serva.

### (b) *Enzyme preparation and reaction conditions*

The diploid strain of *E. coli* (Gerhart & Holoubek, 1967) was grown in a 4000-l fermentation apparatus at the Gesellschaft für Biotechnologische Forschung, Stockheim, and ATCase and catalytic subunit were prepared by a modification (A. M. Foote, R. Leberman,

P. T. Jones & M. F. Moody, unpublished results) of Gerhart & Holoubek's (1967) method. Enzyme solutions were made dimer-free by gel filtration using an LKB Ultragel AcA22 column (Moody *et al.*, 1979). The enzyme was buffered with 50 mM-Tris-borate buffer (pH 8·3), and 0·1 mM-EDTA, 0·1 mM-dithioerythritol, 0·25 mM-phenyl methane sulphonyl fluoride were often added. It was concentrated to 140 mg/ml using an Amicon model 8MC filter unit with a PM 30 membrane, and was cleared by low-speed centrifugation immediately before the experiment. The concentration was determined by spectrophotometry, assuming an extinction coefficient at 280 nm of $0{\cdot}59\,cm^2\,mg^{-1}$ for ATCase (Gehart & Holoubek, 1967).

### (c) *Enzyme solutions for X-ray scattering*

Samples were prepared by taking portions from a stock solution to which the appropriate chemicals were added so as to yield a final protein concentration of 100 mg/ml in every case but one (Fig. 9). The data from all solutions containing carbamyl phosphate were recorded within 2 h of preparation. The initial concentration of carbamyl phosphate was made very high (25 mM) in order to ensure a saturating level in spite of the hydrolysis taking place during that time.

### (d) *X-ray scattering*

X-ray scattering curves were recorded during two sessions at the small-angle scattering installation in the synchrotron radiation laboratory L.U.R.E. The storage ring D.C.I. was operated at 1·72 GeV and 300 mA or 1·85 GeV and 250 mA. The camera has been described in detail by Koch *et al.* (1982). The X-ray beam has a cross-section of 0·5 mm × 1 mm at the sample level and 0·5 mm × 0·7 mm at the detector level.

Two different linear position-sensitive detectors with delay-line readout were used: the data presented in Figs 1, 6 and 8 were obtained with one of them, and all other Figures with the other detector. Each had an entrance window 4 mm wide, and was about 500 mm from the sample. The counting time was between 10 and 30 min, and the temperature was 20°C.

All solutions were studied in the same flattened quartz capillary 0·9 mm thick. The capillary was extensively flushed with buffer to rinse it between 2 successive samples.

Before and after each curve, a carbon-black sample was automatically positioned in the beam, immediately down-beam of the ATCase sample, and its scattering was recorded for 30 s. The intensity scattered by this reference sample, smoothly decreasing as a function of angle, is about 100 times greater than that of the ATCase solution. The number of counts over a large angular range, and from before and after the ATCase measurement, was averaged to give a number (of the order of 500,000 counts) that was used to put all the data on the same scale. This procedure takes into account any slight variations of experimental factors such as beam intensity, detector efficiency or solution transmission. Intensities recorded in 1000 s were of the order of 12,000 counts/channel within the first maximum of the curve of the "C" form, and of the order of 20,000 counts/channel within the first maximum of the curve of the "E" form. A typical channel width was $2 \times 10^{-4}$ Å$^{-1}$/channel.

The scattering parameter $s = 2 \sin \theta/\lambda$, where $2\theta$ is the angle through which the X-rays are scattered, and the wavelength $\lambda = 1{\cdot}608$ Å (K-edge of Co); $i(s)$ is the distribution of scattered intensity. The geometry of the beam yields curves with much better resolution than those obtained with a rotating anode generator (Moody *et al.*, 1979). However, corrections for the width of the detector were done according to Lake's (1967) procedure for the two reference curves. All the other curves shown are raw data, without any correction or background subtraction, with the exception of Fig. 9.

## 3. Results

### (a) *Reference curves*

The curves obtained in the absence or presence of saturating PALA are plotted in Figure 1 in the same way as those in Figure 5 of Moody *et al.* (1979). The positions and intensities at the minima, maxima and crossing-points are given in Table 1. Figure 2 (curves a and b) shows a logarithmic plot of the corresponding raw data. The region of main interest is clear without magnifying the weaker parts of the curves, as is necessary in a linear plot. Intermediate curves can be specified in terms of the extreme forms "C" and "E". When passing from "C" to "E" (i.e. when the molecule is expanding), the first minimum and maximum of the scattering curve move to smaller angles (Fig. 2, curve c).

### (b) *Effects of ATP or CTP on the quaternary structure of ATCase*

#### (i) *In the presence of carbamyl phosphate*

Using the simplest two-state model (Monod *et al.*, 1965), Howlett *et al.* (1977) calculated that, if ATCase obeys this model, the ratio of "C" to "E" forms would shift from 250 : 1 to 7 : 1 (i.e. to 13% "E") if a saturating concentration of carbamyl phosphate were used. They also calculated that from this level it should drop to 2 (i.e. to 33% "E") with ATP; but that with CTP it should rise to 35 (i.e. to less than 3% "E"). All nucleotides should be at saturating concentrations.

The scattering curves of ATCase solutions saturated with carbamyl phosphate (25 mM), and with ATP or CTP (or neither) added to 10 mM, are shown in Figure 3. No significant difference can be detected between these curves. Figure 4 shows three linear combinations of the "C" and "E" curves, corresponding to 3%, 13% and 33% of the "E" form as predicted above. Comparison with Figure 3 shows unambiguously that the enzyme does not behave as predicted by the simple two-state model, although the aspartate saturation curves of our preparations of ATCase are identical to those reported by Howlett & Schachman (1977).

