FEBS Letters 350 (1994) 122–124

FEBS 14359

Crystallisation and crystallographic investigations of cod alcohol dehydrogenase class I and class III enzymes

Ramaswamy S.[a], Mustafa El-Ahmad[b], Olle Danielsson[b], Hans Jörnvall[b,*], Hans Eklund[a]

[a]*Department of Molecular Biology, Swedish University of Agricultural Sciences, S-75124 Uppsala, Sweden*
[b]*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden*

Received 9 June 1994; revised version received 2 July 1994

Abstract

Cod liver alcohol dehydrogenase of class-hybrid properties has been crystallized as an NAD^+–pyrazole complex in the monoclinic space group $P2_1$ with cell dimensions a = 103.3 Å, b = 47.4 Å, c = 80.7 Å, β = 104.6°, and with one dimer in the asymmetric unit. The position of the dimer molecule in the crystal was determined by molecular replacement methods at 3.0 Å resolution. The successful search model was the poly-alanine structure of the horse enzyme. Side chains were then replaced according to the amino acid sequence of the cod enzyme, and the structure has been refined at 2.8 Å to an R-factor of 0.26. Cod liver class III alcohol dehydrogenase crystallizes in the monoclinic space group C2 with cell dimensions a = 127.5 Å, b = 76.6 Å, c = 93.4 Å, β = 99.4° and with probably one dimer in the asymmetric unit.

Key words: Alcohol dehydrogenase; Crystallization; X-ray crystallography; Molecular replacement

1. Introduction

Investigations of protein and gene sequences of alcohol dehydrogenases have shown that they can be grouped in a manner that correlates well with functional differences between the classes of alcohol dehydrogenases [1]. However, the cod liver alcohol dehydrogenase exhibits some hybrid properties: its functional properties are clearly of class I, while its amino acid sequence is more similar to the class III enzymes [2]. An ordinary class III alcohol dehydrogenase is also present as two isozymes in cod liver [3].

For a long time, the only liver alcohol dehydrogenase that was studied by crystallographic methods was that of horse liver class I (the EE isozyme) [4]. These studies provided one of the bases for our understanding of the enzyme. The other basis has been the elucidation of many different classes and highly divergent species variants of the enzyme, providing insight into the family relationships and protein fundamentals, as recently summarized [5].

The horse liver enzyme has been refined in different crystal forms to high resolution ([6,7], and Jones, Ramaswamy and Eklund, manuscript in preparation). The structure of one further mammalian alcohol dehydrogenase, the class I human $\beta_1\beta_1$ isozyme form, has been determined at 2.8 Å [8].

Some of the differences within the class I alcohol dehydrogenases, as well as the differences between three of the classes of alcohol dehydrogenase, have been correlated to function by model building based on the horse enzyme structure [9,10]. Likewise, the amino acid differences between the cod and horse liver class I enzymes were interpreted in a modelling study based on the structure of horse liver alcohol dehydrogenase [2]. Three regions of the class I alcohol dehydrogenase show pronounced species and class differences: residues 50–60, 115–125, and 290–300. All these parts cluster around the middle and outer parts of the substrate binding pocket and involve the domain interaction area.

However, in these regions, where the cod enzyme is different from other alcohol dehydrogenases, the modelling was uncertain and several alternative possibilities existed. This uncertainty and the fact that cod alcohol dehydrogenase is an enzyme with hybrid properties between classes I and III, made a crystallographic investigation of the cod enzyme desirable. We have now crystallized this enzyme and started the determination of its three-dimensional structure by molecular replacement methods. The normal class III enzyme from cod liver has also been crystallized in a form suitable for structure determination.

2. Materials and methods

2.1. Class I alcohol dehydrogenase

2.1.1. Crystallization. Alcohol dehydrogenase of class I activity (ethanol-active) was prepared from cod liver as described [3]. The enzyme was crystallized as an NAD^+–pyrazole complex using the hanging drop method. The reservoir used was 1 ml of 50 mM TES buffer, pH 6.9, 10 mM NAD^+, 1 mM pyrazole, and 15.2% PEG 2000. The 10 μl drop contained 0.5 mg/ml enzyme in the same buffer but with only half the PEG concentration. The initially very small crystals were improved in size by macroseeding [11]. The crystals grew as very thin plates. The average size of the crystals used for data collection was 0.5 × 0.1 × 0.05 mm.

