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# Small-Angle X-Ray Scattering Studies of the Open and Closed Conformations of Aspartate Aminotransferase

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**Summary.** Aspartate aminotransferase was investigated by X-ray small-angle scattering. A small difference was found between the "open" (active) and the "closed" (liganded) conformation of the enzyme. The results were compared with X-ray crystallography data.

**Keywords.** Aspartate aminotransferase; Conformational changes; Small-angle X-ray scattering; Solution structure.

**Untersuchungen zur Röntgenkleinwinkelstreuung der offenen und geschlossenen Konformation von Aspartat-Aminotransferase**

**Zusammenfassung.** Aspartat-Aminotransferase wurde mittels Röntgenkleinwinkelstreuung untersucht. Ein kleiner Unterschied zwischen der „offenen" (aktiven) und der „geschlossenen" (ligandierten) Konformation wurde gefunden. Die Ergebnisse wurden mit Röntgenkristallstrukturdaten verglichen.

*Abbreviations:* AspAT, aspartate aminotransferase.

## Introduction

The structure of aspartate aminotransferase (*L*-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) has been extensively investigated by various chemical and physical methods, including X-ray crystallography [1–3]. This enzyme undergoes a so called "syncatalytic" conformational change during the transamination reaction.

AspAT is a dimeric enzyme with a molecular weight of about 90.000. It contains pyridoxal phosphate as a coenzyme. In the presence of certain active-site ligands (substrate analogs) the small domain has moved towards the active site on the large domain, thus narrowing the wide active-site crevice. The two different conformational states have been designated as the "open" (active) and the "closed" (liganded) form. X-ray crystallography data have been collected and refined recently, enhancing our knowledge of the structural basis for the catalytic activity of AspAT [4].

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Small-angle X-ray scattering is a useful tool for the determination of the size and shape of biological macromolecules in solution. Although not sensitive enough to detect minor changes in local conformation, it proves valuable in the investigation of larger conformational variations caused by relative shifts or rearrangements of whole subunits or domains [5].

## Experimental

Mitochondrial AspAT from chicken has been cloned by the group of Ph. Christen, Biochemisches Institut der Universität Zürich, Switzerland. The preparation method has been reported previously [6].

For the scattering experiments the enzyme solution was dialysed against 50 m*M* sodium phosphate buffer (*pH* 7.5), containing pyridoxal phosphate (0.2 m*M*) and α-ketoglutarate (2 m*M*). To obtain the "closed" conformation α-methylaspartate (35 m*M*) or maleate (25 m*M*) was added.

Scattering curves were recorded stepwise with a conventional Kratky camera [7], in the range $h = 0.09$ to $4.0\,\mathrm{nm}^{-1}$, where $h = (4\pi \sin\theta)/\lambda$ ($2\theta$ = scattering angle, $\lambda = 0.154$ nm, wavelength of the CuKα line). The protein samples were cooled to 4.5°C during the scattering experiments. Enzyme concentrations were in the range 6 mg/ml to 60 mg/ml. Data evaluation and desmearing by indirect Fourier transformation was done as described elsewhere [5].

Theoretical scattering and distance distribution functions were calculated using a finite element method. Models were composed of small identical spheres with a diameter smaller than the resolution of the X-ray small-angle method. The model scattering curves were calculated using Debye's formula, while the distance distribution functions were calculated independently from the coordinates of the spheres, i.e. without Fourier transformation of the scattering curve [8].

Atom coordinates of both conformations of AspAT were supplied by Prof. J. N. Jansonius and Dr. M. G. Vincent, Biozentrum Basel, Switzerland.

The unliganded holoenzyme (pyridoxal form) was investigated in the presence of the coenzyme pyridoxal-5′-phosphate (0.2 m*M*) and the substrate α-ketoglutarate (2 m*M*). Under these conditions the enzyme should be in the active ("open") conformation. To obtain the "closed" conformation AspAT was liganded with two different substrate analoga (inhibitors): α-methyl aspartate (35 m*M*) and maleate (25 m*M*), respectively.

## Results and Discussion

The distance distribution functions (calculated from the experimental scattering data) are shown in Fig. 1. The molecular parameters determined from the experimental scattering data are presented in Table 1. The value of the radius of gyration obtained for the "open" conformation, $2.93 \pm 0.03$ nm, is in good agreement with that obtained by synchrotron radiation ($2.90 \pm 0.05$ nm), reported by Verge et al. [9].

