Crystallization and structure at 3.2 Å resolution of a terbium parvalbumin

J.P. Wéry, O. Dideberg*, P. Charlier and C. Gerday+

Laboratory of Crystallography, Institute of Physics B5, and *+Laboratory of Muscle Biochemistry, Institute of Chemistry B6, University of Liège (Sart Tilman), B-4000 Liège, Belgium*

Received 12 December 1984

Toadfish muscle parvalbumin III_f can bind two Tb^{3+} which displace the two Ca^{2+} normally bound. This terbium derivative was crystallized with space group $P2_12_12$ and lattice constants $a = 56.2$ Å, $b = 59.5$ Å and $c = 27.2$ Å. The structure at 3.2 Å resolution was determined applying a molecular replacement method and using the known co-ordinates of carp Ca^{2+}-parvalbumin. The structure was refined to a conventional R factor of 26% for 1166 reflections in the resolution range 3.2–6 Å. The α-carbon backbone of the structures of toadfish and carp parvalbumins are very similar.

Crystal *X-ray crystallography* *Three-dimensional structure* *Tb^{3+}* *Parvalbumin* *Ca^{2+}-binding site*

1. INTRODUCTION

Parvalbumins are proteins belonging to the so-called family of calcium-modulated proteins [1] which initiate and regulate a considerable number of biological functions. These proteins have been mainly isolated from the fast-twitch muscles of fishes, amphibians and mammals in which they are considered as soluble relaxing factors [2–5]. Indeed, the relation between relaxation speed and parvalbumin concentration in the muscle, suggested by Hamoir et al. and Gerday [6,7] is now well established [8,9]. A typical example is the superfast swimbladder muscle of the toadfish *(Opsanus tau)* which in particular differs from the trunk muscle by a 2–3-fold increase in parvalbumin content [6]. The main parvalbumin (III_f) of this superfast muscle has been purified to homogeneity [10] and its amino acid sequence is known (unpublished). This component has been crystallized and the preliminary X-ray data have been published [10]. To complete the information about the 3-dimensional structure of this protein and to compare it to the published structure of carp muscle parvalbumin [11,12], we were prompted to study this calcium-binding protein by X-ray diffraction. As rare earths can function as heavy atoms we tried to diffuse Tb^{3+} into the native crystal. Such derivatives have been obtained by terbium diffusion into carp parvalbumin crystals [13] and by neodymium diffusion into the vitamin D-dependent calcium-binding protein [14]; in both cases the substitution yielded isomorphous crystals. In our case, however, Tb^{3+} cracked the crystal even at low concentration ($\sim\mu$M). To avoid this problem the substitution of the two Ca^{2+} of native parvalbumin by Tb^{3+} was therefore attempted in solution. With this preparation, a new crystal form was obtained. The determination of this structure at high resolution is interesting for 3 reasons. Firstly, information has been collected using Tb^{3+} and other lanthanides as luminescence probes in carp [15,16] and cod parvalbumin [17–19] through radiationless energy transfer between aromatic amino acids, excited in their UV absorption bands and the lanthanide which, in this way, is sensitized to emit strongly. Secondly, new methods are developing in macromolecular crystallography. They take advantage of the high brilliance and the continuous spectrum of synchrotron radiation to

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30 © 1985 Federation of European Biochemical Societies

optimise the anomalous scattering effects of lanthanide ions [20–26]. Thirdly, two regions of the carp parvalbumin map were difficult to interpret. This new structure may overcome this problem.

2. EXPERIMENTAL

Extraction and purification of the main parvalbumin (III_f) of the toadfish swimbladder muscle been described [10]. The substitution of the two Ca^{2+} by Tb^{3+} was performed using a procedure similar to that described in [15]. The extent of replacement was first followed by fluorescence measurement and titration of a parvalbumin solution (0.08 μM) in 30 mM imidazole, 10 mM NaN_3 (pH 6.6) by a solution 6.4 mM $TbCl_3$ in water. The results showed that the fluorescence enhancement at 544 nm was maximum for a Tb^{3+}/protein ratio of 2. To prepare the crystals, a solution of parvalbumin in the above buffer was treated with an excess of 3 Tb^{3+} per mol protein, chromatographed on a Biogel P_2 column, concentrated on Amicon YM5, and adjusted to 35% saturation with $(NH4)_2SO_4$. The final protein concentration was around 1%. The absence of calcium was checked by atomic absorption and the terbium occupancy was measured by proton activation [27] and found to be essentially complete. The crystals were grown from the above solution using the batch method. Thirty μl of the protein solution were mixed with 7–9 μl of saturated $(NH_4)_2SO_4$ in the buffer. After centrifugation the samples were kept in small tubes at 20°C. The crystals appeared after a few weeks.

