THREE-DIMENSIONAL STRUCTURE AT 3.2 Å RESOLUTION OF THE COMPLEX OF CYTOSOLIC ASPARTATE AMINOTRANSFERASE FROM CHICKEN HEART WITH 2-OXOGLUTARATE

E. G. HARUTYUNYAN, V. N. MALASHKEVICH, S. S. TERSYAN, V. M. KOCHKINA, Yu. M. TORCHINSKY and A. E. BRAUNSTEIN

Institute of Crystallography and *Institute of Molecular Biology, USSR Academy of Sciences, Moscow, USSR*

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1. Introduction

Aspartate aminotransferase (EC 2.6.1.1; L-aspartate: 2-oxoglutarate aminotransferase, AATase) is one of the key enzymes of nitrogen metabolism [1]. The tissues of vertebrates contain 2 homologous isozymic forms of this enzyme: a cytosolic and a mitochondrial form. Both isoenzymes are dimeric proteins, consisting of 2 identical subunits of $M_r \sim 45\,000$. Currently, X-ray structural investigations are underway of chicken cAATase and mAATase [2–4] and of porcine cAATase [5]. Here, we report studies of the three-dimensional structure of chicken cAATase at 3.2 Å resolution. The sequence of its peptide chain is known [6].

2. Methods

Isolation of cAATase from chicken hearts and the procedure for preparation of single crystals of the enzyme have been reported [7,8]. Crystals (space group $P2_12_12_1$ [2]) were transferred into a suspension medium containing $(NH_4)_2SO_4$ (0.6 saturated), 0.3 M phosphate (pH 7.5) and 0.1 M 2-oxoglutarate. Diffraction data were collected on a Syntex $P2_1$ diffractometer. Eight heavy-atom derivatives were obtained and utilized to a different resolution limit (see table 1). The phases obtained by the use of isomorphous derivatives at 3.2 Å resolution were refined by averaging electron densities for the 2 subunits related by non-crystallographic symmetry [9]. The mean figure of merit before and after the refinement was 0.71 and 0.82, respectively.

Abbreviations: cAATase, cytosolic aspartate aminotransferase; mAATase, mitochondrial aspartate aminotransferase; PLP, pyridoxal-5′-phosphate; *p*-CMB, *p*-chloromercuribenzoate; MMA, methylmercuriacetate; DTNB, 5,5′-dithiobis-(2-nitrobenzoate)

3. Results

The electron density map computed at 3.2 Å resolution allowed us to trace the entire polypeptide chain and to determine the coordinates of the C^α atoms. The chicken heart cAATase molecule is an α/β protein, containing 15 α-helices and 13 β-strands/subunit. The topology-packing diagram is shown in fig.1. As seen in this diagram, the subunit of cAATase can be subdivided into 3 parts: the N-terminal part, comprising helices 1 and 2 and β-strands h' and a'; the large, PLP-binding domain, containing a strongly twisted 7-stranded β-sheet surrounded by α-helices, and the C-terminal part, which consists of helices 13,14,15 and β-strands b',c',d'. The N- and C-termini are closely associated, forming a small domain. The electron density in the area of the small domains is somewhat lowered – to a different extent in the 2 subunits; this may be due to differences in their mobilities [4].

Fig.2 shows the course of the polypeptide chain and secondary structure in a cAATase subunit and the disposition of the molecules of PLP and 2-oxoglutarate. The electron densities corresponding to these molecules are of identical intensity and shape in both subunits. The N-terminus of each subunit (residues 1–14) protrudes from the small domain and closely adheres

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Table 1
Heavy-atom derivatives and phase refinement statistics

Derivative	N_{ref}	d_{max} (A)	N_s	E/f_H	R_{st}	R_K	R_c
p-CMB/DTNB*	4444	5.00	4	0.61	0.62	0.10	0.17
p-CMB	11 243	3.65	5	0.81	0.71	0.11	0.17
MMA	15 691	3.20	6	0.53	0.55	0.11	0.20
Mersalyl	4431	5.00	6	0.81	0.74	0.19	0.28
K_2PtCl_4	4450	5.00	10	0.71	0.66	0.11	0.16
$HAuCl_4$	16 535	3.20	6	0.44	0.46	0.10	0.22
K_3IrCl_6	8932	4.00	8	0.57	0.58	0.12	0.23
MMA/DTNB*	8112	4.00	6	0.64	0.60	0.11	0.19

