

Solution X-ray scattering study of pig heart cytosolic aspartate aminotransferase

Daniel Vergé*, Annette Tardieu† and Martine Arrio-Dupont⁺

Laboratoire d'Enzymologie Physico-Chimique et Moléculaire, Bat. 433, Université Paris-Sud, 91405 Orsay and

†Centre de Génétique Moléculaire, CNRS, 91190 Gif sur Yvette and LURE, Bat. 209c Université Paris-Sud, 91405 Orsay, France

Received 2 January 1983; revision received 15 February 1983

Solution X-ray scattering experiments have been performed with apo- and holo-aspartate aminotransferase to detect the conformational change, demonstrated by a variety of techniques, induced by the binding of the coenzyme pyridoxal 5'-phosphate to apoenzyme. The conformational change could not, in fact, be observed by solution X-ray scattering. This suggests that there is no major modification of the overall shape of the enzyme upon coenzyme binding, although there are likely to be variations in the protein-solvent interactions. An accurate value of the radius of gyration, 29.0 ± 0.5 Å has been determined.

Aspartate aminotransferase

*Coenzyme binding
Synchrotron radiation*

Solution X-ray scattering

1. INTRODUCTION

Aspartate aminotransferase (AAT) (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1.), a key enzyme of nitrogen metabolism, catalyzes the reversible transfer of an amino group from L-aspartate or L-glutamate to the α -ketoacids 2-oxoglutarate or oxaloacetate. Distinct cytosolic and mitochondrial isoenzymes have been found in animal tissues. The pig heart cytosolic enzyme (which has been the most extensively studied) is a dimer composed of identical subunits contain-

ing 412 amino acids, whose sequence has been determined [1]. Analysis of the three-dimensional structure of the enzyme is in progress [2,3]. This structure has been found to be closely related to that of other AAT, the structure of which is more advanced: the three-dimensional structure of the chicken heart mitochondrial enzyme is known at 2.8 Å resolution [4] and that of the chicken heart cytosolic enzyme at 3.2 Å resolution [5,6]. The crystalline enzyme is catalytically competent [7] and AAT crystals are being used to search for reaction intermediates [7-10].

Conformational changes between apo- and holoAAT are well known. Several different experimental approaches, mainly performed on cytosolic enzyme, include: stability toward denaturing factors [11-14] and dilution [15], optical rotatory dispersion [16], fluorescence spectroscopy [17], hydrogen-deuterium exchange [18], stopped flow kinetics [19], sedimentation velocity measurements [15] and preliminary experiments using quasi-elastic light scattering [20]. These studies suggest that an important conformational

* Present address: Laboratoire de Technologie appliquée à la Microscopie Electronique, CNRS, 105 Bd Raspail, 75006 Paris, France

⁺ Present address: Laboratoire de Physiologie Cellulaire Cardiaque, INSERM U 241, Bat. 443, Université Paris-Sud, 91405 Orsay, France

Abbreviations: AAT, aspartate aminotransferase; SXRS, solution X-ray scattering; PLP, pyridoxal 5'-phosphate

change is induced by coenzyme binding. However, crystallographic analysis of both forms of the chicken mitochondrial AAT revealed no significant difference [4], except for some local changes located in the active site region [21]. These studies were performed in the presence of P_i . Kinetic experiments of the competition between PLP and phosphate for the binding to apoenzyme now suggest that phosphate alone induces a conformational change in the protein when bound to the coenzyme binding site [22]. Thus, if the changes induced by PLP and phosphate are similar, X-ray diffraction would have failed to detect them. Also, it was not excluded that the behavior of AAT could be different in the crystal and in solution.

To clarify this point, solution X-ray scattering experiments (SXRS) were performed with both apo and holoenzyme from pig heart cytosol (without phosphate). It turned out that no conformational change could be detected. This finding is discussed in reference to previous studies.

2. MATERIALS AND METHODS

2.1. Enzyme

Holoaspartate aminotransferase (α -form) was prepared from pig heart cytosol according to [23] as modified [24]. The apoenzyme, free of phosphate, was prepared according to [25] as in [26]. Enzyme concentrations were determined by absorbance measurements [17]. Transaminase activity was measured according to [27] as in [26].

2.2. Solution X-ray scattering experiments

The experiments were performed with the small-angle camera at the Synchrotron Radiation Laboratory (LURE, Orsay). The camera has been described in [28]. Briefly, X-rays were monochromated using the (111) reflection of a bent germanium crystal [29]. The wavelength was 1.54 Å and the beam cross-section at focus was 1 mm \times 0.4 mm. The synchrotron ring was operated at 1.72 GeV and 100–300 mA. The detector was a linear position-sensitive detector with delay line [30] (as modified by M. Lemonnier). The data were recorded in a Tracor TN 1710 multichannel analyser prior to storage on a PDP disk.