#### (ii) *In the presence of carbamyl phosphate and succinate*

The ATCase reaction involves an ordered mechanism in which carbamyl phosphate binds first and induces a conformational change which promotes the binding of aspartate or its analogues

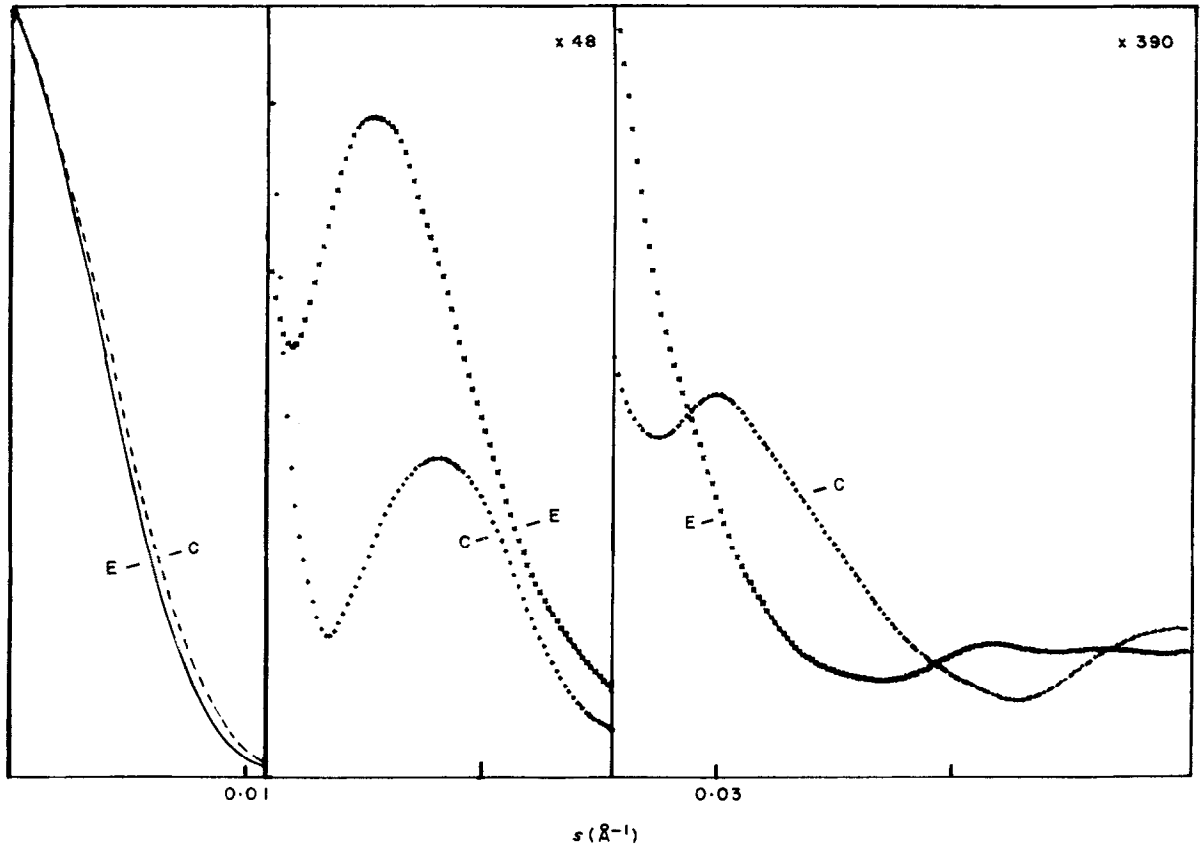


**Figure 1.** X-ray scattering curve of ATCase: E, ligated enzyme with all active sites occupied by PALA; C, unligated enzyme, in the absence of PALA.

(Porter *et al.*, 1969; Collins & Stark, 1969; Hammes *et al.*, 1971; Schaeffer & Stark, 1972; Wedler & Gasser, 1974; Issaly *et al.*, 1982). Accordingly, scattering curves of ATCase solutions were recorded in the presence of saturating amounts of carbamyl phosphate (25 mM), and a subsaturating concentration of succinate (3·57 mM); 10 mM-ATP or 10 mM-CTP (or neither) was then added. The succinate concentration was chosen to give a scattering curve intermediate between "C" and "E", as shown in Figure 2, curve c. ATP causes a shift towards the "E" curve, but CTP produces the reverse change (Fig. 5). Thus, there is a clear effect of nucleotide binding on the quaternary structure of ATCase. Other curves (not shown), recorded at different succinate concentrations with saturating amounts of nucleotide, showed the same behaviour.

ATP can reverse the effect of CTP on substrate binding (Thiry & Hervé, 1978); can it also reverse its effect on the quaternary structure of the protein? We first recorded the scattering curve of an ATCase solution containing 25 mM-carbamyl phosphate, 5·7 mM-succinate and 5 mM-CTP (curve b in Fig. 6). The solution was then made 10 mM in ATP and its scattering curve recorded again. A change towards the "E" curve (curve a in

**Table 1**
*Principal features of the scattering patterns*

| | 1st minimum | 1st maximum | 2nd minimum | 2nd maximum | 3rd minimum | 3rd maximum |
|---|---|---|---|---|---|---|
| *Native enzyme* | *("C")* | | | | | |
| Position | 0·0136 Å$^{-1}$ | 0·0181 Å$^{-1}$ | 0·0274 Å$^{-1}$ | 0·030 Å$^{-1}$ | 0·043 Å$^{-1}$ | 0·050 Å$^{-1}$ |
| | (73·6 Å) | (55·1 Å) | (36·4 Å) | (33·4 Å) | (23·2 Å) | (20·1 Å) |
| Intensity | 0·452 | 1·0 | 0·127 | 0·145 | 0·028 | 0·054 |
| *Enzyme saturated with PALA* | | *("E")* | | | | |
| Position | 0·0120 Å$^{-1}$ | 0·0154 Å$^{-1}$ | — | — | 0·037 Å$^{-1}$ | 0·042 Å$^{-1}$ |
| | (83·1 Å) | (64·9 Å) | | | (26·9 Å) | (24·0 Å) |
| Intensity | 1·45 | 2·21 | — | — | 0·039 | 0·054 |
| *Crossing points* | | | | | | |
| Position | 0·0115 Å$^{-1}$ (87·0 Å) | | 0·0286 Å$^{-1}$ (34·9 Å) | | 0·039 Å$^{-1}$ (25·6 Å) | |

Intensities have been scaled to 1 for the first maximum of the native pattern.

