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Favourable mediation of crystal contacts by cocoamidopropylbetaine (CAPB)

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Crystals of excellent quality are a prerequisite for high-resolution X-ray data. However, in refinement protocols of crystallization conditions it is often difficult to obtain the right combination of, for example, protein concentration, drop size, temperature and additives. A novel approach for optimizing crystal contacts in a most favourable fashion by performing crystallization setups with the zwitterionic surfactant cocoamidoproylbetaine (CAPB) is introduced. In the presence of this surfactant, highly diffracting crystals were obtained. Here, data from a right-handed coiled coil (RHCC) in complex with CAPB at 1.4 Å resolution are presented. The addition of CAPB using otherwise identical crystallization conditions and the same X-ray source caused an improvement in resolution from 2.9 to 1.4 Å.

1. introduction

Many applications based on structural information such as the trapping of catalytic intermediates in enzyme catalysis or analysis of protein–protein interaction mechanisms require high-resolution diffraction data (for a comment, see Harrison, 2004). Thus, the bottleneck for X-ray crystallographers is no longer the *de novo* determination of a protein structure but a methodological approach to high-resolution information of excellent quality. We propose that the addition of the zwitterionic surfactant cocoamidopropylbetaine (CAPB) to an already established protein crystallization setup might mediate favourable crystal contacts, with the consequence of obtaining better diffracting crystals. Surfactants such as CAPB that are capable of micelle formation in aqueous solution are commonly used in household products as ingredients of shampoo, in cosmetics or as surface cleaners for solubilizing amphipathic molecules. CAPB has been shown to have low protein-denaturing effects (Herlofson & Barkvoll, 1996) and very good water solubility. In previous studies, CAPB has already been used to perform crystallization setups of chiral intermediates (data not shown).

As a test system, we have chosen the right-handed coiled-coil domain of tetrabrachion (RHCC) from the archeon *Staphylothermus marinus* (Peters *et al.*, 1995, 1996). The protein serves as a model system for systematic crystallization studies in our laboratory, principally because the protein crystals obtained thus far are very soft (about 75% solvent content; V_M = 5.22 Å^3 Da^{-1}), leading to limited resolution (outer shell 2.9 Å resolution) using in-house X-ray facilities. All attempts to use commercial available non-detergent sulfobetaines (NSDB) to enhance crystal quality failed (Vuillard *et al.*, 1994). The naturally occurring RHCC tetramer forms a bundle of four right-handed amphipathic α-helices supercoiling around each other in a right-handed manner (Stetefeld *et al.*, 2000).

Here, we present the high-resolution structure of the RHCC domain achieved by crystallizing the protein in complex with cocoamidopropylbetaine, which mediates crystal contacts in a most favourable fashion. In addition to leading to a much faster growth of crystals of excellent quality, it allowed data acquisition at 1.4 Å resolution using in-house facilities.

2. Experimental

2.1. Protein crystallization

RHCC was expressed in *Escherichia coli* and purified as described by Stetefeld *et al.* (2000). Crystallization experiments were performed