For X-ray analysis, crystals were mounted by a conventional procedure in a quartz capillary and sealed along with a small volume of buffer 60% saturated with $(NH_4)_2SO_4$. X-ray diffraction photographs were recorded on a precession camera. The space group was found to be $P2_12_12$ whereas the space group of the Ca-parvalbumin was $P2_1$ [10]. A closer examination of the precession photographs of the Ca^{2+} crystals soaked in Tb^{3+} solutions indicated a phase transition $P2_1 \rightarrow P2_12_12$ induced by the lanthanide ions. Twelve strong reflections were centered with a Hilger-Watts diffractometer and cell parameters were refined by the least-squares method. The crystallographic data are listed in table 1. The volume per asymmetric unit 22 714 Å^3 is much smaller than the values found for carp parvalbumin loaded with Ca^{2+}, which are 24 461 and 23 840 Å^3, indicating a more compact structure.

A full data set to 3.2 Å resolution has been collected using Ni-filtered CuKα radiation on a Hilger-Watts diffractometer. An ω-scan technique was used and background intensities were measured on both sides of each reflection. Two reference reflections were regularly monitored during the data collection and the decay was less than 16%. The measurements were corrected for decay and absorption [28]. Two equivalent reflections and their Friedel pairs were collected in shells of increasing θ values, giving a total of 5037 reflections. The $R_M = \Sigma \mid \langle I \rangle - I \mid)/\Sigma I$ was 0.055 for 2886 reflections, from which 1708 unique intensities were calculated.

Table 1

Crystallographic data

	Carp III	Ca^{2+} *O.tau*	Tb^{3+} *O.Tau*
a (Å)	28.7	27.31	56.20
b (Å)	61.1	58.75	59.48
c (Å)	54.7	30.83	27.18
β (°)	94°37′	98°30′	–
Space group	C2	$P2_1$	$P2_12_12$
Z	4	2	4
Volume per asymmetric unit (Å^3)	23 840	24 461	22 714
M_r	11 500	11 869	11 919
Crystallization conditions	75–80% saturated $(NH_4)_2SO_4$ pH 5–8	52–55% saturated $(NH_4)_2SO_4$ pH 7	49–50% saturated $(NH_4)_2SO_4$ pH 6–7

3. RESULTS AND DISCUSSION

The crystal structure was determined using rotation and translation search functions with the known molecular structure of carp III parvalbumin as a model [11,29]. In this model, we have replaced 4 amino acid side chains which are very different in the toadfish parvalbumin sequence by 4 alanine residues. The two Ca^{2+} were also removed.

The orientation of the Tb-parvalbumin molecule within the unit cell of the crystal was found by applying the rotation function [30,32]. The highest peak in the rotation function in 10° intervals in angle space (θ_+, θ_2, θ_-) *was 7.12 σ(R),* and 5.9 *σ(R)* for the second highest one. A fine scan in 2° steps was then performed and yielded the final Eulerian angles $\theta_1 = 353°$, $\theta_2 = 50°$, $\theta_3 = 333°$. The model was rotated according to these angles. The translation parameters were determined using a translation function similar to the function T_1 [33]. The 3 Harker sections of the translation functions showed many peaks, but only two solutions could be found for the translation parameters. For the first solution the relative position of the peak was 1, 2, 2 and for the second solution 5, 3, 1, respectively for the $X = 1/2$, $Y = 1/2$ and $Z = 0$ sections. The R value of the two models was not very different, but the packing of the molecules showed clearly that the first solution was incorrect. The R value calculated with the translation (0.215, 0.325, 0.275) was 0.45 in the 10–4.5 Å resolution range (576 reflections) after a few cycles of rigid body refinement [34]. The 6 helices were refined separately as a rigid body and the R factor droped to 0.41 (10–4.1 Å resolution). For this solution, we have calculated all the interatomic vectors for the heavy atoms, assuming Tb^{3+} and Ca^{2+} have the same positions. In the anomalous dispersion difference Patterson map, we found high peaks for only one terbium.

A restrained least-squares procedure [35] was performed and after 8 cycles, the R factor was 0.26 (6–3.2 Å resolution) for 1166 reflections. The stereochemistry of the model was not allowed to diverge (r.m.s. deviation from ideal bond lengths was 0.03 Å).

Fourier and difference maps were inspected and electron density appeared in the two EF loops, which may indicate the presence of the Tb^{3+}. After refinement, we compared the α-carbon backbone of carp parvalbumin and toadfish Tb-parvalbumin and we observed an r.m.s. discrepancy of 0.67 Å over all the α-carbon positions. The largest values were found in the segment (2–4). This region of the molecule has a low electron density in the carp parvalbumin map and the interpretation was difficult. We found no similarity in the packing of carp parvalbumin [11] and this protein.

Because the Tb^{3+} is luminescent in aqueous solution and generally retains this luminescence when bound to complex ligand systems, terbium is a particularly attractive ion [36]. The X-ray data will be collected at high resolution and the structure will be refined. We expect interesting results by comparing the two parvalbumin structures.

ACKNOWLEDGEMENTS

This work was supported by the Fonds National de la Recherche Scientifique Médicale, no. 3.4515.81 (to the Laboratory of Muscle Biochemistry) and the Fonds National de la Recherche Scientifique, no. 1.5.624.84 (to O.D.). We thank Professor G. Hamoir who made available for us the parvalbumin. Thanks are also due to a research grant of the Fonds National de la Recherche Scientifique no. MB 13050 Ex. 81/82, and the hospitality of the Duke Marine Laboratory.