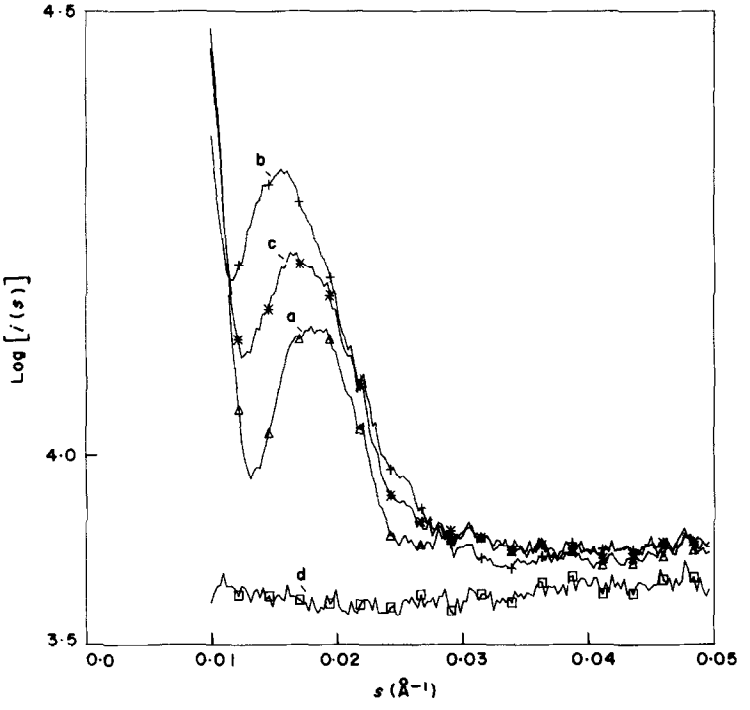


**Figure 2.** X-ray scattering curves of ATCase: log plot of raw data. Curve a, unligated enzyme (△); curve b, enzyme fully ligated with PALA (+); curve c, enzyme ligated with 25 mM-carbamyl phosphate and 3·25 mM-succinate, giving an effect close to the mid-transition (*); curve d, background (scattering curve of buffer) (□).

Fig. 6) demonstrates that ATP does indeed reverse the effect of CTP on the quaternary structure.

(iii) *In the presence of PALA*

ATCase solutions were prepared with PALA at a concentration giving a scattering curve intermediate between two extremes. The scattering curves of such a solution in the presence of 10 mM-ATP or 10 mM-CTP (or neither) are shown in Figure 7. Unlike the situation with succinate, ATP has no detectable influence on the "C" : "E" ratio, and CTP has only a very small effect. Similar

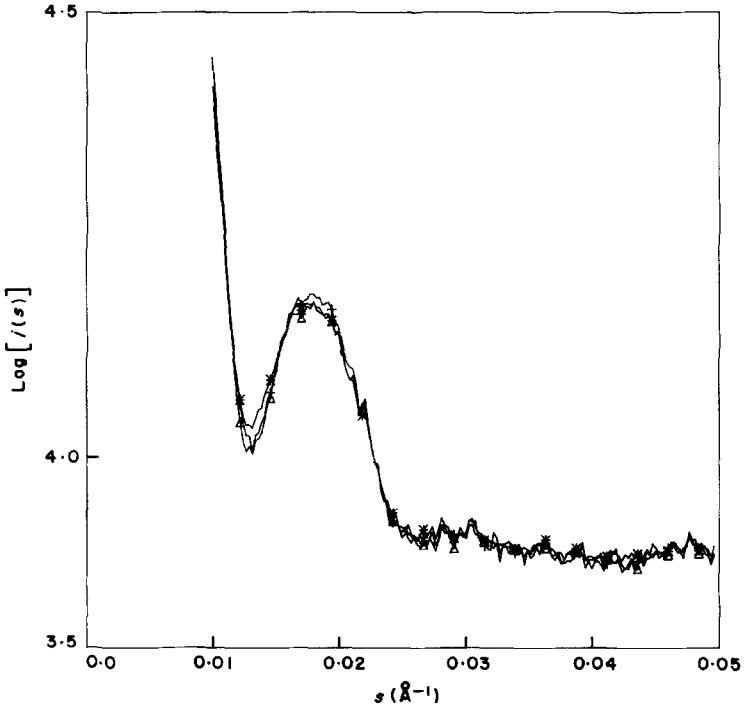


**Figure 3.** X-ray scattering curves of ATCase in the presence of 25 mM-carbamyl phosphate. (△) In the absence of nucleotides; (+) with 10 mM-ATP; (*) with 10 mM-CTP.

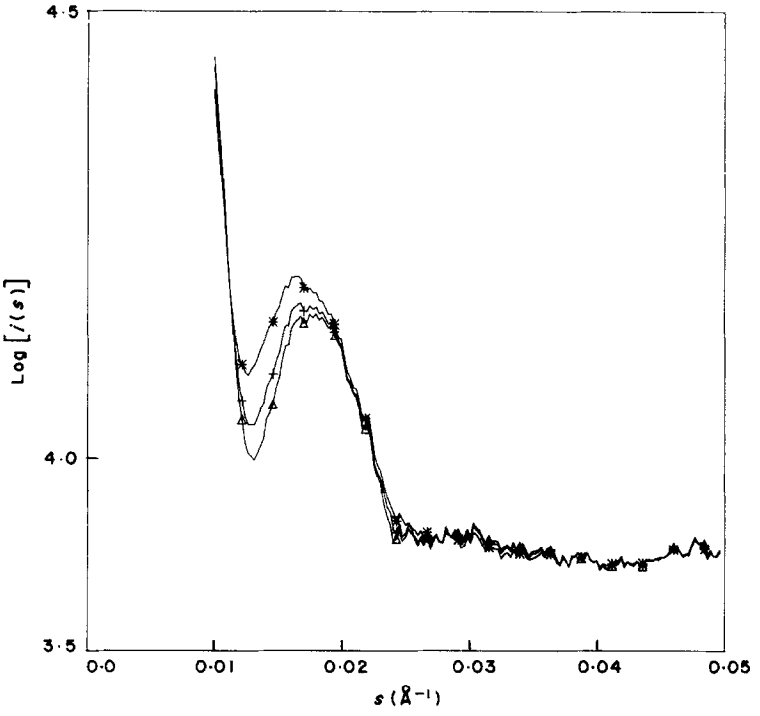


**Figure 4.** Linear combinations of the 2 extreme scattering curves of ATCase (see the text for explanation). "C", unligated enzyme; "E", fully ligated enzyme. (△) 0·97 "C"+0·03 "E"; (+) 0·87 "C"+0·13 "E"; (*) 0·67 "C"+0·33 "E".

curves (not shown) have been recorded at different PALA concentrations and show the same results. Thus, when PALA is used to produce an intermediate scattering curve, the effect of nucleotides is drastically reduced from that observed in the presence of carbamyl phosphate and succinate.

