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Crystallization and preliminary crystallographic analysis of the NAD(H)-binding domain of *Escherichia coli* transhydrogenase

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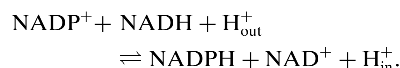
Transhydrogenase is a proton-pumping membrane protein that is required for the cellular regeneration of NADPH. The NAD(H)-binding domain (domain I) of transhydrogenase from *Escherichia coli* was crystallized using the hanging-drop vapour-diffusion technique at room temperature. The crystals, which were grown from PEG 4000 and ammonium acetate in citrate buffer, belong to the triclinic space group $P1$, with unit-cell parameters $a = 38.8$, $b = 66.8$, $c = 76.4$ Å, $\alpha = 67.5$, $\beta = 80.8$, $\gamma = 81.5°$. X-ray diffraction data were collected to 1.9 Å resolution using synchrotron radiation. The crystals contain one dimer of transhydrogenase domain I per asymmetric unit.

1. Introduction

Nicotinamide nucleotide transhydrogenase (EC 1.6.1.1) is an integral membrane protein that is present in the inner membrane of animal mitochondria and the cytosolic membrane of most bacteria (Hoek & Rydström, 1988; Jackson *et al.*, 2002). The function of transhydrogenase (TH) is to provide the cell with NADPH for detoxification purposes; it has been shown that deletion mutants of both *Caenorhabditis elegans* (Arkblad, 2002) and *Rhodobacter sphaeroides* (Hickman *et al.*, 2002) TH have a decreased resistance towards oxidative stress. Furthermore, TH is a provider of NADPH for biosynthesis (Ambartsoumian *et al.*, 1994). TH couples the reversible reduction of $NADP^+$ by a hydride-ion equivalent from NADH with the translocation of a proton across the membrane according to the following equation:



$$NADP^+ + NADH + H^+_{out} \rightleftharpoons NADPH + NAD^+ + H^+_{in}.$$

The ratio between hydrides transferred and protons translocated is 1.

Structurally, THs are composed of three domains: domains I, II and III. Of these, domain II (dII) is the membrane-spanning domain, whereas domains I (dI) and III (dIII) are hydrophilic and harbour NAD(H) and NADP(H), respectively. dII contains the proton-conducting channel, while dI and dIII are responsible for the direct hydride transfer between the substrates. Domains I, II and III contain approximately 400, 400 and 200 residues, respectively. X-ray structures have been reported for the isolated dI from one species, *R. rubrum* (Buckley *et al.*, 2000; Prasad *et al.*, 2002), and for two isolated dIIIs, namely those from bovine heart (Prasad *et al.*, 1999) and human heart (White *et al.*, 2000). In addition, two NMR structures of dIII have been reported: one for *R. rubrum* (Jeeves *et al.*, 2000) and a global fold from *Escherichia coli* (Johansson *et al.*, 1999). An X-ray structure of the cocrystallized dI–dIII complex from *R. rubrum* has also been reported (Cotton *et al.*, 2001). In all published dI structures, this domain assembles into a dimer. For this reason, dI is believed to be the unit responsible for dimerization of the intact functional enzyme. The entire transhydrogenase complex has a molecular weight of ~210 kDa, but the structure of the intact enzyme has yet to be determined.

While the dIII structures of four species have been determined, structural information on dI is limited to one species, *R. rubrum*. In *R. rubrum* and in several other bacteria dI is expressed as a separate polypeptide chain, whereas in animal and the other bacterial type of TH (*e.g.* that from *E. coli*), the TH polypeptide is continuous or divided into two parts, respectively (Jackson *et al.*, 2002). A crystal structure of dI from a TH with a different chain organization is therefore likely to significantly enhance our understanding of the function of this class of enzymes.

