X-ray studies on triclinic crystals of fatty acid binding protein

Example of an extremely X-ray-resistant protein

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Fatty acid binding protein (pI 7.0) from bovine liver cytosol was crystallized using polyethylene glycol 4000 and 6000 as precipitating agents. The crystals are triclinic, space group P1. One molecule of 14 kDa occupies the unit cell with constants a = 33.5 Å, b = 39.4 Å, c = 30.6 Å, α = 113.6°, β = 113.8°, γ = 88.8°. Crystal diffraction extends to at least 2.25 Å resolution and the crystals are stable in the X-ray beam for more than 450 h. One native data set to 2.5 Å resolution has been collected.

Crystallization *Fatty acid binding protein* *X-ray analysis*

1. INTRODUCTION

From bovine liver cytosol two fatty acid binding proteins (FABP) have been isolated [1]. They differ by one pH unit in their isoelectric points of 6.0 and 7.0, respectively, and have similar amino acid compositions, both lacking tryptophan. The molecular mass of either FABP has been determined by SDS-PAGE as 11.8 ± 1 kDa in accordance with the characteristic features of FABP from rat liver [2,3]. However, amino acid [4] and cDNA [5] sequencing of the latter protein gives a molecular mass of 14.187 kDa. The complexing of various lipophilic ligands by either FABP has been studied in detail [1]. Upon binding of organic anions by these proteins, such as fatty acids having 16–20 carbon atoms with 0–4 double bonds as well as their CoA-esters, characteristic pI-shifts into the acidic range are observed. Methyl esters of long-chain fatty acids and cholesterol bind only to pI 7.0-FABP, and not to pI 6.0-FABP and do so non-stoichiometrically. Fatty acids occurring naturally in the cell are complexed by pI 7.0-FABP in a 2:1 ratio, whereas 16-(9′-anthroyloxy)palmitic acid is bound in a 1:1 stoichiometry. Together with other competitive binding experiments [1,6] this indicates that there is a single binding site for fatty acids.

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Part IV of series 'Fatty acid binding proteins' (Part III is [6])

2. MATERIALS AND METHODS

The pI 7.0-FABP used for crystallization trials, was isolated as in [1]. PEG 6000 and 4000 (Merck, Darmstadt) were used as precipitants along with sodium cacodylate and Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) as buffers. Crystals were grown by the vapour diffusion technique [7] using the hanging drop variant [8] in 24 well tissue culture trays (Costar, Cambridge, MA). In a typical experiment, 5 µl protein were pipetted onto siliconized microscope cover slides to

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which 5 μl of reservoir were added without subsequent mixing of the drops. The cover slides were sealed onto wells with apiezon vacuum grease.

3. RESULTS

Crystals of p*I* 7.0-FABP were grown from a 3% (w/v) solution of the protein in water using PEG 6000 in buffer containing 100 mM Hepes (pH 7.1) as precipitant. These crystals grow to their final size (~0.4 mm^3) within 8 days. Crystals used for the X-ray analysis described here (fig.1) were obtained using the same buffer system with PEG 4000 (pH 7.1) as precipitant. These grow to their maximum size (1.0 × 0.8 × 0.2 mm^3) within 11 days. Crystals with the same morphology are obtained in the pH range 6.7–7.1 with both precipitants, though in the case of PEG 6000 they developed rough surfaces on standing. With sodium cacodylate as buffer medium small needle crystals, unsuitable for X-ray investigation, are also obtained.

One crystal (see fig.1) was used for all the X-ray work described here. Precession photography (GX6 and GX20 rotating anodes operating at 40 kV/40 mA and 40 kV/70 mA, respectively) revealed the crystal system as being triclinic. The resolution on these photographs extends to 2.25 Å. The hk0-layer is shown in fig.2. Accurate cell dimensions (see table 1) were calculated by the least squares refinement of 15 reflections ($7.5° < \theta < 10°$) centered on a modified [9] STOE four circle diffractometer (40 kV/35 mA, Ni-filtered CuK_α radiation) equipped with a helium tunnel. These are consistent with one molecule of 14 kDa per asymmetric unit and a V_m-value of 2.38 $Å^3$/Da. It appears that the actual molecular mass of this hepatic FABP is indeed higher than indicated by SDS-PAGE.

Three-dimensional intensity data were collected with the orientation matrix calculated as above covering the unique volume of reciprocal space to a resolution of 2.5 Å (see table 2). Three intensity and orientation standards were monitored periodically and showed insignificant fluctuations during the experiment. Of the total number of reflections measured, 64% may be considered observed at the 2σ-level on *F*.