## 4. Discussion

### (a) *Validity of the experiments*

Since this is the first of a series of articles describing the application of X-ray scattering to study the quaternary structure of ATCase, we should discuss the validity of this method.

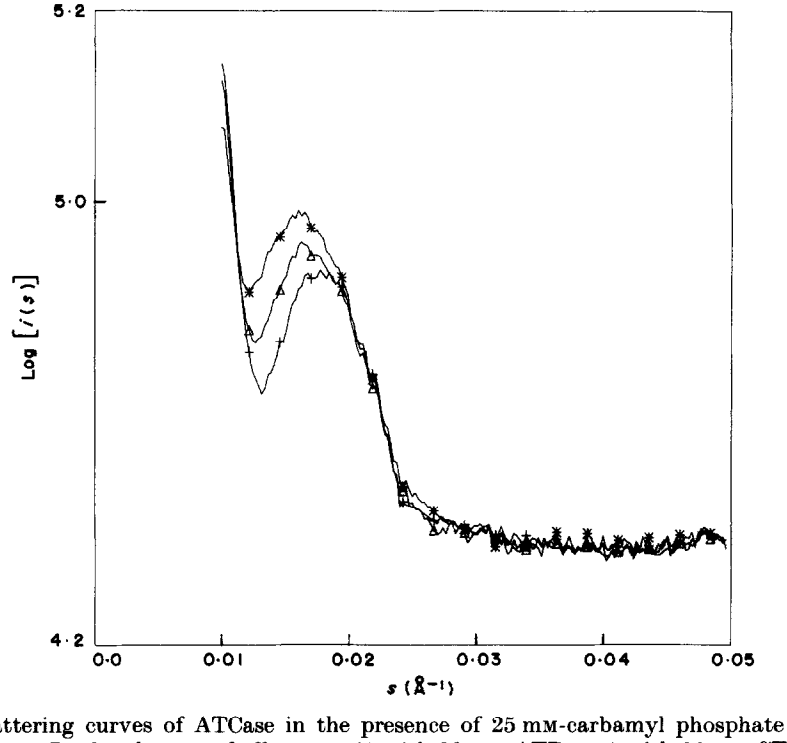


**Figure 5.** X-ray scattering curves of ATCase in the presence of 25 mM-carbamyl phosphate and 3·6 mM-succinate (total concentrations). (△) In the absence of effectors; (*) with 10 mM-ATP; (+) with 10 mM-CTP.

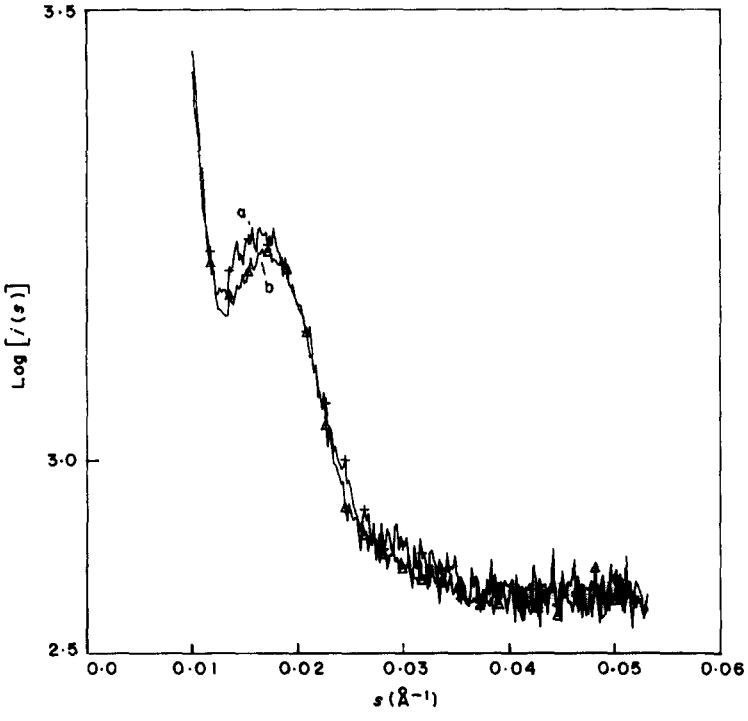


**Figure 6.** X-ray scattering curves of ATCase in the presence of 25 mM-carbamyl phosphate and 5·7 mM-succinate (total concentrations). Curve b, with 5 mM-CTP (△); curve a, after adding ATP solution up to 10 mM to the previous sample (+), scaled to the same enzyme concentration as in curve b.

(1) How reproducible are our curves, i.e. what is the smallest statistically significant difference between two curves? In the course of a long session at L.U.R.E. we recorded, on four different days, seven curves of native ATCase in the absence of substrate. All solutions were made from the same stock solution, the concentration being adjusted immediately before use. We then determined the average and standard deviation of scattered intensity for each scattering angle. The average curve is plotted plus and minus the standard deviation, illustrating the reproducibility of our data (Fig. 8).

(2) Could our method be sensitive to aggregation?