The data were recorded in two different series of experiments. Low angle data, 1.4×10^{-3}

$\text{\AA}^{-1} < s < 1.4 \times 10^{-2} \text{\AA}^{-1}$ ($s = \sin \theta / \gamma$) were recorded with a sample to detector distance of 850 mm. Higher angle data, $4 \times 10^{-3} \text{\AA}^{-1} < s < 4 \times 10^{-2} \text{\AA}^{-1}$, were recorded with a sample-to-detector distance of 640 mm. Absolute scale measurements were obtained with reference to scattering from a pre-calibrated black carbon sample. After solvent subtraction, the data were corrected for collimation distortions as in [31].

The samples were contained in quartz capillary tubes ≈ 1 mm diam. About 20 μ l were used for each experiment. All samples were prepared in 0.05 M triethanolamine-HCl buffer (pH 8.3). The solutions were concentrated by ultrafiltration on ultragaines UH 100/25 (Schleicher and Schull) and centrifuged before use. Samples were 7–20 mg/ml for low angle experiments. Exposure was for 5 min. Samples were 20–100 mg/ml for higher angle experiments and exposure varied from 10–20 min. The enzymatic activity was found to be identical before and after the experiments.

3. RESULTS

For the analysis of low angle data, we made use of Guinier plots, namely the logarithm of intensity vs s^2 . Such plots yield straight lines whose slope is proportional to the square of the radius of gyration of the particle and whose extrapolation to the

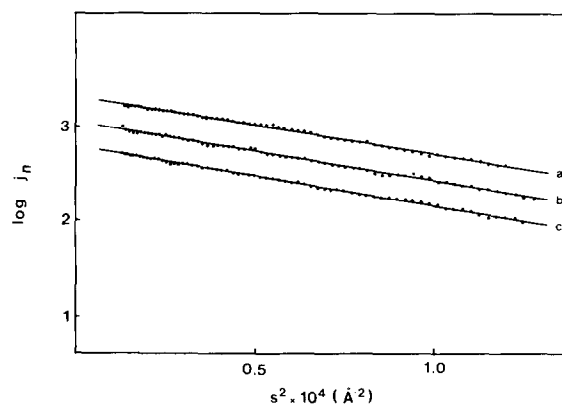


Fig. 1. Examples of Guinier plots for AAT: (a) holoenzyme, 15.2 mg/ml; (b) holoenzyme, 9.0 mg/ml; (c) apoenzyme, 9.8 mg/ml. The intensities are given in arbitrary units. They are corrected for background and detector response but otherwise are unsmoothed and uncorrected for collimation distortions. Note that these plots are linear over an extended range.

origin permits the determination of the particle M_r [32]. Typical curves are shown in fig. 1.

We found M_r $97\,000 \pm 8000$ in excellent agreement with the value calculated from the primary sequence: $93\,000$ [1]. This result, together with the observed linearity of the Guinier plots, indicates

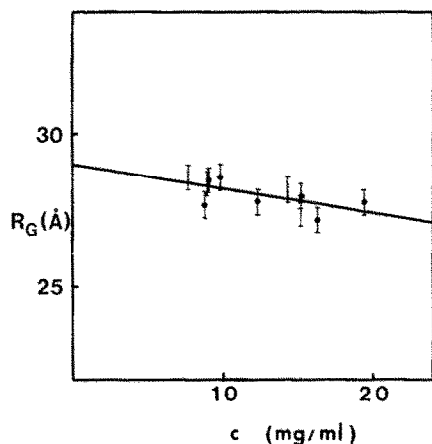


Fig. 2. Variation of the radius of gyration as a function of concentration for the (●) apo- and (○) holoenzyme. The values are corrected for collimation distortions. Given the quality of the data (fig. 1), the error bars derive mainly from the uncertainty ($\pm 2\%$) in the coefficient of the background subtracted.

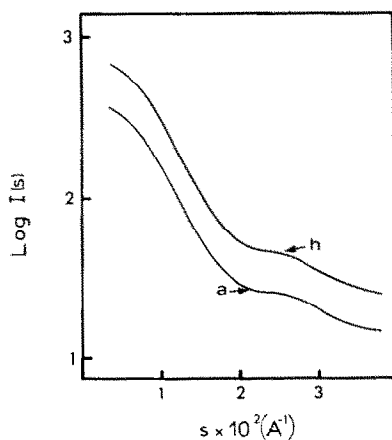


Fig. 3. Example of extended scattering curves of (upper) holo- and (lower) apoenzyme. The intensities are given in arbitrary units and are vertically shifted to facilitate comparison. The data are uncorrected for background or detector response. They were recorded under exactly the same concentration conditions for both apo- and holoenzyme (43 mg/ml).

that the enzyme is not contaminated with aggregates in solution.