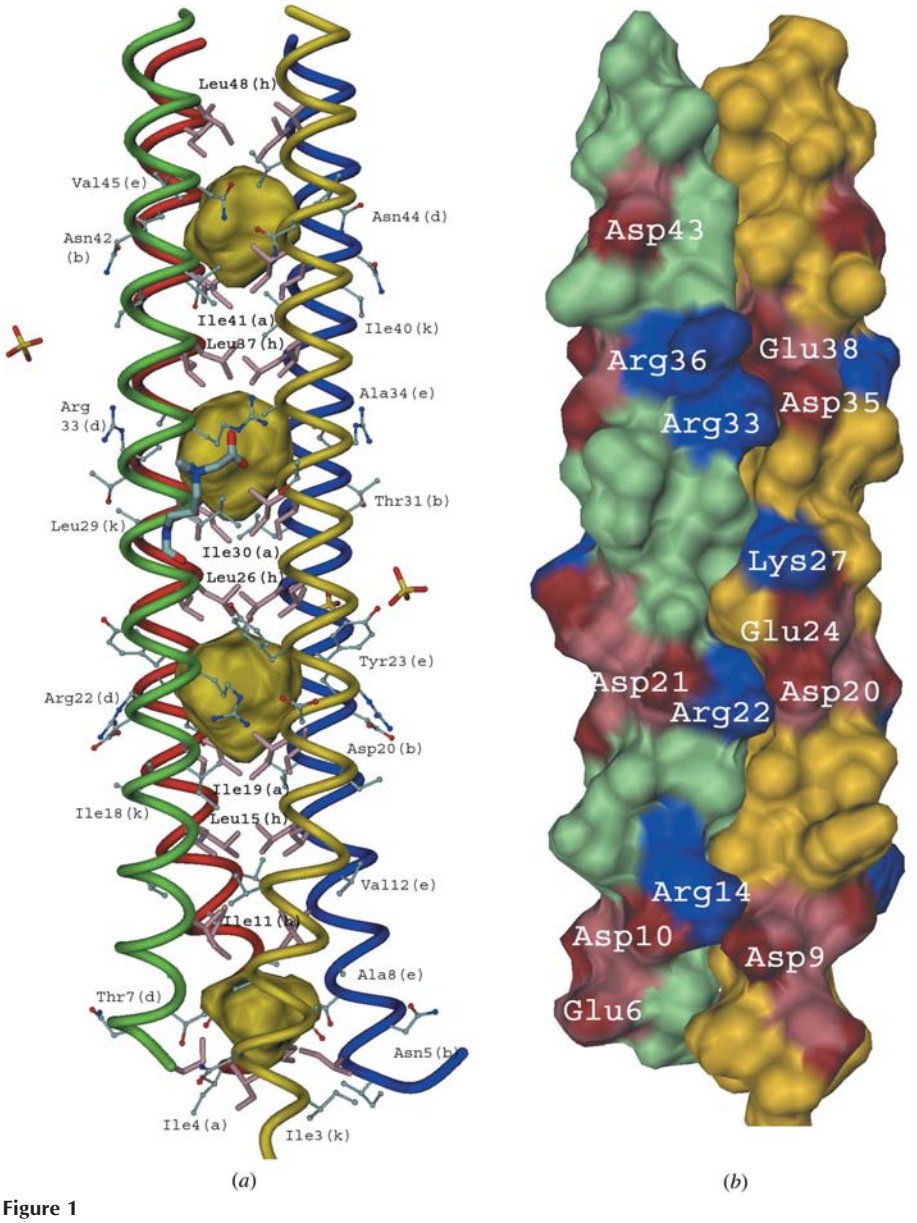

Figure 1
Overall structure of RHCC with CAPB. (*a*) Side view of the tetrameric channel. The N-terminus is at the bottom and the helical backbone is shown in ribbon representation with different colours for each chain. The colour scheme for separate helices is maintained throughout the manuscript. Amino-acid residues forming knobs-into-holes interactions according to the 7,4-motif of the undecad repeat are shown in pink; in contrast, residues involved in interhelical interface formation are coloured by atom type. Positions of each residue according to the undecad repeat pattern are indicated in parentheses. Sulfate ions and truncated cocoamidopropylbetaine are shown. The four large cavities are drawn in yellow and numbered sequentially I–IV from the N-terminus. (*b*) Surface presentation of complex charged residues. The portion of of two helices is shown by van der Waals spheres. The molecular surface is shown in light green for helix *C* and yellow for helix *D*. For both helices, positively and negatively charged residues are colored red and blue, respectively.

at 277 K by vapour diffusion. Sitting drops were made by mixing 4 µl protein solution (13 mg ml^{-1}) with 8 µl 2 *M* ammonium sulfate and 200 m*M* Tris–HCl pH 7.9. Protein samples were co-crystallized performing a set of dilution series with CAPB in the range 0.5–4%(*w*/*v*). A 30%(*w*/*v*) stock solution of cocoamidopropylbetaine from Goldschmidt AG, Essen, Germany was used.

2.2. Structure determination

The crystals belong to space group $P3_121$, with unit-cell parameters $a = b = 110.81$, $c = 71.12$ Å. The asymmetric unit contains one tetramer ($V_M = 5.17$ Å^3 Da^{-1}). The native data set was collected at 100 K on an 18 cm MAR imaging-plate detector using monochromated Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å) from an Elliott GX-20 rotating-anode generator. Diffraction images were processed using the *MOSFLM* program suite (Leslie, 1994) and the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Positional refinement was performed with *CNS* using the maximum-likelihood method (Brünger *et al.*, 1998). Refinement with *CNS* was alternated with manual electron-density refitting of side chains and terminal regions using *MAIN* (Turk, 1992). The target parameters of Engh and Huber, overall anisotropic *B*-factor scaling and bulk-solvent corrections were utilized without applying non-crystallographical symmetry restraints (Engh & Huber, 1991). Water molecules were added chosen by distance criteria and hydrogen-bonding geometry and were tested for position in spherical density, reasonable temperature factors, real-space *R* values and improvement of the *R* factors. The refined crystallographic *R* factor is 21.1 and R_{free} is 22.6. Real-space *R* factors calculated for the final model using *CNS* show excellent correlation between the protein model and the electron density (real-space correlation coefficient is 0.87; real-space *R* factor is 0.16). In contrast, the real-space correlation coefficient and real-space *R* factor for CAPB are 0.63 and 0.34, respectively. Analysis of several difference Fourier maps (scaled at different σ levels) did not give any further suggestion for improving the CAPB model. Performing an occupancy-refinement protocol in *CNS* reveals an overall occupancy of 0.83 for the betaine. The final model includes 204 amino-acid residues, three sulfate ions, 518 waters and one CAPB molecule.