* The derivatives *p*-CMB/DTNB and MMA/DTNB were obtained by soaking of the crystals first in DTNB solution and then in solutions of *p*-CMB or MMA

N_{ref}, number of reflections measured; d_{max}, resolution limit; N_s, number of heavy atom sites per dimer; E, RMS lack of closer error; f_H, RMS heavy atom structure factor

$$R_{st} = \Sigma||F_{PH}\,(exp)| - |F_{PH}\,(calc)||/\Sigma||F_{PH}\,(exp)| - |F_P||$$

$$R_K = \Sigma||F_{PH}\,(exp)| - |F_{PH}\,(calc)||/\Sigma|F_{PH}\,(exp)|$$

$$R_c = \Sigma||F_P| - |F_{PH}\,(exp)||/\Sigma|F_P|$$

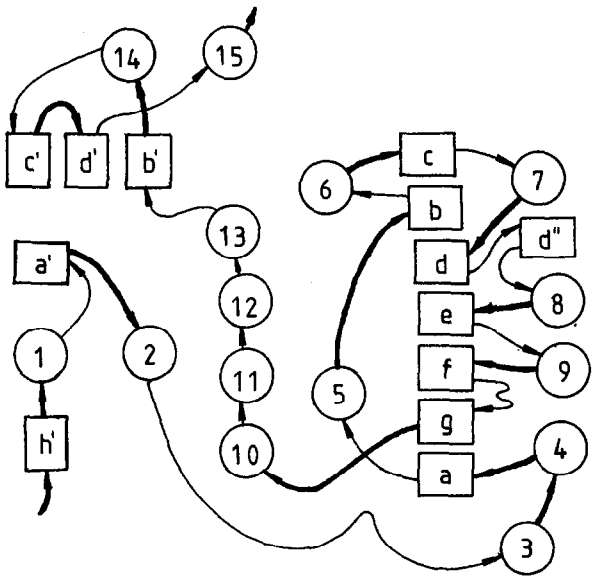

Fig.1. Topology-packing diagram of a subunit of cAATase. α-Helices and β-strands are denoted as in [4]: (○) helices; (□) β-strands.

to the surface of the neighbouring subunit's large domain.

The active center is situated in a deep cleft formed by the walls of the large and small domains of one subunit and a border of the adjacent subunit's large domain. β-Strand h' and the first turns of helix 1 of the small domain partly overlay the entrance from solvent into the active center cavity. Several amino acid residues of the adjacent subunit participate in formation of the active center.

The A side of the coenzyme's pyridine ring faces the β-sheet of the large domain, and the B side faces the solvent and the Trp-140 residue situated above the ring (the faces of the ring are designated as in [4]). The plane of the pyridine ring is inclined towards the molecular axis under an angle of ~35°. The N^1 atom of the ring is bonded to the Asp-222 residue in β-strand *e* belonging to the β-sheet. The phosphate 'handle' of PLP points towards the molecular axis and is situated at ~10 Å distance from the latter; the $C^{5'}$–O bond is nearly parallel to the molecular axis,

Fig.2. Schematic drawing of a cAATase subunit. The molecular axis is perpendicular to the plane of the drawing; its position is designated by (●); (– – –) stretches of polypeptide chain from the adjacent subunit; helices are shown in the form of cylinders; β-strands in the form of arrows. The zigzag line near the PLP ring denotes 2-oxoglutarate.

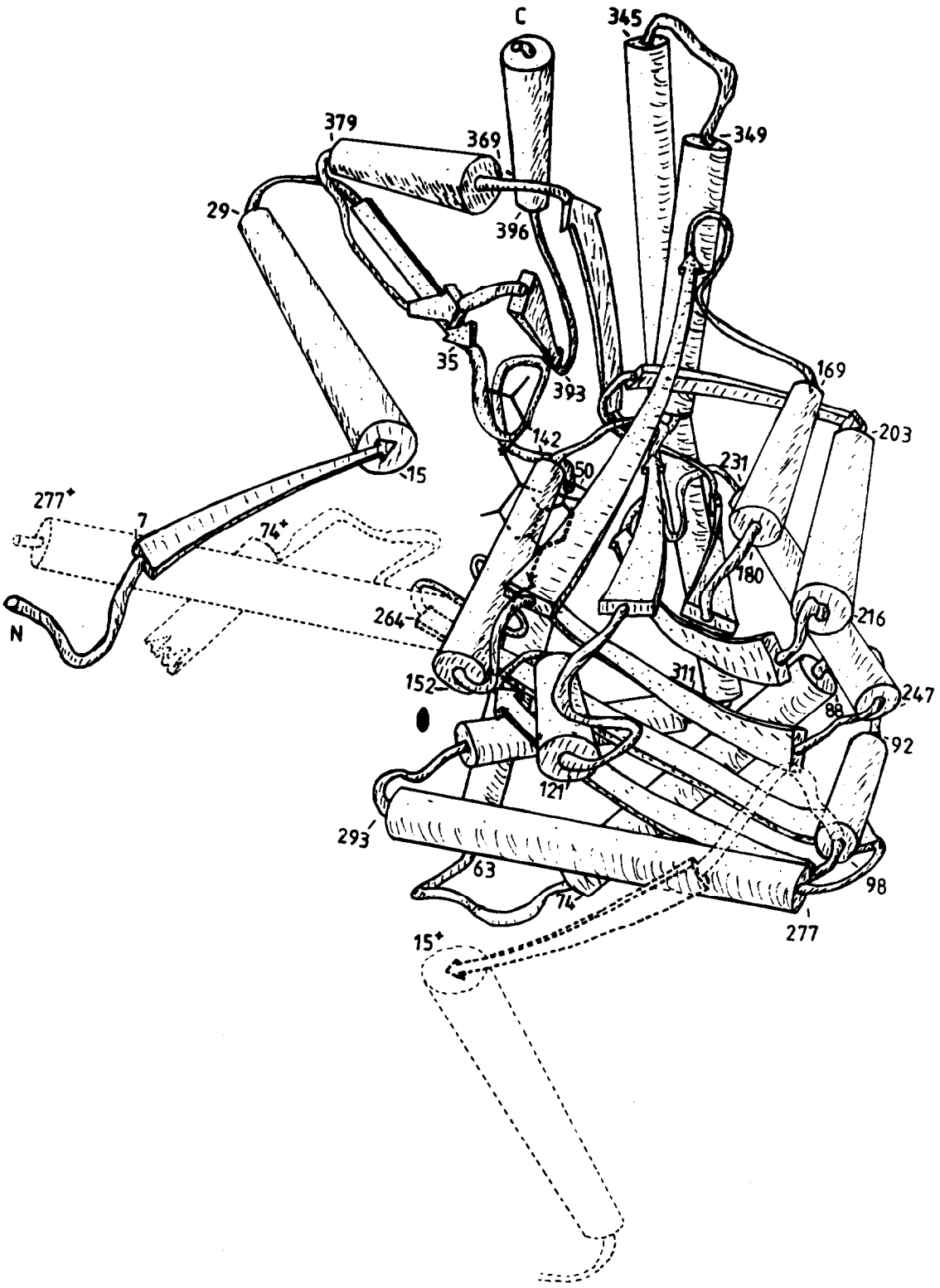
C
345
379
369
349
29
396
35
393
169
203
142
50
231
15
277+
7
74+
180
264
216
N
311
152
247
88
92
121
293
63
74
98
277
15+

Fig.2

and forms an angle of 30–35° with the plane of the pyridine ring. The phosphate group is located in a pit formed by residues Gly-107, Gly-108, Thr-109, Ser-255, Ser-257, Arg-266, Tyr-70* and, possibly, Ser-296* (*residues belonging to the adjacent subunit). The Lys-258 residue, forming the internal aldimine bond with PLP, is situated in the loop between β-strands *f* and *g*. Residue Tyr-225 closely adjoins the coenzyme in proximity of atom $O^{3'}$ and the aldimine bond.

The electron density corresponding to the 2-oxoglutarate molecule is located between the cationic side chains of Arg-386 and Arg-292*, and in close proximity to the coenzyme ring (at the level of its lower border, i.e., of the $O^{3'}$ atom). Besides the two arginine residues, Val-17, Phe-18, Val-37, Asn-194, Phe-360 and Met-382 are also present at the substrate binding site.

Comparison of our data with those in [4] testifies to a high degree of homology between the three-dimensional structures of chicken cAATase and mAATase. Between these structures there are minor differences, mainly in regard to the lengths of some elements of secondary structure, as well as the presence of 2 short additional β-strands, h' and d'' in cAATase.

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