2.1.2. Data collection and data processing. Diffraction data were collected from three crystals on an R-axis imaging plate detector on a Rigaku rotating anode and one crystal at the synchrotron in Hamburg on the X11 line using a MAR image plate system. All data sets were

*Corresponding author. Fax: (46) (8) 337 462.

SSDI 0014-5793(94)00746-2

processed using Denzo [12]; 212 frames, each of 1° oscillation, were collected from the four crystals. Data were generally weak as the crystals are thin, but the best crystals diffract to 2.4 Å resolution. However, useful data could be collected only to 2.8 Å resolution.

All the individual frames were merged together using the program SCALEPACK [12] with an *R*-merge for all reflections of 16.1% (for 16,558 unique reflections and 170,831 observed reflections). However, when only reflections with $I/\sigma(I) > 1$ were used (132,844 observed reflections and 13,345 unique reflections) the *R*-merge was 13.5%. The amplitudes were computed from intensities using the program TRUNCATE in the CCP4 suite (Daresbury, UK). The total completeness for reflections in the range 8–2.8 Å is 87%; 71% at highest resolution, between 2.9 and 2.8 Å.

2.1.3. Molecular replacement. The structure was solved by molecular replacement using the program XPLOR [13]. A poly-alanine chain based on the dimer of the refined structure of the horse liver alcohol dehydrogenase holoenzyme [7] was used as a search model. Although the peaks in rotation function were not very well resolved, subsequent Patterson correlation refinement revealed that the two highest peaks were those corresponding to the two different orientations of the dimer. Translation function calculations using the highest peak gave a single clear solution. Rigid body refinement of the positioned poly-alanine dimer improved the *R*-factor to 46%.

At this stage, a model of the cod enzyme based on the horse enzyme [2] was superposed on the poly-alanine according to the molecular replacement solution. This full chain model was used for further refinement.

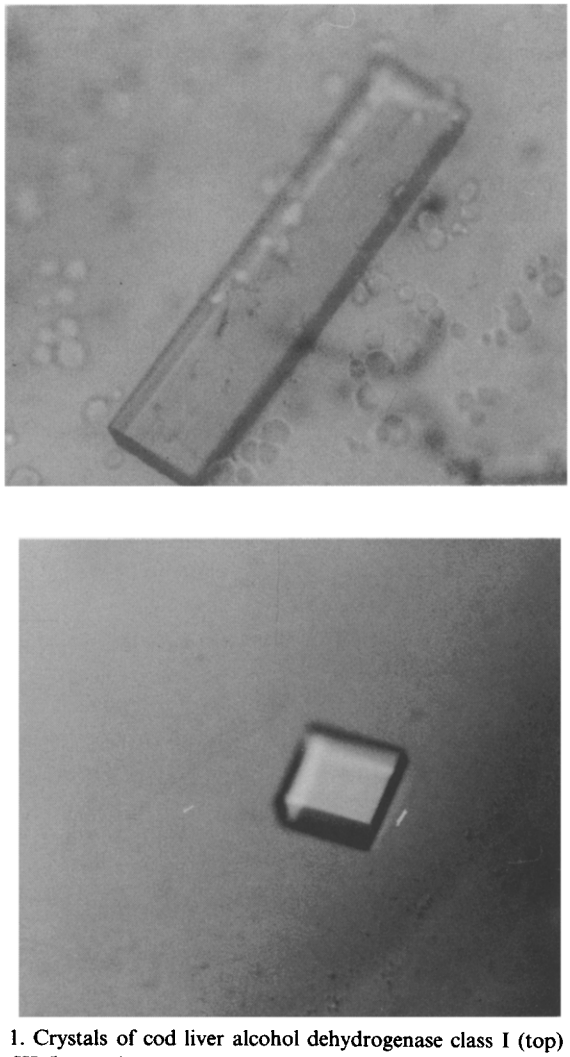

Fig. 1. Crystals of cod liver alcohol dehydrogenase class I (top) and class III (bottom).