3. Results and discussion

3.1. Crystals and X-ray diffraction

Initial setups where RHCC crystals grew in the presence of CAPB showed that the crystallization behaviour differed significantly from the previous conditions. Despite the fact that the crystal size, morphology and space group did not change, the growth rate was accelerated dramatically. In contrast to control setups, which produce full-size crystals within 4–6 weeks, single RHCC crystals co-crystallized with CAPB grow fully within 1–3 d. Test series of different dilutions of the additive were not indicative of a concentration limitation. The best diffracting data were obtained with 1.2–1.5%(*w*/*v*) CAPB. Remarkably, there is no need to soak RHCC crystals in cryoprotection solutions in order to perform X-ray experiments at 100 K. The diffraction limit on the in-house X-ray source was about 1.2 Å and a

complete high-quality data set was collected at 1.4 Å resolution. To perform a proof of concept, a total of ten crystals of RHCC were handled with CAPB and showed comparable properties. The data-collection statistics are given in Table 1.

3.2. Overall structure

The structure of RHCC co-crystallized with CAPB was solved and refined at 1.4 Å resolution using native RHCC (PDB code 1fe6) as the search template (Fig. 1). Table 1 summarizes the crystallographic refinement statistics. The electron density for the refined structure is well defined and the final model consists of 1618 protein atoms, 518 water molecules, three sulfates and one CAPB molecule. The rather low $B_{\rm overall}$ for protein atoms only (18.8 Å^2) indicates a rigid protein structure. Remarkably, only Asp21 and Ser25 in subunits *A*, *B* and *D* show alternative conformations, which is suggestive of a certain degree of flexibility within this region. A C$^\alpha$-atom superposition with the previously determined RHCC structure (PDB code 1fe6) reveals structural identity (r.m.s.d. of 202 C$^\alpha$ atoms < 0.2 Å).

The RHCC chain fragment forms a parallel right-handed coiled-coil tetramer with an average length and diameter of 72 and 25 Å, respectively (Fig. 1). Four large cavities ranging in volume between 145 and 300 Å^3 are placed at regular distances along the strictly hydrophobic channel of the RHCC tetramer. Two types of knobs-into-holes interactions at positions *a* and *h* correspond to a 7,4-motif of core residues. In addition, the RHCC structure shows strictly or predominantly hydrophobic amino acids in positions *e* and *k* (Fig. 1). Together, they form a very extensive hydrophobic interhelical interface. Of the large number of charged residues within the RHCC sequence (about one third of all amino acids), all are involved in salt-bridge formation, either intrahelically or interhelically (Stetefeld *et al.*, 2000), with two exceptions (Asp6 and Glu43). The salt bridges are organized into three complex networks forming layers that surround the tetrameric cylinder at the height of the cavities (Fig. 1).

3.3. CAPB-mediated crystal contacts

RHCC has been shown to form a regular mesh-like arrangement in the hexagonal setting of the crystal lattice. RHCC crystals have only 25% protein content and are consequently very loosely packed. The crystal contact face between symmetry-related molecules is extremely selective. Less than 5% of the solvent-accessible surface area is involved in crystal contacts. This suggested the addition of CAPB into the crystallization mix with the aim of optimizing the crystal contacts between symmetry-equivalent molecules. As shown in Fig. 2, the zwitterionic betaine is located at the interface between two RHCC portions. Remarkably, CAPB molecules could only be detected in the contact regions between two symmetry-equivalent molecules. Despite the fact that only a truncated CAPB could be interpreted in the electron-density map owing to its flexible aliphatic tail, both ionic and aliphatic mediator interactions could be observed. The terminal carboxy group of the betaine forms ionic interactions with Arg33, Thr31 and Arg36′, while the quarternary amine group is involved with both methylene groups in van der Waals contacts with Leu29 and Ile40′.

The following aliphatic tail is involved in a number of contacts, including Leu29, Leu32, Lys27 and Asp43′. Finally, the amide group including the carbonyl function forms network interactions with Asn28, Tyr25 and Asp43′. Although this part of the CAPB molecule could not be detected within the electron-density map, the molecule reveals a rather low *B* factor (33.3 Å^2) in comparison to water (41.3 Å^2) and sulfate ions (35.4 Å^2). This is indicative for a tight and selective protein–CAPB interaction.