Furthermore, the sequence identity between *R. rubrum* and *E. coli* dI is only 40%. Therefore, the surface properties of dI from *R. rubrum* and *E. coli* are expected to differ considerably. Since there is no covalent linkage between *R. rubrum* domains I and II, *R. rubrum* dI has to bind more strongly to the other components in order to maintain the integrity of the enzyme. Indeed, it has been shown that *R. rubrum* dI has a much higher affinity for *R. rubrum* dIII and concomitantly this complex shows a higher hydride-transfer rate than *E. coli* domains I and III (Diggle *et

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al., 1996; Fjellström *et al.*, 1999). Remarkably, *R. rubrum* dI even has a higher affinity for *E. coli* dIII than does *E. coli* dI. The dI–dIII interaction necessary to carry out hydride transfer between NADH and $NADP^+$ is probably mediated by residues in the *E. coli* system that are more hydrophobic, as indicated by studies by Bergkvist *et al.* (2000). At present, there is little structural information on how the hydrophilic domains dI and dIII interact with the proton-conducting channel in dII. Hence, the difference in the surface-exposed residues in dI might provide additional insight into the coupling of hydride transfer and proton translocation.

We report here the crystallization and preliminary X-ray diffraction studies of the NAD(H)-binding domain from *E. coli* transhydrogenase.

2. Crystallization

Expression, characterization and purification of *E. coli* TH dI have been described elsewhere (Fjellström *et al.*, 1997). Crystallization was performed in 24-well tissue-culture plates (Göteborgs Termometerfabrik, Sweden) using the hanging-drop vapour-diffusion technique at 293 K. Initial screening of crystallization conditions was performed with Structure Screen I from Molecular Dimensions (Molecular Dimensions Ltd, England). Each drop contained 1.5 µl protein solution (concentrated to 30 mg ml^{-1} in 10 m*M* sodium phosphate pH 7 and 100 m*M* sodium chloride), supplemented with one molar equivalent of NAD^+, and 1.5 µl reservoir solution. Drops were suspended over 700 µl reservoir solutions. Crystals suitable for diffraction experiments appeared over a weekend in 0.2 *M* ammonium acetate, 0.1 *M* trisodium citrate dihydrate pH 5.6 and 30% PEG 4000 (Fig. 1).

Figure 1
A crystal of TH domain I from *E. coli*. The dimensions of the crystals are approximately 50 × 30 × 20 µm.

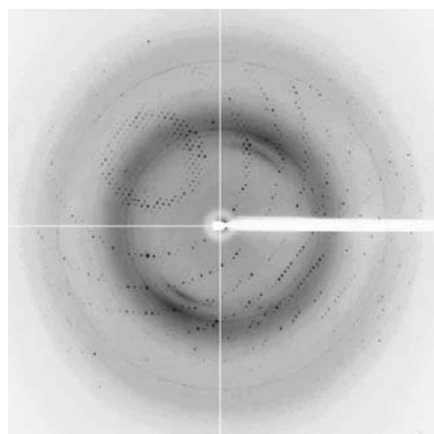

Figure 2
Diffraction pattern of TH domain I crystal.

3. Data collection and processing

Crystals were flash-frozen in liquid nitrogen after transfer to a cryoprotectant consisting of the reservoir solution supplemented with 15% glycerol. Data collection on a pre-frozen crystal was performed at 100 K on beamline ID14-1 at the ESRF. 200 frames were collected with an oscillation angle of 1° at a wavelength of 0.933 Å. The crystal-to-detector distance was set to 161.2 mm. Diffraction data were collected to 1.9 Å using an ADSC Q4R CCD detector. A diffraction image of TH dI is shown in Fig. 2.

The data were indexed and integrated using *DENZO* and scaled using *SCALEPACK* (*HKL* package; Otwinowski & Minor, 1997). Data do not obey any higher symmetry and are consistent only with the triclinic space group *P*1. The Matthews coefficient (V_M; Matthews, 1968) of 2.1 $Å^3$ Da^{-1}, corresponding to a solvent content of 42.1%, indicated two monomers of dI per asymmetric unit. Data-collection statistics are summarized in Table 1.