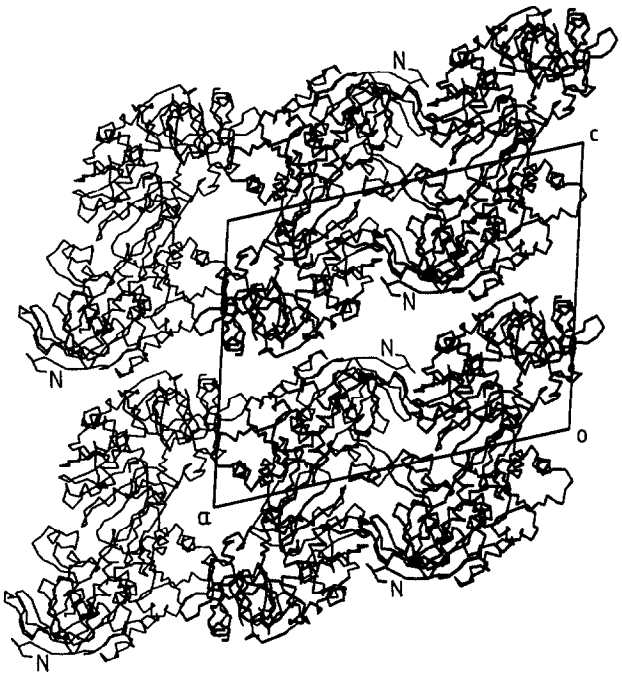

Fig. 2. A Cα representation of the packing of cod ADH class I. Direction of view along the monoclinic *b* axis; *a* and *c* indicate the other axes, o the origin, and N the N-terminus of each protein chain.

2.1.4. Refinement. In all subsequent refinements, only one monomer (the non-crystallographic asymmetric unit) was used. The relationship between the two monomers was computed using the LSQ option in O [14], while a map was computed using the full dimer (CCP4 suite, Daresbury, UK). The non-crystallographic symmetry operators were improved using a map correlation calculation between the two subunits using the program RAVE (Kleyweigt, Uppsala, manual). This improved, non-crystallographic symmetry operator was then used in the XPLOR refinement. The monomer was first refined as a rigid body, followed by conventional positional refinement. This model was then subjected to averaging using RAVE (Kleyweigt, Uppsala, manual).

Further refinement progressed by an iteration of the following steps: (i) Slowcool and Powell minimization using XPLOR, (ii) averaging using RAVE, and (iii) model building and improvement in O.

2.2. Class III ADH

2.2.1. Crystallization. The alcohol dehydrogenase class III *II* isozyme was prepared from cod liver as described ([3], and Danielsson et al., manuscript in preparation). The protein was crystallized as an NAD^+ complex using the hanging drop method. The reservoir used was 1 ml of 50 mM TES buffer, pH 6.9, 100 mM NaCl, 10 mM NAD^+, and 23.6% PEG 4000. The 10 μl drop contained 1 mg/ml enzyme in the same buffer but with only half the PEG concentration. The average size of the crystals used for data collection was $0.4 \times 0.3 \times 0.075$ mm.

2.2.2. Data collection and data processing. Diffraction data were collected from one crystal on an *R*-axis imaging plate detector on a Rigaku rotating anode and processed using Denzo [12]. The crystals diffract to 2.8 Å resolution. The individual frames were merged together using the program SCALEPACK [12].

3. Results and discussion

Both class I and class III cod alcohol dehydrogenases have been crystallized by the hanging drop method. The class I crystals grow as very thin plates and the average size of the crystals used for data collection was 0.5×0.1

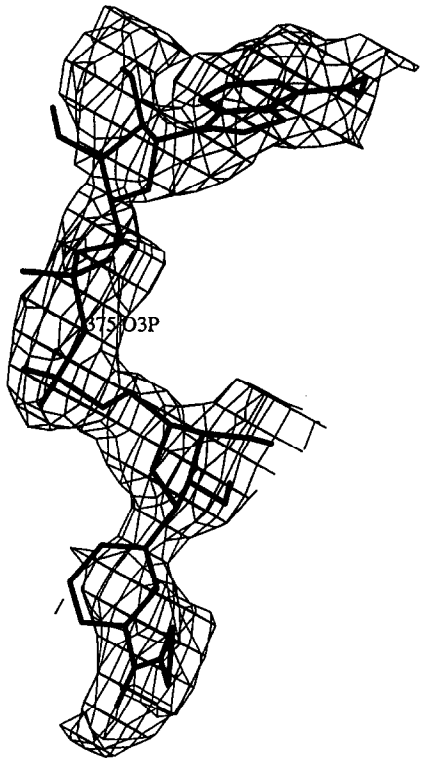

Fig. 3. Electron density map, $2|F_{observed}|-|F_{calculated}|$, after the first round of averaging using the polyalanine search model. NAD^+ was not part of the structure factor calculation.

mm, and less than 0.1 mm along the longest crystallographic cell axis (Fig. 1). The crystals belong to the monoclinic space group $P2_1$ with cell dimensions $a = 103.3$ Å, $b = 47.4$ Å, $c = 80.7$ Å, $\beta = 104.6°$. A $V_m = 2.4$ Å^3/Da, with one dimer per asymmetric unit, corresponds to a solvent content of 48%.

