PRELIMINARY X-RAY INVESTIGATION ON A NEW CRYSTALLINE VARIETY OF PORCINE PANCREATIC α-AMYLASE

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Received 28 March 1977

1. Introduction

Among the properties of an enzyme the ability to crystallize has been intensively explored, formerly by biochemists and nowadays by the crystallographers. However, the crystallization process still remains mysterious and unpredictable. Porcine pancreatic α-amylase is one of the first enzyme which was crystallized [1,2] but no extensive crystallographic study has yet been undertaken. An initial analysis was published [3]: a crystalline variety with 2 molecules in the asymetric unit was obtained but the corresponding diffraction patterns show rather a poor resolution. Recently, thin needle-like crystals have been obtained in the presence of polyethylene glycol [4] and the author claims a resolution of 2.8 Å. That seems surprising owing to the quality of the micrographs of the crystals.

We report here a study on the crystallization of pancreatic α-amylase and on the preparation of a new crystalline variety suitable for an high resolution X-ray analysis.

The reasons which lead us to consider this enzyme as a potential candidate for a three-dimensional analysis include the fact that the molecular properties of α-amylase are now well known. This enzyme (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) exists in two molecular forms having a similar amino acid composition and an identical specific activity but two different isoelectric points: 5.9 and 5.4 for amylase I and amylase II, respectively [4]. Both molecular forms consist of one single polypeptide chain, mol. wt 53 000 and contain a C-terminal leucine residue [6]. The N-terminal of the chain is blocked by an acetyl group [7]. Amylase contains one calcium ion which is essential for its activity [8] and 2 sulfhydryl groups which are masked by calcium [9].

2. Materials and methods

Porcine pancreatic α-amylase was prepared as described by Marchis-Mouren and Pasero [10] and both molecular forms I and II were crystallized. Crystals were grown from solutions of amylase in 10 mM Tris–HCl buffer.

The method of Noelting and Bernfeld [11] was used to check the specific activity. The reactivity of the SH groups allowed us to prepare 2 derivatives: amylase–$(SHg)_2$ and amylase–S–Hg–S [12] which were crystallized in the same conditions as native amylase.

For X-ray diffraction study the crystals were sealed in thin-walled glass capillaries in the usual manner. The X-ray photographs were taken on a Buerger precession camera using Ni-filtered Cu radiation at room temperature.

3. Results and discussion

Protein solutions (10 mg/ml buffer) were crystallized in presence of $CaCl_2$ 1 mM. In order to grow large crystals (about 1 mm) a long period of 1 or 2 months is required. During that time the loss of activity in solution is less than 50% of the initial value. According to the temperature 2 crystalline varieties were obtained: the variety A appears at 4°C and the variety B at 25°C (fig.1a, b).

Both crystalline varieties A and B can be obtained either with amylase I or amylase II, however a slight difference between them can be pointed out. Concerning the low temperature variety A, large crystals can only be grown with amylase II whereas amylase I solutions give rise to needles which are similar to those found by McPherson-Rich [3]. In contrast both molecular forms led to crystals B displaying same morphology and quality and identical diffraction patterns.

