manuscript be5111 for review

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449
Editors: **E. N. Baker and Z. Dauter**

Coenzyme and His-tag induced crystallisation of octopine dehydrogenase from the adductor muscle of Pecten maximus

Sander H. J. Smits, Andre Mueller, Manfred K. Grieshaber and Lutz Schmitt*

Category: *crystallization papers*

Co-editor:
Professor E.N. Baker
School of Biological Sciences, University of Auckland, Private Bag 92-019, Auckland, New Zealand
Telephone: 64 9 373 7599
Fax: 64 9 373 7619
Email: ted.baker@auckland.ac.nz

Contact author:
Lutz Schmitt
Institute of Biochemistry, Heinrich Heine University, Universitaetstr. 1, Duesseldorf, 40225, Germany
Telephone: +49-211-81-10773
Fax: +49-211-81-13510
Email: lutz.schmitt@uni-duesseldorf.de

international union of crystallography
referee's report

Journal: Acta Crystallographica Section D: Biological Crystallography

Paper reference: be5111

Title: Coenzyme and His-tag induced crystallisation of octopine dehydrogenase from the adductor muscle of Pecten maximus

Author(s): Sander H. J. Smits, Andre Mueller, Manfred K. Grieshaber and Lutz Schmitt*

Please either fax this form or e-mail your comments to the Co-editor. If you cannot review this manuscript by the desired deadline, please contact the Co-editor immediately so that it can be sent to another referee. This form is provided for your convenience. If preferred, your report may be returned in any other format.

1. Is the manuscript worthy of publication?

   Yes, as it is _____;

   Yes, with minor revision _____;

   Yes, but with major revision _____;

   No, not at all _____

2. Is the work technically sound? _____

   Does the evidence presented justify the conclusions drawn? _____

   If not, please specify.

3. Is the presentation as efficient as possible? _____

   Can any material be omitted or deposited? _____

   Please specify.

4. Is the choice of journal correct? _____

   (Not relevant to conference proceedings.)

5. Have the requirements of *Notes for Authors* been met? _____

6. Is the Literature adequately cited? _____

7. Revisions to be made by the author.

   If it is necessary to continue on another sheet, please enclose an extra unsigned copy.

Signature _____ Date _____

Coenzyme and His-tag induced crystallization of octopine dehydrogenase

Sander H. J. Smits,[a] Andre Mueller,[b] Manfred K. Grieshaber[b] and Lutz Schmitt[a*]

[a]Institute of Biochemistry, Heinrich Heine University Duesseldorf, Germany, and [b]Institute of Zoophysiology, Heinrich Heine University, Germany. E-mail: lutz.schmitt@uni-duesseldorf.de

Synopsis The crystal structure of octopine dehydrogenase revealed a specific role of the His_5-tag in inducing crystal contacts that were required for successful crystallization.

Abstract Over the last decade protein purification became more efficient and standardized through the introduction of affinity tags. Choice and position of the tag, however, might directly influence the process of protein crystallization. Octopine dehydrogenase (OcDH) without His-tag and tagged protein constructs such as OcDH-His_5 and OcDH-LEHis_6 have been investigated for their crystallizability. Only OcDH-His_5 yielded crystals, which, however, were multiple. To improve crystal quality, the cofactor NADH was added resulting in single crystals suitable for structure determination. As shown by the structure, the His_5-tag protrudes into the cleft between the NADH and L-arginine binding domains and is mainly fixed by water molecules. The protein is thereby stabilised to such an extent that the formation of crystal contacts can proceed. Together with NADH the His_5-tag obviously locks the enzyme in a specific conformation, which induces crystal growth.

Keywords: His-tag; coenzyme; octopine; crystal contact

1. Introduction

The NAD(P)H-dependent reductive condensation of an amino group of an amino acid and an α-keto acid is catalyzed by a family of enzymes referred to as opine dehydrogenases (Grieshaber *et al.*, 1994). The reaction products, the so called opines (Thompson & Donkersloot, 1992), have two asymmetric centers and, in nature, opines may exhibit either (L, L) or (D, L) stereochemistry (Storey & Dando, 1982). Only recently the structure of the octopine dehydrogenase (OcDH) from adductor muscle of the great scallope, *Pecten maximus*, *(*Smits *et al.,* 2008) allowed to elucidate the reaction mechanism of this enzyme family, although the structure of CENDH from *Arthrobactersp. strain 1C* (Britton *et al.*, 1998) has been solved already in the late nineties.