The orientation of the molecule in the cell was determined by molecular replacement methods using a poly-alanine chain based on the dimer of the refined structure of the horse liver alcohol dehydrogenase holoenzyme [7] as a search model. Translation function calculations using the highest peak gave a single clear solution. Rigid body refinement of the positioned poly-alanine dimer improved the *R*-factor to 46%. The packing of the molecules in the unit cell is shown in Fig. 2. As a test for the correctness of the molecular replacement solution, NAD^+ was not originally included in the refined model. The map at the end of averaging clearly indicated the presence of the coenzyme NAD^+ (Fig. 3). NAD^+ was then built into the density and included in the refinement.

At this stage, a model of the cod enzyme based on the horse enzyme [2] was superposed on the poly-alanine according to the molecular replacement solution. This full chain model was used for further refinement. The present *R*-factor is 26.1% for all reflections in the resolution range 8–2.8 Å.

The crystals of the cod class III alcohol dehydrogenase (Fig. 1) belong to the monoclinic space group C2 with cell dimensions $a = 127.5$ Å, $b = 76.6$ Å, $c = 93.4$ Å, $\beta = 99.4°$. A $V_m = 2.8$ Å^3/Da, with one dimer per asymmetric unit, corresponding to a solvent content of 56%. Other crystal forms of this enzyme were also obtained and investigated but not found suitable for further studies.

Acknowledgements: Grants from the Swedish Medical Research Council (13X-3532), the Swedish Natural Science Research Council, the Swedish Alcohol Research Fund (88/12:6), and Karolinska Institutet, are gratefully acknowledged.

References

[1] Jörnvall, H., Danielsson, O., Eklund, H., Hjelmqvist, L., Höög, J.-O., Parés, X. and Shafqat, J. (1993) in: Enzymology and Molecular Biology of Carbonyl Metabolism, vol. 4 (Weiner, H., Crabb, D.W. and Flynn, T.G. eds.) pp. 533–544, Plenum, New York.
[2] Danielsson, O., Eklund, H. and Jörnvall, H. (1992) Biochemistry 31, 3751–3759.
[3] Danielsson, O. and Jörnvall, H. (1992) Proc. Natl. Acad. Sci. USA 89, 9247–9251.
[4] Eklund, H. and Brändén, C.-I. (1987) in: Alcohol Dehydrogenase: Biological Molecules and Assemblies, vol. 3 (Jurnak, F. and McPherson, A. eds.) pp. 73–142, Wiley, New York.
[5] Danielsson, O., Atrian, S., Luque, T., Hjelmqvist, L., Gonzàlez-Duarte, R. and Jörnvall, H. (1994) Proc. Natl. Acad. Sci. USA 91, 4980–4984.
[6] Al-Karadaghi, S. (1993) PhD thesis, Stockholm University.
[7] Ramaswamy, S., Eklund, H. and Plapp, B.V. (1994) Biochemistry 33, 5230–5237.
[8] Hurley, T.D., Bosron, W.F., Hamilton, J.A. and Amzel, L.M. (1991) Proc. Natl. Acad. Sci. USA 88, 8149–8153.
[9] Eklund, H., Horjales, E., Vallee, B.L. and Jörnvall, H. (1987) Eur. J. Biochem. 167, 185–193.
[10] Eklund, H., Müller-Wille, P., Horjales, E., Levina, O., Holmquist, B., Vallee, B.L., Höög, J.-O., Kaiser, R. and Jörnvall, H. (1990) Eur. J. Biochem. 193, 303–310.
[11] Thaller, C., Weaver, L.H., Eichele, G., Wilson, E., Karlsson, R. and Jansonius, J.N. (1981) J. Mol. Biol. 147, 465–469.
[12] Otwinowski, Z. (1991) Denzo, manual.
[13] Brünger, A.T., Kuriyan, J. and Karplus, M. (1987) Science 235, 458–460.
[14] Jones, T.A., Zou, J.Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Cryst. 47, 110–119.