Fig.1. Crystal grown from PEG 4000 in Hepes buffer (pH 7.1) used for unit cell determination and data collection.

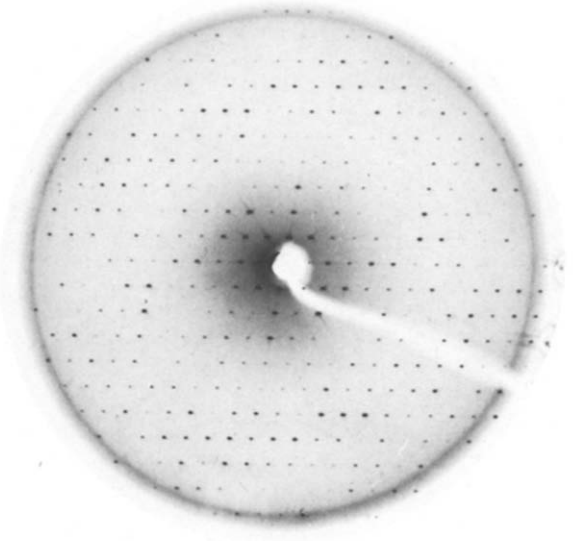

Fig.2. hk0-layer, taken 240 h after first X-ray exposure. μ, 15°; *f*, 100 mm; exposure time, 19 h; GX20 40 kV/70 mA, Ni-filtered CuK_α radiation.

Table 1

Crystallographic data for pI 7.0-FABP

Space group P1
a = 33.47(1) Å, b = 39.36(8) Å, c = 30.56(6) Å, α = 113.6(1)°, β = 113.8(1)°, γ = 88.8(1)°, V = 33290 Å^3, Z = 1, $\bar{V}$ = 2.38 Å^3/Da, $V_{solvent}$ = 48%

Standard deviations are given in parentheses

Table 2

Data collection and reduction

Crystal dimensions	1.0 × 0.8 × 0.2 mm^3
Scan width	60 steps of 0.02°
Scan mode	ω/θ
Aperture	3.5 mm/3.5 mm
Maximum scan time	2.0 s/step
Minimum I/σ	2.0
Maximum I/σ	20.0
% $F > 2\sigma$	64.0

4. DISCUSSION

Crystallographic data on fatty acids bound to a non-enzymic soluble protein, i.e., albumin and β-lactoglobulin, are not available. The first crystallization of a FABP, as reported here, offers a chance to eventually understand the noncovalent binding at the atomic level. The most striking feature of these crystallographic results is the remarkable radiation stability (more than 450 h) observed for p*I* 7.0-FABP. To the best of our knowledge there are only two other examples of proteins with similar radiation resistance: the RNase T_1/GpG complex examined in this laboratory (unpublished) which survived 470 h without noticeable decay and crambin [10] with a reported stability of more than 300 h X-ray exposure. The extremely low solvent content (28%) in the case of the latter may explain this observation. However, for p*I* 7.0-FABP with 48% solvent we suggest that the stability is rather a function of a quite stable tertiary structure. This is in agreement with the observed heat stability of this protein [1]. Preliminary experiments with p*I* 7.0-FABP/fatty acid complexes are under way, as is the search for heavy atom derivatives.

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REFERENCES

[1] Haunerland, N., Jagschies, G., Schulenberg, H. and Spener, F. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 365–376.
[2] Ockner, R.K., Manning, J.A. and Kane, J.P. (1982) J. Biol. Chem. 257, 7872–7878.
[3] Trulzsch, D. and Arias, I.M. (1981) Arch. Biochem. Biophys. 209, 433–440.
[4] Takahashi, K., Odani, S. and Ono, T. (1982) FEBS Lett. 140, 63–66.
[5] Gordon, J.I., Alpers, D.H., Ockner, R.K. and Strauss, A.W. (1983) J. Biol. Chem. 258, 3356–3363.
[6] Reers, M., Elbracht, R., Rüdel, H. and Spener, F. (1984) Chem. Phys. Lip. 36, 15–28.
[7] Davies, D.R. and Segal, D.M. (1971) in: Methods in Enzymology (Jacoby, W.B. ed.) vol.22, pp.266–269, Academic Press, New York.
[8] McPherson, A. (1982) in: Preparation and Analysis of Protein Crystals, pp.96–97, Wiley, New York.
[9] Steifa, M. (1984) 13th International Congress of Crystallography, Hamburg Collected Abstracts, P. C-409 (abstract 16.2-6).
[10] Teeter, M.M. and Hendrickson, W.A. (1979) J. Mol. Biol. 127, 219–223.