In recent years, His-tagged proteins have been extensively used for rapid and efficient protein purification. The role of the His-tag in protein crystallization, however, is under considerable debate ever since (Carson *et al.*, 2007). On the one hand, a His-tag can inhibit crystallization, which can be overcome by cleaving off the His-tag via an engineered protease site. Conversely, in most cases the His-tag does not interfere with crystal formation, although and due to its flexibility the poly-histidines can rarely be detected in the electron density map. Here, we describe a His-tag induced crystallization of OcDH, which was dependent on the length of the His-tag.

2. Materials and Methods

2.1. Expression and purification of recombinant OcDH

Cloning of tagless OcDH (OcDH-tagless) and the His_5-tagged OcDH (OcDH-His_5) were performed as described previously (Muller *et al.*, 2007). Additionally, a His_6-tagged OcDH variant (OcDH-LE-His_6) was cloned. Expression and purification of OcDH-His_5 as well as OcDH-LEHis_6 were performed as previously described (Muller *et al.*, 2007).

2.2. Crystallization, data collection and structure determination of OcDH-His_5

Purified OcDH-tagless, OcDH-His_5, and OcDH-LEHis_6 were dialyzed against 10 mM Hepes pH 7.0, 1 mM EDTA and 1 mM DTT. Protein samples were concentrated to 20 mg mL^{-1} and optimized crystals of OcDH-His_5 were grown as described (Smits *et al.*, 2008). Datasets of OcDH-His_5 were collected at the BW7A or X12 beamlines at the EMBL outstation, DESY, Hamburg.

3. Results and Discussion

3.1. Overexpression and purification of OcDH

Three different constructs were cloned for crystallization purposes (OcDH-tagless, OcDH-His_5, and OcDH-LEHis_6). The His-tags were placed at the C-terminus, since the important NADH binding site is encoded in the first N-terminal 20 amino acids. In OcDH-LEHis_6 two additional amino acids, leucine and glutamate are located between OcDH and the His-tag as encoded on the plasmid.

OcDH-His_5 and OcDH-LEHis_6 were purified by Ni-NTA chromatography yielding almost 20 mg homogenous enzyme per liter cell culture (purity larger than 98%).

The purification of OcDH-tagless required several chromatographic steps, yielding 3-5 mg per liter cell culture, with a purity of >98%. In terms of activity, the three constructs were undistinguishable (data not shown) and comparable to OcDH purified directly from *Pecten maximus* (van Thoai *et al.*, 1969).

3.2. Crystallization

Although extensively tried, OcDH-tagless and OcDH-LEHis_6 never yielded crystals. Probably OcDH can adopt multiple conformations which prevented crystal formation. The purified OcDH-His_5, however, yielded small crystals, which already by optical examination appeared multiple (Figure 1A). They diffracted to a resolution of 2.6Å with multiple lattices on one diffraction image and neither the XDS nor the DENZO (Otwinowski & Minor, 1997) program packages were able to process the data. Any effort to improve these crystals using for example seeding, temperature ramping or various crystallization conditions failed or produced only crystals of mediocre quality.

Finally, the coenzyme NADH was added (final concentration 0.8 mM) prior to crystallization. This produced crystals under conditions similar to those in the absence of NADH (Figure 1B). In particular the incubation temperature appeared to be critical and had to be kept at 12°C. The crystals obtained were single and diffracted to 2.1Å, which allowed processing of the data and subsequent structure determination (Smits *et al.*, 2008).

3.3. Structure of OcDH

The three dimensional structure of OcDH from *Pecten maximus* was recently reported (Smits *et al.*, 2008). Already in the sequence two distinct domains can be found in OcDH, a NADH-dependent glycerol-3-phosphate dehydrogenase like domain (domain I) and an octopine dehydrogenase specific domain (domain II; interpro database). Each domain comprises approximately half of the protein, with domain I containing the classic Rossman fold of dinucleotide binding proteins (Rossmann *et al.*, 1974, Schulz, 1992).

3.4. His-tag and crystal contacts

Surprisingly, the His_5-tag protrudes in the cleft between domain I and II (Figure 3). From the histidines of the His-tag, His402 directly interacts with the side chain of Val307, whereas His403 interacts with the 3' OH moiety of the ribose of NADH. Position and orientation of the other histidines inside the His-tag are stabilized via a complex water network (Figures 2 and 3). The water molecules were picked automatically using ARP WARP (Perrakis *et al.*,

1999) and manually checked for proper density. His400 interacts via 2 water molecules with Val307 and His401, His401 via two water molecules with Tyr325, His402 via one water molecule with Tyr325, His403 via two water molecules with Thr143, Leu 116, Pro116 as well as Tyr235 and His404 interacts via one water molecule with Lys163, Tyr 235, Tyr282, Tyr283, and Tyr303. The complete His-tag thereby opens up both domains to a certain extent, and the structure is fixed in this conformation. The electron density surrounding the His_5-tag is of high quality (Figure 3).