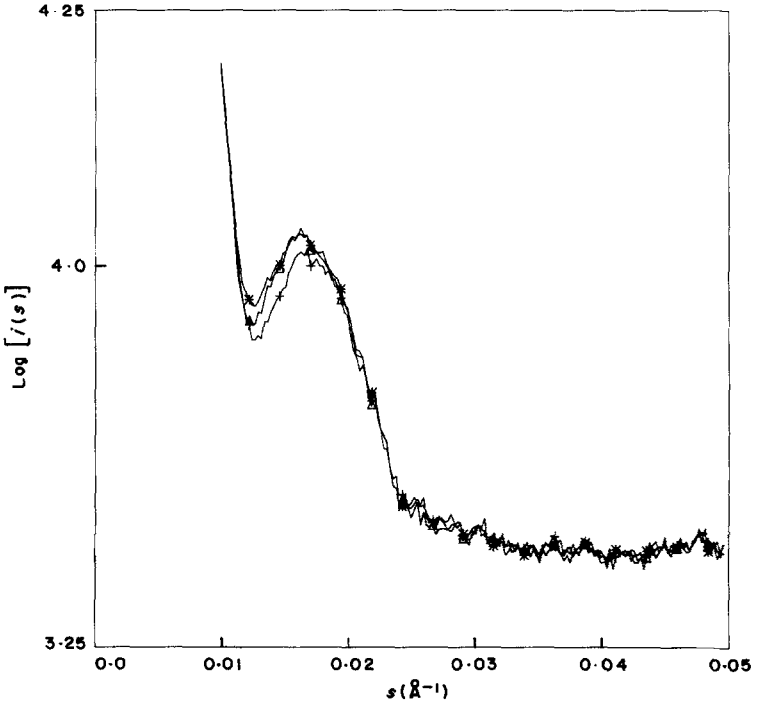


**Figure 7.** X-ray scattering curves of ATCase in the presence of 0·6 mol of PAPA/mol of active site. (△) In the presence of nucleotide; (*) with 10 mM-ATP; (+) with 10 mM-CTP.

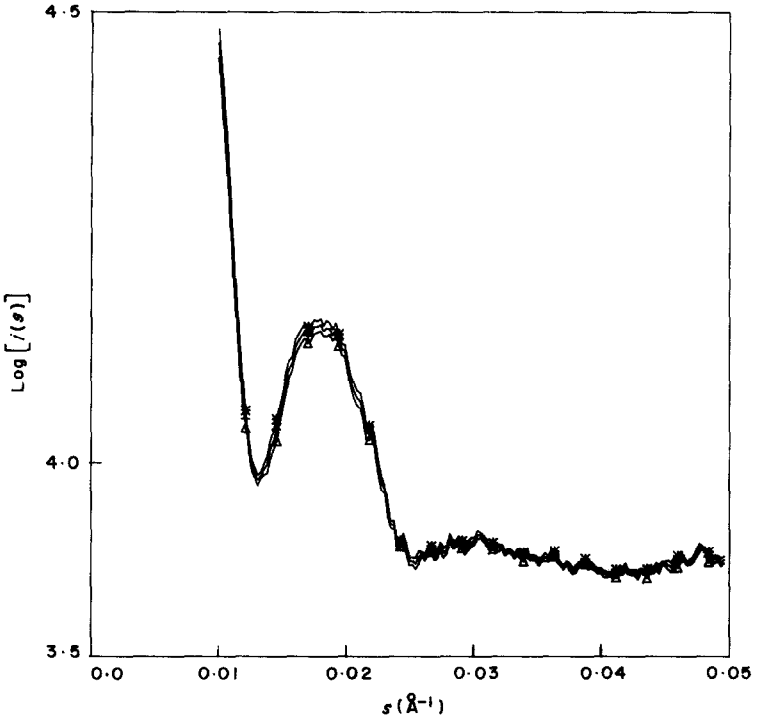


**Figure 8.** The distribution obtained from 7 X-ray scattering curves of ATCase in the absence of ligand. The middle curve (+) shows the average and the upper (*) and lower (△) curves show this plus or minus one standard deviation, respectively.

ATCase is known to form small amounts of stable dimers (Griffin *et al.*, 1972; Kerbiriou & Hervé, 1973; Yang *et al.*, 1974; Moody *et al.*, 1979), which would be favoured at high enzyme concentration or in the presence of nucleotides (Cook & Milne, 1977). However, all ATCase solutions used in this work were made dimer-free shortly before use (see Materials and Methods, section (b)). The X-ray samples were analysed by polyacrylamide gradient gel electrophoresis after the scattering experiments

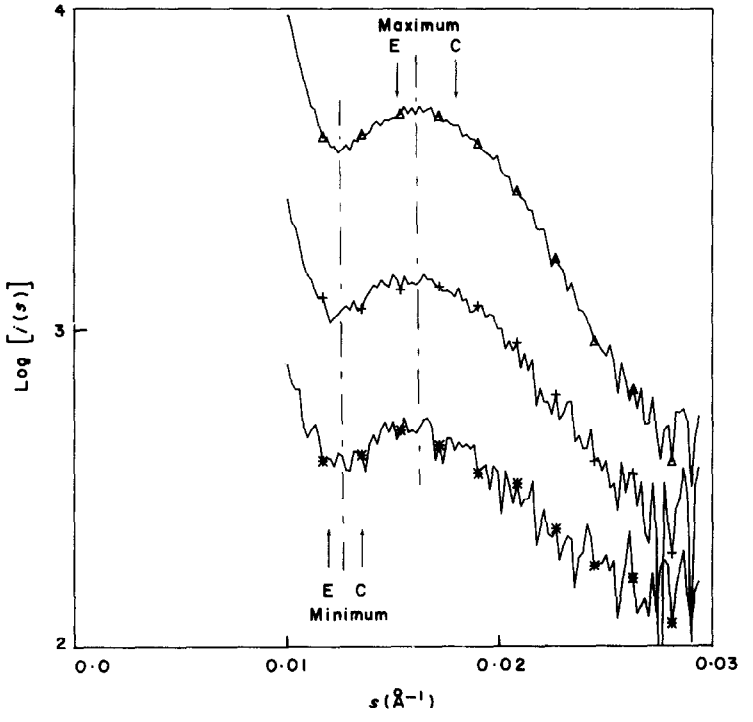


**Figure 9.** X-ray scattering curves of ATCase in the presence of 0·4 mol of PALA/mol of active site at different enzyme concentrations. The background scattering has been subtracted from all 3 curves. Upper curve, 100 mg/ml; middle curve, 25 mg/ml; bottom curve, 10 mg/ml. The arrows with the letters C and E show the positions of the first minima and maxima in the extreme states.

and showed less than 1% contamination by dimer. In any case, the effect of aggregates is limited to the very small-angle region and, in the region of interest, the dimer scattering curve is practically identical with that of the monomer (Vachette *et al.*, unpublished results).