Molecular replacement was carried out using the program *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). We first attempted to use the X-ray structure of the dimer of domain I from *R. rubrum* (PDB code 1l7e) as a search model, but without success. However, when we turned to the monomer as a search model, a solution was found for two monomers related by a local twofold axis with a correlation coefficient of 31.3% and an *R* factor of 50.6%. In the subsequent rigid-body refinement, we split the dI monomer into its two subdomains, which decreased the *R* factor to 48.6%. Cycling with *ARP*/*wARP* (Perrakis *et al.*, 1999) resulted in *R* and R_{free} values of 21.9 and 30.5%, respectively. At this point, we could not observe any electron density from bound substrate molecules. Refinement using the program *REFMAC*5 (Murshudov *et al.*, 1997) and model building with the program *O* (Jones *et al.*, 1991) is currently under way.

Table 1
Data-collection statistics for TH domain I.

Values in parentheses refer to the highest resolution shell (1.97–1.90 Å).

Space group	*P*1
Unit-cell parameters (Å, °)	$a = 38.8$, $b = 66.8$, $c = 76.4$, $\alpha = 67.5$, $\beta = 80.8$, $\gamma = 81.5$
Resolution range (Å)	40.0–1.90
No. of total reflections	102767
No. of unique reflections	52855
Data completeness (%)	96.6 (95.2)
R_{merge}† (%)	3.5 (17.3)
Average I/σ (I)	12.8 (3.6)

† $R_{merge} = \sum |I_i - \langle I \rangle| / \sum I_i$.

In parallel to the refinement, we are performing soaks and cocrystallization experiments of TH dI with its natural substrates, NAD^+ and NADH, at higher concentrations. We hope soon to complement the structural analysis of the apo-domain I with the substrate-bound structures.

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References

Ambartsoumian, G., D'Ari, R., Lin, R. T. & Newman, E. B. (1994). *Microbiology*, **140**, 1737–1744.

Arkblad, E. (2002). PhD Thesis. Chalmers University of Technology, Göteborg, Sweden.

Bergkvist, A., Johansson, C., Johansson, T., Rydström, J. & Karlsson, B. G. (2000). *Biochemistry*, **39**, 12595–12605.

Buckley, P. A., Jackson, J. B., Schneider, T., White, S. A., Rice, D. W. & Baker, P. J. (2000). *Structure*, **8**, 809–815.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Cotton, N. P., White, S. A., Peake, S. J., McSweeney, S. & Jackson, J. B. (2001). *Structure*, **9**, 165–176.

Diggle, C., Bizouarn, T., Cotton, N. P. & Jackson, J. B. (1996). *Eur. J. Biochem.* **241**, 162–170.
Fjellström, O., Bizouarn, T., Zhang, J. W., Rydström, J., Venning, J. D. & Jackson, J. B. (1999). *Biochemistry*, **38**, 415–422.
Fjellström, O., Johansson, C. & Rydström, J. (1997). *Biochemistry*, **36**, 11331–11341.
Hickman, J. W., Barber, R. D., Skaar, E. P. & Donohue, T. J. (2002). *J. Bacteriol.* **184**, 400–409.
Hoek, J. B. & Rydström, J. (1988). *Biochem. J.* **254**, 1–10.
Jackson, J. B., White, S. A., Quirk, P. G. & Venning, J. D. (2002). *Biochemistry*, **41**, 4173–4185.
Jeeves, M., Smith, K. J., Quirk, P. G., Cotton, N. P. & Jackson, J. B. (2000). *Biochim. Biophys. Acta*, **1459**, 248–257.
Johansson, C., Bergkvist, A., Fjellström, O., Rydström, J. & Karlsson, B. G. (1999). *FEBS Lett.* **458**, 180–184.
Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst.* D**53**, 240–255.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Perrakis, A., Morris, R. & Lamzin, V. S. (1999). *Nature Struct. Biol.* **6**, 458–463.
Prasad, G. S., Sridhar, V., Yamaguchi, M., Hatefi, Y. & Stout, C. D. (1999). *Nature Struct. Biol.* **6**, 1126–1131.
Prasad, G. S., Wahlberg, M., Sridhar, V., Sundaresan, V., Yamaguchi, M., Hatefi, Y. & Stout, C. D. (2002). *Biochemistry*, **41**, 12745–12754.
Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.
White, S. A., Peake, S. J., McSweeney, S., Leonard, G., Cotton, N. P. & Jackson, J. B. (2000). *Structure*, **8**, 1–12.