The stable conformation of OcDH, induced by the His_5-tag created crystal contacts which are located at the bottom of both domains (Figure 4B). Here Phe35, Glu38, Asp37, Glu40, and Arg41 of domain I of monomer A interact with Gly173, Thr174, Ala175, and Lys176 of domain I of a symmetry-related monomer B. The crystal contacts in domain II are mediated by Val313, Asp314, and Ala315 of monomer A interacting with Thr373, Gly374, and Lys 375 of monomer B. A disruption of any of these interactions by a further opening of the cleft between the two domains would loosen or even diminish crystal formation as corroborated by the observation that crystals without coenzyme were multiple and of less quality. Any prolongation of the His_5-tag will probably result in a similar orientation of the tag inside the cleft, but would minimize the contacts between the protein inside the crystal thereby inhibiting crystal growth. This might explain why OcDH-LEHis_6 did not yield any crystals under the same conditions. The two monomers are probably too far apart to generate similar protein-protein contacts. This specific role of the His_5-tag could explain why OcDH-tagless did not crystallize; the two domains are too flexible and the stable conformation suited for crystallization can only be induced by the His_5-tag inserted into the cleft in combination with the addition of NADH.

Chimeric proteins are utilized in the forefront of protein science for many applications. Especially His-tags (Smith *et al.*, 1988) have gained great popularity over the last decades as a purification tool for recombinant proteins. The often unspoken assumption pretends that these tags have no effect on the structure and function of the protein (Chant *et al.*, 2005). OcDH is one example where not only the presence, but also the length of the His-tag is crucial for the crystallization process, as it induces additional and important crystal contacts. A similar observation was made in the structure of a 116-residue protein (PDB code 1v30). Here, a long C-terminal helix, which includes the His_6-tag, protrudes outside the molecule and packs with another molecule along the crystallographic twofold axis (Tajika *et al.*, 2004). The authors noted that they were unable to obtain crystals of the wild-type sequence (no His-tag) under the same crystallization conditions.

In summary, the length of the His-tag in combination with the cofactor appeared crucial in the structure determination of OcDH, although all three constructs appeared identical in terms of purification and activity, only one construct yielded crystals from which the X-ray structure could be determined. This might also suggest that not only black and white answers (His-tag or no His-tag) should be given for proteins "difficult to crystallize", but that also variations in the length of the His-tag should be considered in such crystallization efforts.

Figure 1 Crystals of apo OcDH-His5 (A) and of OcDH-His_5 with bound coenzyme NADH (B).

Figure 2 His_5-tag mediated crystal contacts. (B): Overall structure of OcDH (green) and the His_5-tag (red) pointing into the cleft between the two domains of a symmetry related molecule (white). Red circles indicate protein-protein mediated crystal contacts. (A and C): Blow-up of protein-protein mediated crystals contacts. The top of domain I interacts with the bottom of domain I of a neighbouring molecule. An identical arrangement is observed for domain II.

Figure 3 Interactions of the His_5-tag (magenta) with the surrounding water molecules (blue dots). Highlighted are side chains (white), which interact in first or second line with the His_5-tag via a water network. The coenzyme NADH is colored in green. The grey mesh shows a $1F_o$-F_c omit map of the His_5-tag contoured at 2σ.

Acknowledgements

We thank Matthew Grooves and Paul Tucker for excellent support at the beamlines of the EMBL Outstation Hamburg (Germany). This work was supported by the DFG (grant GR456/20-4 to M.K.G.).

References

Britton, K. L., Asano, Y. & Rice, D. W. (1998). *Nat Struct Biol,* **5**, 593-601.

Carson, M., Johnson, D. H., McDonald, H., Brouillette, C. & DeLucas, L. J. (2007). *Acta Crystallographica Section D,* **63**, 295-301.

Chant, A., Kraemer-Pecore, C. M., Watkin, R. & Kneale, G. G. (2005). *Protein Expr Purif,* **39**, 152-159.

Grieshaber, M. K., Hardewig, I., Kreutzer, U. & Portner, H. O. (1994). *Rev Physiol Biochem Pharmacol,* **125**, 43-147.

Muller, A., Janssen, F. & Grieshaber, M. K. (2007). *Febs J,* **274**, 6329-6339.

Otwinowski, Z. & Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode, Vol. 276, Meth. Enzymol., edited by C. W. Carter & R. M. Sweet, London: Academic Press.

Perrakis, A., Morris, R. & Lamzin, V. S. (1999). *Nat Struct Biol,* **6**, 458-463.