(3) Could the enzyme structure or the proportions of different forms be altered by the high concentration (100 mg/ml) necessary for X-ray solution scattering experiments? To test this, scattering curves were recorded from an ATCase solution containing PALA at 40% saturation, at concentrations from 100 mg/ml down to 10 mg/ml (Fig. 9). Although the signal becomes weaker at lower concentrations, the positions of the first minimum and of the first maximum (which are very different in the "C" and "E" curves: Fig. 2) can still be located. They remain the same from 100 mg/ml down to 10 mg/ml, so the structure of ATCase and the composition of the solution are independent of the enzyme concentration over that range.

(4) Is the change in scattering curve specifically sensitive to quaternary structure changes, or might a significant part of it be accounted for by local tertiary structure changes? The largest changes (radius of gyration, position and intensity of the first minimum and maximum) occur at scattering angles corresponding to distances >50 Å, which are dominated by inter-subunit vectors. The specific sensitivity of the scattering curve to quaternary structure changes is seen by comparing our experimental curves with scattering curves calculated from the crystallographic atomic coordinates. The native structure in the absence of ligand was experimentally determined (Honzatko *et al.*, 1982), while that of the fully ligated form was obtained by considering the *r* and *c* chains as rigid domains, and determining which of their possible movements give the best fit to the diffraction data from the PALA-ligated crystals (Ladner *et al.*, 1982). The scattering curves computed from the two models show most of the features of the experimental ones, even though no structural changes had been introduced below the quaternary structure level (Altman *et al.*, 1982; Rey & Dumas, 1984).†

X-ray solution scattering seems to have several advantages over hydrodynamic measurements. (1) The scattering curves are sensitive to many structural parameters: from the formula of Luzzati (1980), 14 independent terms would be needed to represent the part of the curve sensitive to substrate-analogue binding, i.e. out to 0·05 $Å^{-1}$. (2) The signal/background ratio is very high: parasitic scattering is very low and flat in the region of maximal change (Fig. 2), while the conformation-induced changes amount to 100% in the region of the first minimum and maximum (Fig. 2). Even a partial change of conformation can be detected by visual inspection of raw data (see Figs 4 and 5). (3) The effect on the scattering curve of substrate or effector binding *per se* is less than 1% over the whole of the angular range, very small compared with the 100% change observed. Scattering curves can thus be compared directly, without any correction. (4) The measurements were made without any perturbations due to pressure, shearing, etc.

(b) *Effect of ATP or CTP*

As explained earlier, we cannot measure the effects of ATP or CTP unless we have first brought the proportions of "C" : "E" forms near equality by adding substrate-analogues. This has been done using either PALA or carbamyl phosphate/succinate as the substrate-analogues. As shown in Results, section (b), the quaternary structure of ATCase is sensitive to the nucleotide effectors in the presence of carbamyl phosphate and succinate but not in the presence of PALA. This could not be the case if effectors acted directly on the quaternary structure, because some part at least of this direct effect would then be seen irrespective of the nature of the substrate-analogue. Instead, the sensitivity to the precise nature of the substrate-analogues implies that the effectors are acting directly on the sites that recognize those analogues. Since the most relevant property of the analogues is their strength of binding to the active site, it appears that part of the binding energy of the effectors must be altering the binding of the substrate-analogues.

When half the active sites bind succinate, the free succinate concentration is in the millimolar range. ATP binding (to saturation) reduces the free succinate concentration several-fold. This will alter the proportion of active sites binding substrate-analogues, and hence will change the quaternary structure equilibrium, as observed. On the other hand, the free PALA concentration, when half the active sites bind PALA, is well below a micromole. ATP will increase this affinity but, even if the consequence were that all the free PALA were to become bound, this could increase the concentration of saturated sites by only a fraction of a micromole per litre. Since the enzyme concentration is nearly 2 mM in active sites, the change in active site occupancy would be negligible.

Thus, the major effect of nucleotide binding is not a direct one on the equilibrium between quaternary structural states, but rather an altered affinity of the active sites for substrates (Thiry & Hervé, 1978; Tauc *et al.*, 1982). This will change the number of sites binding substrate, and it is the active site occupancy that determines the quaternary structure.

The independence of the effector–substrate (heterotropic) and the substrate–substrate (homotropic) signal transmission mechanisms probably

† Comparison of the computed curves given by Altman *et al.* (1982) with our new data (Fig. 1 and Table 1) shows an improved agreement in the region from 0·03 to 0·055 $Å^{-1}$, with crossing points at both ends of the range. This confirms their suggestion that "some ambiguity in the relative backgrounds of the two experimental curves" existed in this region.

occurs in other allosteric enzymes. This is suggested by the fact that when these enzymes are chemically cross-linked into either the low or high affinity state, they no longer exhibit homotropic co-operative interactions but they retain at least part of the heterotropic interactions. Besides ATCase (Enns & Chan, 1978; Chan & Enns, 1979), this is true for yeast phosphofructokinase (Laurent *et al.*, 1979), rabbit muscle phosphofructokinase (Lad & Hammes, 1974), glycogen phosphorylase (Wang & Tu, 1970) and *Acinetobacter* citrate synthase (Mitchell & Weitzman, 1983). In this last case the uncoupling is especially spectacular, since the chemical treatment abolishes homotropic co-operative interactions not only between the catalytic sites but also between the regulatory sites without altering heterotropic interactions.

(c) *Mechanism of signal transmission*

It has long been clear that activation of ATCase by substrate analogues involves a large change of shape, so that substrate–substrate signals are transmitted by a mechanism that involves a quaternary structure change. This sort of mechanism is familiar through the example of haemoglobin. However, in this paper we have confirmed the interpretation given by Thiry & Hervé (1978) and Tauc *et al.* (1982), that effector–substrate signals proceed by an additional and quite different mechanism, in which quaternary structure change is either absent (with ATP) or very small (with CTP). This situation raises two major problems, the distance over which this new type of signal is transmitted, and its coupling with the quaternary structure signals at their common site of action.