The variation of the radius of gyration as a function of concentration, for both apo- and holoenzyme, is shown in fig. 2. It is clear from the figure that no significant difference in the radius of gyration can be detected between holo- and apoenzyme. The value extrapolated to zero concentration from the whole of the experimental data is equal to 29.0×0.5 Å.

Experiments at higher concentration were performed in the attempt to detect a possible difference between apo- and holoenzyme in the higher angle part of the scattering curves. Scattering curves, under identical concentration conditions, were also found to be similar (e.g., fig. 3). Consequently, the data were not analyzed further.

4. DISCUSSION

From the X-ray scattering study [33], a value of 32.6 ± 0.9 Å was reported for the radius of gyration, definitely higher than the value we have measured (29.0 ± 0.5 Å). Possible explanations for such a discrepancy are the lower accuracy in [33] or the presence of a small amount of aggregates, or, more simply, that the preparation in [33] was a mixture of several subforms which are known to have different sugar contents [24,34,35]. Here, the good agreement obtained for the M_r and perfect linearity of the Guinier plots argues for the absence of aggregates; moreover, the pure α -form (free of sugars) of AAT was used.

The results obtained here can also be compared to the crystallographic data. According to them, the AAT molecule can be approximated at low resolution by an ellipsoid of dimensions $105 \times 60 \times 50$ Å [4]. The calculated radius of gyration of such an ellipsoid is 29.3 Å which compares well with our experimental value. A sphere occupying the same volume would have a radius of gyration of 26.3 Å.

The fact that we did not detect conformational changes induced by the binding of the coenzyme does not mean that they do not occur. This result suggests that conformational changes determined by spectroscopic experiments are local and do not correspond to large rearrangements of the molecule. They probably reflect the changes around the active site demonstrated by crystallographic

analysis. Such local changes would not be detected in these experiments. However, other changes could also occur, that would remain unnoticed with SXRS. For instance, as the coenzyme binding site possesses several charged groups, removal of the coenzyme could lead to differences in protein-solvent interactions. A difference in the internal motions of the molecule could also occur, as suggested by the lower stability of the apoenzyme toward denaturation [11-14], the facilitated dissociation of apoenzyme into subunits upon dilution [15,36] and by hydrogen-deuterium exchange experiments [18].

Thus, we are led to conclude, in agreement with diffraction experiments, that the conformational change between the apo- and holoenzyme does not correspond to a major modification of the overall shape of the enzyme. However, this conformational change can involve local changes and, in solution, may be associated with modifications of the protein-solvent interactions and/or with larger structural fluctuations of the apoenzyme compared to holoenzyme.

ACKNOWLEDGEMENTS

We thank J. Carrette and B. Krop for excellent technical assistance.

REFERENCES

- [1] Ovchinnikov, Y.A., Egorov, T.A., Aldanova, N.A., Feigina, M.Y., Lipkin, V.M., Abdulaev, N.G., Grishin, E.V., Kiselev, A.P., Modyanov, N.N., Braunstein, A.E., Polyanovsky, O.L. and Nisikov, V.C. (1973) *FEBS Lett.* 29, 31-34.
- [2] Arnone, A., Rogers, P.H., Schmidt, J., Han, C.N., Harris, C.M. and Metzler, D.E. (1977) *J. Mol. Biol.* 112, 509-513.
- [3] Arnone, A., Briley, P.D., Rogers, P.H., Hyde, C.C., Metzler, C.M., Metzler, D.E. (1981) *Fed. Proc. FASEB* 40, 1757.
- [4] Ford, G.C., Eichele, G. and Jansonius, J.N. (1980) *Proc. Natl. Acad. Sci. USA* 77, 2559-2563.
- [5] Borisov, V.V., Borisova, S.N., Sosfenov, N.I. and Vainshtein, B.K. (1980) *Nature* 284, 189-190.
- [6] Harutyunyan, E.G., Malashkevich, V.N., Tersyan, S.S., Kochkina, V.M., Torchinsky, Y.M. and Braunstein, A.E. (1982) *FEBS Lett.* 138, 113-116.
- [7] Eichele, G., Karabelnik, D., Halonbrenner, R., Jansonius, J.N. and Christen, P. (1978) *J. Biol. Chem.* 258, 5239-5242.
- [8] Metzler, C.M., Metzler, D.E., Martin, D.S., Newman, R., Arnone, A. and Rogers, P. (1978) *J. Biol. Chem.* 253, 5251-5254.
- [9] Mozzarelli, A., Ottonello, S., Rossi, G.L. and Fasella, P. (1979) *Eur. J. Biochem.* 98, 173-179.
- [10] Makarov, V.L., Kochkina, V.M. and Torchinsky, Y.M. (1980) *FEBS Lett.* 114, 79-82.
- [11] Schlegel, H. and Christen, P. (1974) *Biochem. Biophys. Res. Commun.* 61, 117-123.
- [12] Ivanov, V.I., Volkenstein, M.V., Karpeisky, M.Y., Moras, S. and Okina, E.I. (1973) *Eur. J. Biochem.* 40, 519-526.
- [13] Relimpio, A., Iriarte, A., Chlebowsky, J.F. and Martinez-Carrion, M. (1981) *J. Biol. Chem.* 256, 4478-4488.
- [14] Gartosio, A., Salerno, C., Franchetta, F. and Turano, C. (1982) *J. Biol. Chem.* 257, 8163-8170.
- [15] Cournil, I., Barba, J.M., Vergé, D. and Arrio-Dupont, M. (1975) *J. Biol. Chem.* 250, 8564-8568.
- [16] Torchinsky, Y.M. and Kogan, G.A. (1970) *Mol. Biol.* 4, 860-865 (Rus); 696-700 (Engl).
- [17] Arrio-Dupont, M. (1978) *Eur. J. Biochem.* 91, 369-378.
- [18] Abaturon, L.V., Polyanovsky, O.L., Torchinsky, Y.M. Varshavsky, Y.M. (1968) in: *Pyridoxal Catalysis: Enzymes and Model Systems* (Snell, E.E. et al. eds) pp. 171-177, Wiley-Interscience, London, New York.
- [19] Vergé, D. and Arrio-Dupont, M. (1981) *Biochemistry* 20, 1210-1216.
- [20] Vergé, D., Jullien, M., Tardieu, A. and Arrio-Dupont, M. (1981) *Cong. Soc. Franc. de Phys., Clermont-Ferrand*, p. 321.
- [21] Eichele, G. (1980) PhD Thesis, Basel University.
- [22] Vergé, D. (1981) PhD Thesis, Université de Paris-Sud, Orsay.
- [23] Martinez-Carrion, M., Turano, C., Chiancone, E., Bossa, F., Giartosio, A., Riva, F. and Fasella, P. (1967) *J. Biol. Chem.* 242, 2397-2409.
- [24] Bossa, F., John, R.A., Barra, D. and Fasella, P. (1968) *FEBS Lett.* 2, 115-117.
- [25] Wada, H. and Snell, E.E. (1962) *J. Biol. Chem.* 237, 127-132.
- [26] Arrio-Dupont, M. and Vergé, D. (1982) *J. Mol. Biol.* 157, 383-394.
- [27] Karmen, A. (1955) *J. Clin. Invest.* 34, 131-133.
- [28] Koch, M.H.J., Stuhmann, H.B., Tardieu, A. and Vachette, P. (1983) in: *Uses of Synchrotron Radiation in Biology* (Stuhmann, H.B. ed) Academic Press, New York, in press.
- [29] Lemonnier, M., Fourme, R., Rousseaux, F. and Kahn, R. (1978) *Nucl. Instrum. Meth.* 152, 173-177.
- [30] Gabriel, A. (1977) *Rev. Sci. Instrum.* 48, 1303-1305.

- [31] Lake, J.A. (1967) *Acta Crystallogr. A* 23, 191-194.
- [32] Luzzati, V. (1960) *Acta Cryst.* 13, 939-945.
- [33] Esipova, N.G., Dembo, A.T., Tumanyan, V.G. and Polyansky, O.L. (1968) *Molekul. Biol.* 2, 527-535 (Rus); 424-430 (Engl).
- [34] Denisova, G.F. and Polyansky, O.L. (1973) *FEBS Lett.* 35, 129-132.
- [35] Arrio-Dupont, M., Carrette, J., Fournet, B. and Montreuil, J. (1981) *Biochimie* 63, 975-977.
- [36] Polyansky, O.L. (1968) in: *Pyridoxal Catalysis: Enzymes and Model systems*, (Snell, E.E. et al. eds) p. 155, Wiley-Interscience, London, New York.