REFERENCES

[1] Kretsinger, R.H. (1980) CRC Crit. Rev. Biochem. 8, 119–171.
[2] Gerday, C. and Gillis, J.M. (1976) J. Physiol. 258, 96–97.
[3] Blum, H.E., Lehky, P., Kohler, L., Stein, E.A. and Fischer, E. (1977) J. Biol. Chem. 252, 2834–2838.
[4] Pechère, J.F., Derancourt, J. and Haiech, J. (1977) FEBS Lett. 75, 111–114.
[5] Gillis, J.M., Thomason, D., Lefevre, J. and Kretsinger, R.H. (1982) J. Muscle Res. Cell. Motil. 3, 377–398.
[6] Hamoir, G., Gerardin-Otthiers, N. and Focant, B. (1980) J. Mol. Biol. 143, 155–160.
[7] Gerday, C. (1982) Mol. Physiol. 2, 63–87.
[8] Heizmann, C.W., Berchtold, M.W. and Rowlerson, A.M. (1982) Proc. Natl. Acad. Sci. USA 79, 7243–7247.
[9] Klug, G., Reichmann, H. and Pette, D. (1983) FEBS Lett. 152, 180–182.

[10] Hamoir, G., Dideberg, O. and Charlier, P. (1981) J. Mol. Biol. 153, 487-489.
[11] Kretsinger, R.H. and Nockolds, C.E. (1973) J. Biol. Chem. 248, 3319-3326.
[12] Moews, P.C. and Kretsinger, R.H. (1975) J. Mol. Biol. 91, 210-228.
[13] Sowadski, J., Cornick, G. and Kretsinger, R.H. (1978) J. Mol. Biol. 124, 123-132.
[14] Szebenyi, D.M.E., Obendorf, S.K. and Moffat, K. (1981) Nature 294, 327-332.
[15] Donato, H. and Martin, R.B. (1974) Biochemistry 13, 4575-4579.
[16] Miller, T.L., Cook, R.M., Nelson, D.J. and Theoharides, A.D. (1980) J. Mol. Biol. 141, 223-226.
[17] Moo-Jhong Rhee, Sudnick, D.R., Arkle, V.K., and Horrocks, W.D. (1981) Biochem., 20, 3328-3334.
[18] Horrocks, W.D. and Collier, W.E. (1981) J. Am. Chem. Soc. 103, 2856-2862.
[19] Horrocks, W.D. and Sudnick, D.M. (1981) Acc. Chem. Res. 14, 384-392.
[20] Templeton, L.K., Templeton, D.H. and Phizackerley, R.P. (1980) J. Am. Chem. Soc. 102, 1185.
[21] Karle, J. (1980) Int. J. Quantum Chem., Quantum Biology Symposium 7, 357-367.
[22] Narayan, R. and Ramaseshan, S. (1981) Acta Crystallogr. A37, 636-641.
[23] Kahn, R., Fourme, R., Bosshard, R., Dideberg, O. and Charlier, P. (1982) Eleventh Eur. Cryst. Meeting, Jerusalem, p. 137.
[24] Kahn, R., Fourme, R., Bosshard, R., Chiadmi, M., Risler, J.L., Dideberg, O. and Wéry, J.P. (1984) submitted.
[25] Greenhough, T.J. and Helliwell, J.R. (1983) Prog. Biophys. Mol. Biol. 41, 67-123.
[26] Miake-Lye, R.C., Doniach, S. and Hodgson, R.O. (1983) Biophys. J. 41, 287-292.
[27] Weber, G., Robaye, G., Delbrouck, J.M., Roelandts, I., Dideberg, O., Bartsch, P. and Depauw, M.C. (1980) Nucl. Instrum. Methods 1968, 551-556.
[28] North, A.C.T., Philips, D.C. and Mathews, F.S. (1968) Acta Crystallogr. A24, 351-359.
[29] Bernstein, F.C., Koetzle, T.F., Williams, C.B., Meyer, E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535-542.
[30] Rossmann, M.G. and Blow, D.W. (1962) Acta Crystallogr. 15, 24-31.
[31] Lattman, E.E. and Love, W.E. (1970) Acta Crystallogr. 26B, 1854-1857.
[32] Lattman, E.E. (1972) Acta Crystallogr. B28, 1065-1069.
[33] Crowther, R.A. and Blow, D.M. (1967) Acta Crystallogr. 23, 544-548.
[34] Vallino, M. (1969) J. Org. Chem., 1-10.
[35] Hendrickson, W.A. and Konnert, J.H. (1980) in: Biomolecular Structure, Function, Conformation and Evolution (Srinivasan, R. ed.) vol. I, pp. 43-57, Pergamon Press, Oxford.
[36] Richardson, F.S. (1982) Chem. Rev. 82, 541-552.