Any mechanism of signal transmission within proteins must solve the major problem of attenuation. The transmission of the contraction signal in the T4 bacteriophage sheath, which occurs over nearly 1000 Å, is an exceptional case since the signal receives regular amplification, employing the energy of an irreversible conformational change. This would not be possible for signal transmission in a molecule at equilibrium, as is the case with effector and substrate-analogue binding to ATCase. How could such attenuation be minimized? Presumably there must be specialized pathways whereby the distortion that carries the signal is decoupled from other possible displacements. In the quaternary structure change (whose existence can be understood from the need to connect all six active sites) the attenuation would be minimized, since there are many gaps between adjacent subunits of ATCase (see Fig. 7 of Connally, 1983). In this way a relatively large movement could occur without excessive activation energy, so the transition takes only 10 to 20 milliseconds at 4°C (Kihara *et al.*, 1984).

But the second transmission mechanism, since it proceeds by tertiary changes, would appear to lose this important advantage. This is a problem only because of the distance over which it acts: signal transmission by relatively local chain movements is well-established in various enzyme molecules (reviewed by Janin & Wodak, 1983; Bennett & Huber, 1984). However, in ATCase, effector-induced signal transmission extends over a distance of at least 50 to 70 Å, and crosses polypeptide chain boundaries. Considerable attenuation of the distortion seems to occur over this distance, since CTP-induced chain movements, which are large near the effector binding site, cannot be detected over the catalytic subunit (Honzatko & Lipscomb, 1982). Unfortunately, no crystal structure is available for the form that would be most relevant to understanding this transmission mechanism, i.e. ATCase that has been complexed with ATP prior to crystallization.

The second problem concerns how these two quite different signal transmission mechanisms are coupled to affect substrate binding. It is not clear exactly where this coupling takes place, presumably somewhere between the active site and the zinc domain of the regulatory subunit. But wherever it is, two classes of movement must take place there after substrates bind: those that couple to the effector site, and those that couple to the quaternary structure change. Only the first class of active site movement would be affected by, or lead to, effector binding changes. However, the details of either set of movement will become clear only when the crystallographic structure of the PALA-ligated enzyme becomes available (Krause *et al.*, 1985).

The lack of quaternary structure changes during transmission of the effector signal makes it difficult to study this further by X-ray solution scattering, so subsequent papers of this series will concern only homotropic (substrate–substrate) co-operativity. Further information about the effector signal will come not only from crystallography, but also from functional studies of molecules altered in the $c : r$ bonding region. For example, 2-thiouracil ATCase (Kerbiriou & Hervé, 1972, 1973; Kerbiriou *et al.*, 1977; Kantrowitz *et al.*, 1977), a modified enzyme which is locked in an active state, lacks homotropic co-operative interactions and also shows loosened $c : r$ interactions (Bothwell & Schachman, 1980). The structural importance of this region of the molecule is under further study using mutations located in the regulatory chain.

We thank Dr W. Wania of the Gesellschaft für Biotechnologische Forschung, Stockheim, for running the large fermentation, and A. M. Foote for help with this; Dr G. R. Stark for a gift of PALA; the controllers of the storage ring D.C.I. (Laboratoire de l'Accelerateur Linéaire d'Orsay); and Dr Henri Buc for stimulating discussions.

This work was supported in part by the C.N.R.S. and la Mission des Biotechnologies du Ministère de la Recherche et de l'Industrie, contrat no. 82.V.1287.

## References


Allen, C. M. Jr & Jones, M. E. (1964). *Biochemistry*, **3**, 1238–1247.

Altman, R. B., Ladner, J. E. & Lipscomb, W. N. (1982). *Biochem. Biophys. Res. Commun.* **108**, 592–595.

Bennett, W. S. & Huber, R. (1984). *CRC Crit. Rev. Biochem.* **15**, 290–384.

Bothwell, M. A. & Schachman, H. K. (1980). *J. Biol. Chem.* **255**, 1962–1970.

Chan, W. W.-C. & Enns, C. A. (1979). *Canad. J. Biochem.* **57**, 798–805.

Changeux, J.-P. & Rubin, M. M. (1968). *Biochemistry*, **7**, 553–560.

Changeux, J.-P., Gerhart, J. C. & Schachman, H. K. (1968). *Biochemistry*, **7**, 531–537.

Collins, K. D. & Stark, G. R. (1969) *J. Biol Chem.* **244**, 1869–1877.

Connally, M. L. (1983). *Science*, **221**, 709–713.

Cook, R. A. & Milne, J. A. (1977). *Canad. J. Biochem.* **55**, 346–358.

Dickerson, R. E. & Geis, I. (1983). *Hemoglobin: Structure, Function, Evolution, and Pathology*. Benjamin/Cummings Publ. Co., Inc., Menlo Park, Calif.

Dubin, S. B. & Cannell, D. S. (1975). *Biochemistry*, **14**, 192–195.

Enns, C. A. & Chan, W. C. (1978). *J. Biol. Chem.* **253**, 2511–2513.

Gerhart, J. C. (1970). *Curr. Top. Cell. Regul.* **2**, 275–325.

Gerhart, J. C. & Holoubek, H. (1967). *J. Biol. Chem.* **242**, 2886–2892.

Gerhart, J. C. & Pardee, A. B. (1962). *J. Biol. Chem.* **237**, 891–896.

Gerhart, J. C. & Schachman, H. K. (1968). *Biochemistry*, **7**, 538–552.

Griffin, J. H., Rosenbusch, J. P., Weber, K. & Blout, E. R. (1972). *J. Biol. Chem.* **247**, 6482–6490.

Hammes, G. G., Porter, R. W. & Stark, G. R. (1971). *Biochemistry*, **10**, 1046–1050.

Honzatko, R. B. & Lipscomb, W. N. (1982). *J. Mol. Biol.* **160**, 265–286.