While the differences in the two conformations are hardly visible in the scattering curves, they are more evident in the distance distribution functions. The shift of the maximum to lower distances $r$ indicates that the molecule in the "closed" form might be somewhat more compact, but the maximum diameter remains unchanged (10 nm). The "tightening" of the molecule is also reflected by a decrease of the radius of gyration (−2%), the radius of gyration of the cross section (−2%), and the hydrated volume (−3%). The two substrate analoga (maleate and α-methyl aspartate) seem to have the same effect on AspAT.

Model calculations were based on atom coordinates obtained by X-ray crystallography [4]. The models were composed of identical spheres (radius 0.34 nm),

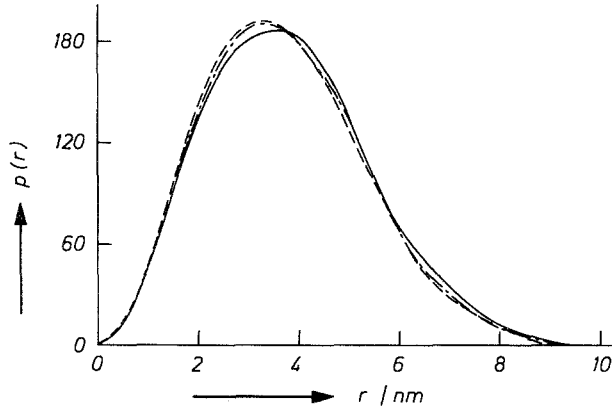


**Fig. 1.** Comparison of experimental distance distribution functions of AspAT (liganded with two different substrate analoga) with that of the unliganded enzyme; (– · –) AspAT + maleate, (– – –) AspAT + α-methylaspartate, (———) unliganded AspAT

**Table 1.** Molecular parameters of aspartate aminotransferase determined from the experimental scattering and distance distribution functions [$R$ radius of gyration (nm), $R_c$ radius of gyration of the cross section (nm), $V$ hydrated volume (nm$^3$)]

| | $R$ | $R_c$ | $V$ |
|---|---|---|---|
| AspAT ("open") | 2.93 ± 0.03 | 1.97 ± 0.02 | 155 ± 5 |
| AspAT + maleat ("closed") | 2.89 ± 0.03 | 1.93 ± 0.02 | 150 ± 5 |
| AspAT + α-methyl aspartate ("closed") | 2.87 ± 0.03 | 1.93 ± 0.02 | 150 ± 5 |

each sphere representing one amino acid. The center of the sphere was placed at the center of gravidity of the corresponding amino acid.

Model fitting was done by comparing the theoretical scattering and distance distribution functions with the experimentally determined curves, and the molecular parameters calculated from these functions (Table 2). The models are shown in Fig. 2.

It turned out that the bulk movement of the small domains (Fig. 2 b) has indeed been responsible for the shift of the maximum of the distance distribution function (Fig. 1). Thus it has been proved that the "syncatalytic" change proposed by crystallography data can also be observed in solution, i.e. in the natural environment of the enzyme.

**Table 2.** Molecular parameters of aspartate aminotransferase determined from theoretical scattering and distance distribution functions [$R$ radius of gyration (nm), $R_c$ radius of gyration of the cross section (nm)]

| | $R$ | $R_c$ |
|---|---|---|
| AspAT, "open" | 2.92 | 1.92 |
| AspAT, "closed" | 2.87 | 1.88 |

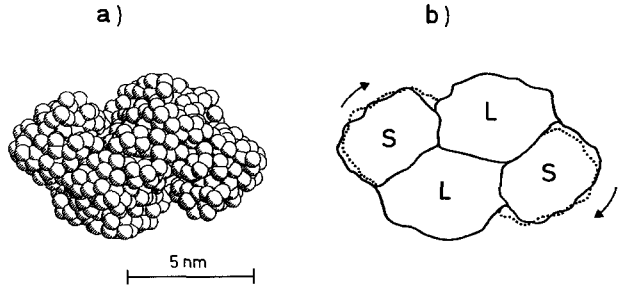


**Fig. 2. a** Model for AspAT in the "open" conformation, calculated from X-ray crystallography data. Each sphere represents one amino acid. **b** Schematic representation of the bulk movement of the small domain. *L*: large domain; *S*: small domain; solid: small domain in the "open" conformation; dotted: small domain in the "closed" conformation. Reconstructed from X-ray crystallography data [4]

In both cases ("open" and "closed") the crystal dimensions had to be expanded slightly to fit the experimentally found values of the radii of gyration. Considering the errors of the experimental values (about ±1%, cf. Table 1), the difference is not very significant, but a swelling in solution of 3–4% can be assumed for both conformations of aspartate aminotransferase.

Again it has been shown that scattering methods – though low in resolution – can be used to investigate minor rearrangements of macromolecule domains. Conformational models proposed on the basis of crystal data can thus be tested for their validity in solution.

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