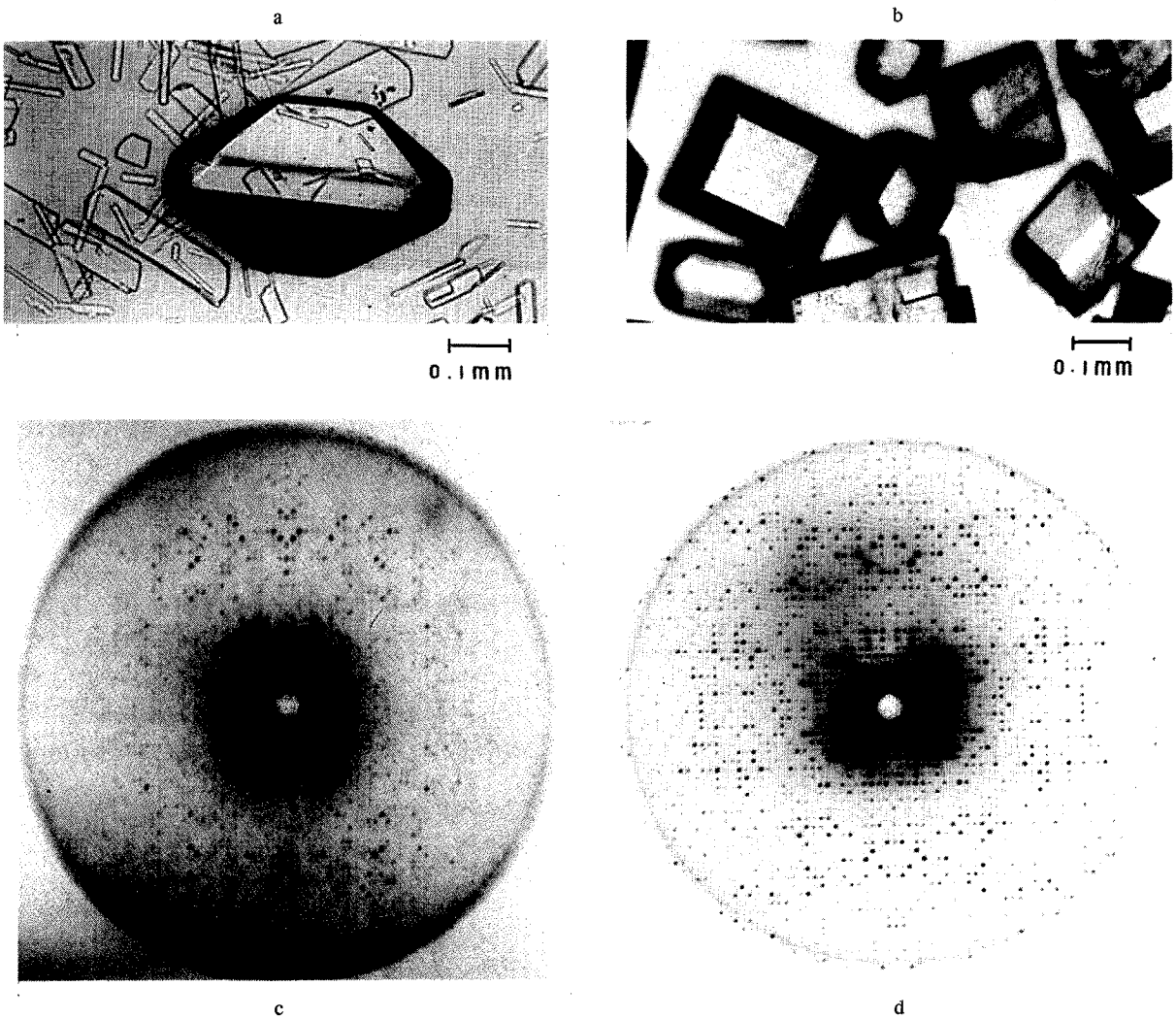

Fig.1. Photographs of crystals of porcine pancreatic α-amylase II. (a) Variety A obtained at 4°C. (b) Variety B obtained at 25°C. Precession photographs (16°) of (o k 1) zones. (c) Variety A crystal. (d) Variety B crystal of porcine pancreatic α-amylase II. Identical diffraction patterns were obtained with amylase I.

Table 1
Crystal data of porcine pancreatic amylase

	McPherson-Rich [3]	Variety A	Variety B
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell parameters:			
a	70 Å	70.6 Å	56.0 Å
b	110 Å	114.7 Å	88.3 Å
c	117 Å	118.5 Å	104.1 Å
v	9.01×10^5 Å^3	9.6×10^5 Å^3	5.14×10^5 Å^3
No. molecules/ asymetric unit	2	2	1
V_M (crystal vol./unit mol. wt) [13]	2.13 Å^3/dalton	2.26	2.42
Solvent cell content	42%	45%	49%
Resolution	3–4 Å	3.0 Å	2.5 Å

The crystal data of the 2 varieties are reported on table 1 and the diffraction patterns are given in fig.1c, d. The crystals appear to be orthorhombic (space group: $P2_12_12_1$). The new crystalline variety B is a priori more attractive for the crystallographers: with only one molecule/asymetric unit, instead of two in that of the variety A, it appears that the determination of the tertiary structure is now a highly reduced-size problem. Of course that is very important for a molecule of mol. wt 53 000. In addition the procession photographs for the variety B extend at least to 2.5 Å resolution.

The search for heavy atom derivatives is in progress. Three derivatives have already been obtained for the variety A: two by crystallization of the amylase–$(SHg)_2$ and amylase–S–Hg–S and one by soaking native crystals in Hg–acetate 1 mM. The amylase–$(SHg)_2$ and amylase–S–Hg–S derivatives have also been crystallized at 25°C and are perfectly isomorphous with the native amylase as shown by the X-ray pictures.

4. Conclusion

The study of the three-dimensional structure of α-amylase is under way and the quality of the variety B crystals allows a high resolution. At the moment data sets for the native protein and for one derivative have been collected at 5.0 Å resolution.

Moreover the sequence determination has been undertaken by Marchis-Mouren group. For that purpose amylase was cleaved by CNBr and the resulting 9 peptides were purified and identified [14]. The ordering of the peptides within the sequence was achieved through an isotopic technique based upon pulse labelling [15].

Acknowledgements

We are grateful to Mrs Pasero and Mazzei (CNRS–LA No. 202) for the preparation of amylase and M. Frey, R. Haser and F. Payan for helpfull discussion.

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