Honzatko, R. B., Crawford, J. L., Monaco, H. L., Ladner, J. E., Edwards, B. F. P., Evans, D. R., Warren, S. G., Wiley, D. C., Ladner, R. C. & Lipscomb, W. N. (1982). *J. Mol. Biol.* **160**, 219–263.

Hoover, T. A., Roof, W. D., Foltermann, K. F., O'Donovan, G. A., Bencini, D. A. & Wild, J. R. (1983). *Proc. Nat. Acad. Sci., U.S.A.* **80**, 2462–2466.

Howlett, G. J. & Schachman, H. K. (1977). *Biochemistry*, **16**, 5077–5083.

Howlett, G. J., Blackburn, M. N., Compton, J. G. & Schachman, H. K. (1977). *Biochemistry*, **16**, 5091–5099.

Issaly, I., Poiret, M., Tauc, P., Thiry, L. & Hervé, G. (1982). *Biochemistry*, **21**, 1612–1623.

Jacobson, G. R. & Stark, G. R. (1973). In *The Enzymes* (Boyer, P. D., ed.), vol. 9, pp. 225–308, Academic Press, New York and London.

Janin, J. & Wodak, S. J. (1983). *Prog. Biophys Mol. Biol.* **42**, 21–78.

Johnson, R. S. & Schachman, H. K. (1983). *J. Biol. Chem.* **258**, 3528–3538.

Kantrowitz, E. R., Jacobsberg, L. B., Landfear, S. M. & Lipscomb, W. N. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 111–114.

Kantrowitz, E. R., Pastra-Landis, S. C. & Lipscomb, W. N. (1980*a*). *Trends Biochem. Sci.* **5**, 124–128.

Kantrowitz, E. R., Pastra-Landis, S. C. & Lipscomb, W. N. (1980*b*). *Trends Biochem. Sci.* **5**, 150–153.

Ke, H., Honzatko, R. B. & Lipscomb, W. N. (1984). *Proc. Nat. Acad. Sci., U.S.A.* **81**, 4037–4040.

Kerbiriou, D. & Hervé, G. (1972). *J. Mol. Biol.* **64**, 379–392.

Kerbiriou, D. & Hervé, G. (1973). *J. Mol. Biol.* **78**, 687–702.

Kerbiriou, D., Hervé, G. & Griffin, J. H. (1977). *J. Biol. Chem.* **252**, 2881–2890.

Kihara, H., Barman, T. E., Jones, P. T. & Moody, M. F. (1984). *J. Mol. Biol.* **176**, 523–534.

Koch, M. H. J., Stuhrmann, H. B., Vachette, P. & Tardieu, A. (1982). In *Uses of Synchrotron Radiation* (Stuhrmann, H. B., ed.), pp. 223–253, Academic Press, London.

Konigsberg, W. H. & Henderson, L. (1983). *Proc. Nat. Acad. Sci., U.S.A.* **80**, 2467–2471.

Krause, K. L., Volz, K. W. & Lipscomb, W. N. (1985). *Proc. Nat. Acad. Sci., U.S.A.* **82**, 1643–1647.

Lad, P. M. & Hammes, G. G. (1974). *Biochemistry*, **13**, 4530–4537.

Ladner, J. E., Kitchell, J. P., Honzatko, R. B., Ke, H. M., Volz, K. W., Kalb, A. J., Ladner, R. C. & Lipscomb, W. N. (1982). *Proc. Nat. Acad. Sci., U.S.A.* **79**, 3125–3128.

Lake, J. A. (1967). *Acta Crystallogr.* **23**, 191–194.

Laurent, M., Seydoux, F. J. & Dessen, P. (1979). *J. Biol. Chem.* **254**, 7515–7520.

Luzzati, V. (1980). In *Imaging Processes and Coherence in Physics, Lecture Notes in Physics* (Schlenker, M., Fink, M., Goedgebuer, J. P., Malgrange, C., Vienot, J. C. & Wade, R., eds), pp. 209–215, Springer-Verlag, New York.

Mitchell, C. G. & Weitzman, P. D. J. (1983). *FEBS Letters*, **151**, 260–264.

Monod, J., Wyman, J. & Changeux, J.-P. (1965). *J. Mol. Biol.* **12**, 88–118.

Moody, M. F., Vachette, P. & Foote, A. M. (1979). *J. Mol. Biol.* **133**, 517–532.

Perutz, M. F. & Fermi, G. (1981) *Haemoglobin and Myoglobin, Atlas of Molecular Structures in Biology*, vol. 2, Clarendon Press, Oxford.

Porter, R. W., Modebe, M. O. & Stark, G. R. (1969). *J. Biol. Chem.* **244**, 1846–1859.

Rey, F. A. & Dumas, C. (1984). *Biochimie*, **66**, 43–48.

Schachman, H. K., Pauza, C. D., Navre, M., Karels, M. J., Wu, L. & Yang, Y. R. (1984). *Proc. Nat. Acad. Sci., U.S.A.* **81**, 115–119.

Schaeffer, M. H. & Stark, G. R. (1972). *Biochem Biophys. Res. Commun.* **46**, 2082–2086.

Tauc, P., Leconte, C., Kerbiriou, D., Thiry, L. & Hervé, G. (1982). *J. Mol. Biol.* **155**, 155–168.

Thiry, L. & Hervé, G. (1978) *J. Mol. Biol.* **125**, 515–534.

Wang, C., Yang, Y. R., Hu, C. Y. & Schachman, H. K. (1981). *J. Biol. Chem.* **256**, 7028–7034.

Wang, J. H. & Tu, J.-I. (1970). *J. Biol. Chem.* **245**, 176–182.

Weber, K. K. (1968). *Nature (London)*, **218**, 1116–1119.

Wedler, F. C. & Gasser, F. J. (1974). *Arch. Biochem. Biophys.* **163**, 57–68.

Yang, Y. R. & Schachman, H. K. (1980). *Proc. Nat. Acad. Sci., U.S.A.* **77**, 5187–5191.

Yang, Y. R., Syvanen, J. M., Nagel, G. M. & Schachman, H. K. (1974). *Proc. Nat Acad. Sci., U.S.A.* **71**, 918–922.


*Edited by V. Luzzati*