Rossmann, M. G., Moras, D. & Olsen, K. W. (1974). *Nature,* **250**, 194-199.

Schulz, G. E. (1992). *Cur. Opion. Struc. Biol.,* **2**, 61-67.

Smith, M. C., Furman, T. C., Ingolia, T. D. & Pidgeon, C. (1988). *J Biol Chem,* **263**, 7211-7215.

Smits, S. H. J., Mueller, A., Schmitt, L. & Grieshaber, M. K. (2008). *Journal of Molecular Biology,* **318**, 200- 211.

Storey, K. B. & Dando, P. R. (1982). *Comp Biochem Physiol B,* 521-528.

Tajika, Y., Sakai, N., Tamura, T., Yao, M., Watanabe, N. & Tanaka, I. (2004). *Proteins,* **57**, 862-865.

Thompson, J. & Donkersloot, J. A. (1992). *Annu Rev Biochem,* **61**, 517-557.

van Thoai, N., Huc, C., Pho, D. B. & Olomucki, A. (1969). *Biochim Biophys Acta,* **191**, 46-57.

Figure 1:

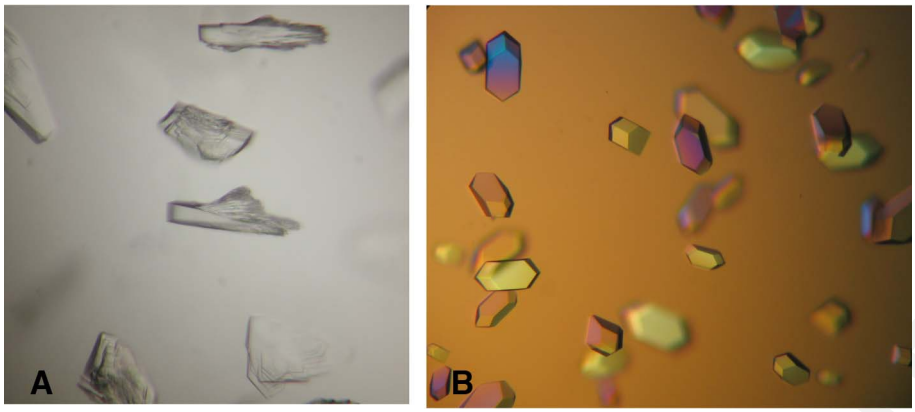

Figure 2:

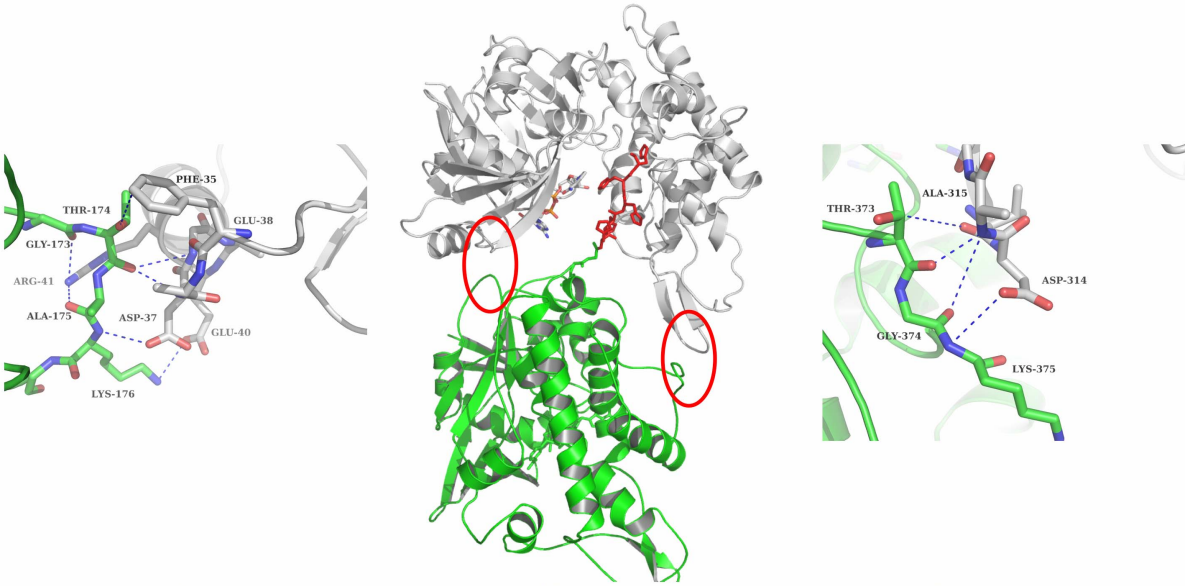

Figure 3: