

Molecular Structure of Taka-Amylase A

I. Backbone Chain Folding at 3 Å Resolution

Yoshiki MATSUURA, Masami KUSUNOKI, Wakako HARADA,
Nobuo TANAKA, Yuichi IGA, Noritake YASUOKA,
Hiroko TODA, Kozo NARITA, and Masao KAKUDO

Institute for Protein Research, Osaka University, Suita, Osaka 565

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The crystal structure of Taka-amylase A was studied by an X-ray diffraction method at 3 Å resolution. A total of 452 amino acid residues were found from the electron density map at the present stage. The four disulfide bonds and the branched carbohydrate were also located on the map. The difference electron density map of the maltotriose-soaked crystal showed that a maltose unit was bound in the active center cleft. The binding of iodine atoms to the enzyme was also studied.

Taka-amylase A [EC 3.2.1.1], an α -amylase produced by *Aspergillus oryzae*, has been extensively studied by both biochemical and physicochemical methods. This protein crystallizes in space group $P2_1$ with cell dimensions of $a=91.9$, $b=133.3$, $c=94.3$ Å and $\beta=102.7^\circ$, and three molecules in the asymmetric unit. A low resolution X-ray structure analysis of this protein has already been reported (1), and in this paper we will report the results of further analysis which was extended to a resolution of 3 Å with the three heavy atom derivatives.

Intensity data for the native and the derivative crystals were collected with a Rigaku automated four-circle diffractometer with the Ni-filtered $\text{Cu-K}\alpha$ radiation from a rotating anode generator (40 kV, 200 mA). A set of independent reflections were obtained from 25 to 30 crystals for each of the native, $\text{UO}_2(\text{NO}_3)_2$, HgCl_2 , and $\text{KAu}(\text{CN})_2$ derivatives, respectively. The number of independent reflections included in the 6 to 3 Å resolution range was about 39,600. The refinement of heavy atom parameters was performed by means

of the usual alternating phase determination and least-squares method with 9,166 reflections selected so as to include nearly equal numbers of reflections in the ten subdivided shells of $\sin\theta/\lambda$. The results of the refinement are summarized in Table I. The best electron density distribution at 3 Å resolution was calculated with the phases obtained from the low resolution analysis and the present analysis. The averaged figure of merit was 0.67 in the present range of resolution, 0.73 for the whole range within 3 Å. As discussed in the previous paper, the calculated electron density map was converted to maps of the three independent molecules referring to each molecular coordinate system. The averaged electron density map calculated from these three maps was particularly useful for tracing the peptide chain folding. By use of a Richard's optical comparator, a skeletal model of the protein (1 Å=2 cm) was constructed from the averaged electron density map while occasionally comparing it with the individual molecular maps. A total of 452 peptide units were connected without too much trouble through

TABLE I. Summary of refinement.

Resolution	Averaged figure of merit $\langle m \rangle$	Number ^a of reflections observed	Heavy atom reagents soaked	Kraut's R (R _k)	Centric R (R _c)
∞ —7.4	0.91	3,200	UO ₂ (NO ₃) ₂	0.116	0.592
			HgCl ₂	0.105	0.501
			KAu(CN) ₂	0.098	0.506
			AgNO ₃	0.074	0.486
			K ₂ PdCl ₄	0.053	0.596
			K ₂ PtCl ₄	0.128	0.628
			K ₂ Pt(CN) ₄	0.076	0.628
7.4—6.0	0.82	2,700	UO ₂ (NO ₃) ₂	0.125	0.657
			HgCl ₂	0.112	0.483
			AgNO ₃	0.112	0.541
			K ₂ PdCl ₄	0.085	0.708
6.0—3.0	0.67	39,600	UO ₂ (NO ₃) ₂	0.104	0.685
			HgCl ₂	0.096	0.646
			KAu(CN) ₂	0.119	0.727
∞ —3.3	0.73	45,500			

the continuous electron density hump. However, it still remains to be determined in detail whether or not some residues, in particular, those of both terminal ends are smeared out to the solvent region in the density map, because the number of residues found in the map is somewhat less than that deduced from the chemical analysis (2, 3). Therefore, the sequence number for each residue is not given in this paper. The skeletal model and an illustration of the backbone chain are shown in Figs. 1 and 2, respectively. As an example of the electron density distribution, the H₈ helix is shown in Fig. 3, which was displayed by the Vector General graphics system developed here. About 20% of the total residues are involved in the α -helices (H₁ to H₈). There are some β -pleated sheets as well, as shown in Fig. 2. Four disulfide bonds were clearly recognized at S₁ to S₄ on the electron density map. A carbohydrate group (4) was found near an asparagine residue as a branched electron density, projecting towards the solvent region and smeared out gradually in the solvent. The illustration of the whole chain folding indicates some characteristic features of enzyme structure which have so far been recognized in general. The molecule is composed of two domains: the main and the C-terminal domains which are connected to each other by a single polypeptide chain. The topology of the main domain is quite similar to that of triose phosphate

isomerase (5) and is classified as class IV defined by Levitt and Chothia (6). On the other hand, the C-terminal domain corresponds to class II which has only β -sheets. One can easily distinguish the cleft as a particular region from other parts of the molecule in the upper right of the illustration. The cleft¹, about 25 $\times$ 15 Å in length and width on the molecular surface, is walled by several curving corners of the chain folding, so as to provide some functional side chains towards the active site. The whole architecture around the cleft is protected by the thick core of chain folding which contains most of the α -helices and β -sheets. The inhibitor molecules were found in the cleft and in the present analysis, the difference Fourier map using a derivative of maltotriose, a poor substrate, at 4 Å resolution gave an electron density hump corresponding to two glucose units in size at the same site as inhibitor site II in the cleft. We may conclude that the bond cleaving reaction of the α -1,4-glycosidic linkage occurs around this site. This site is located near the end of the cleft, and some potential residues for hydrolysis reaction, such as His, Asp, Lys, and Glu, are close to it, referring to the amino acid sequence map.

¹ The term 'hollow' was used in the low resolution study (1) to express the feature around the active center, but the term 'cleft' seems to be more suitable at this resolution.

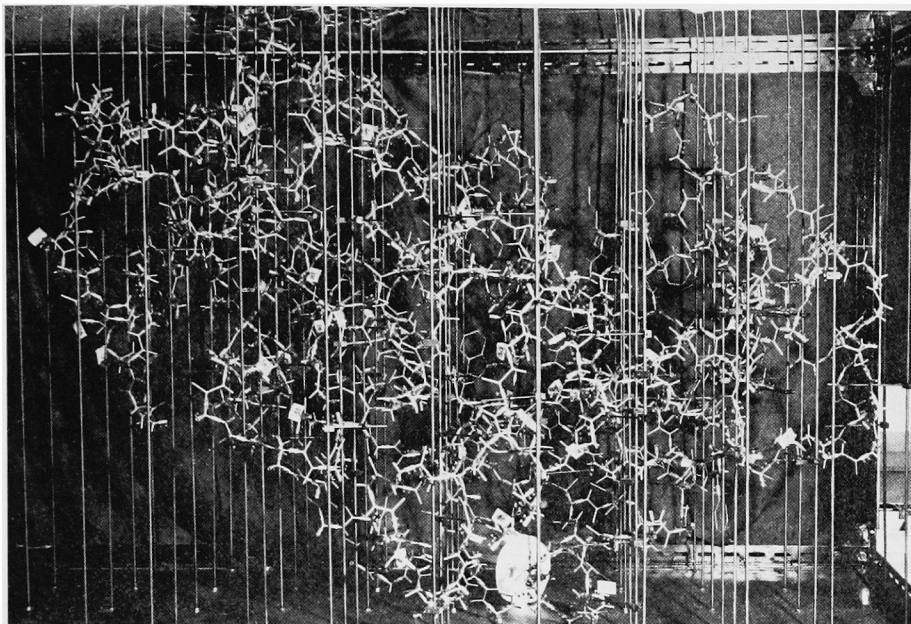


Fig. 1. Overall view of the skeletal model of Taka-amylase A at the scale of $1 \text{ \AA} = 2 \text{ cm}$; The model was constructed from main chain units and some distinguishable residues. A cleft is seen in the upper right.

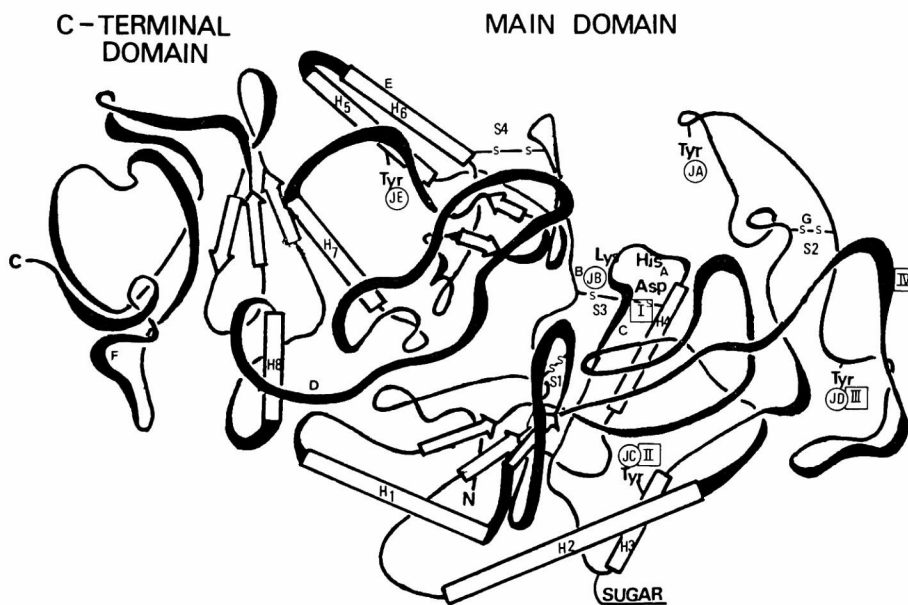


Fig. 2. Illustrative figure of the chain folding of the molecule.

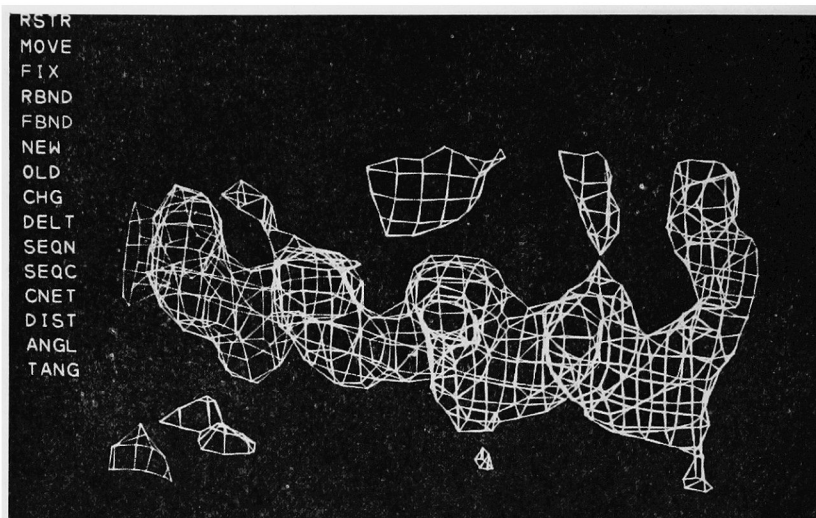


Fig. 3. The electron density distribution in the region of the α -helix, H_8 , which was displayed by the Vector General system.

In addition to the above mentioned analysis, an iodine derivative (1 mM I_2 and 5 mM KI, 75% satd. ammonium sulfate, pH 5.5) was prepared according to Schramm (7). A difference Fourier map at 7.4 Å resolution of this crystal showed five sites of iodine, for each molecule. As shown in Fig. 2, one of the iodine atoms was found near the disulfide bridge, S_3 , which is located at the back of the molecule. Other atoms were found to be bound to the aromatic rings of the tyrosinyl residues, one of which hangs out over the active site and may have something to do with the enzyme activity.

The position of the essential calcium ion may be assumed to be near both site C and the maltose residual peaks in the difference Fourier map mentioned above. This peak is surrounded by some atoms of side groups and a peptide chain, and probably by water molecules. At this position, the calcium ion may play a role in tightening the catalytic sites suitable for binding amylose and/or for supplying water molecules to the reaction. A more detailed structure including the sequence of whole amino acid residues, and a plausible mechanism of the hydrolysis reaction of this protein will be presented later.

The computation involved in this analysis was performed at the Crystallographic Research Center of this Institute.

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