4. Conclusion

The aim of this study was to explore the ability of the surfactant CAPB as a potential mediator of favourable crystal contacts. CAPB shows several properties that are attractive for crystallization

Table 1
Data-collection and refinement statistics of RHCC at 1.4 Å (PDB code 1ybk; with CAPB) using the in-house rotating-anode generator compared with 1.8 Å resolution data from DESY beamline X11 (PDB code 1fe6; without CAPB; Stetefeld *et al.*, 2000).

All data sets were measured at 100 K. Values in parentheses are for the final shell.

Data-collection statistics		
X-ray source	Elliot GX-20	Synchrotron
Resolution (Å)	1.4 (1.49–1.40)	1.8 (1.87–1.80)
Observed reflections	804598	854694
Unique reflections	86287	46116
Completeness	96.6 (96.6)	100 (100)
$R_{\rm sym}$†	6.3 (37.0)	5.2 (28.7)
Wilson *B* factor (Å^2)	22.19	24.16
$\langle I/\sigma(I)\rangle$	14.6 (2.7)	12.1 (3.4)
Refinement statistics		
R factor‡ (%), no σ cutoff	21.1 (28.9)	19.77 (23.3)
$R_{\rm free}$ (%), no σ cutoff	22.6 (29.9)	21.65 (26.1)
Mean *B* factor (Å^2)		
Protein atoms	18.8	27.98
Water molecules	41.3	40.24
Sulfate ions	35.4	—
CAPB	33.3	—
R.m.s.d. bonds (Å)	0.004	0.004
R.m.s.d. angles (°)	0.7	0.72
Ramachandran plot§	100/0/0/0	100/0/0/0

† $R_{\rm sym} = \sum|I - \langle I\rangle|/\sum I$. ‡ *R* factor $= \big||F_{\rm obs}| - |F_{\rm calc}|\big|/\sum|F_{\rm obs}|$. § Percentage of residues in most favoured/additional allowed/generously allowed/disallowed regions of the Ramachandran plot.

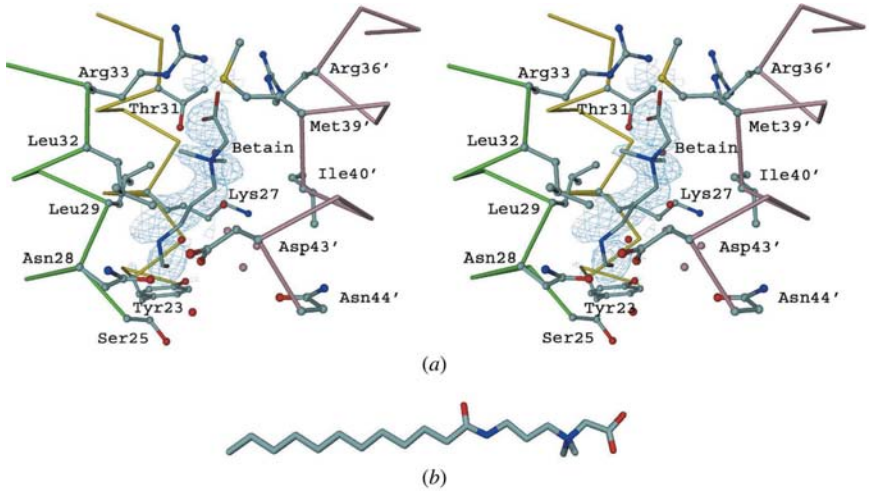

Figure 2
Superposition of the final refined $2DF_{\rm o} - mF_{\rm c}$ map at 1.4 Å resolution (1.5σ contour level) onto the crystal contact area mediated by cocoamidopropylbetaine. (*a*) The aliphatic tail of the betaine molecule could not be detected. Side-chain atoms are coloured by atom type. Water molecules are drawn as red balls. The backbones of helices *C* and *D* are shown in green and yellow, respectively. Backbone atoms and water molecules of symmetry-equivalent molecules are shown in pink. Residues of the symmetry-equivalent molecule are denoted with ′. (*b*) The chemical structure of CAPB coloured according to atom type.

purposes. The zwitterionic molecule is not sensitive towards oxygen, displays a high stability, is highly soluble in water and does not alter the physico-chemical properties of precipitating agents and buffer systems (*e.g.* pH, viscosity or absorbance). It has to be mentioned, if used in large-scale approaches, that CAPB is cheap and can be easily obtained. A possible explanation of the data reported in this work is that CAPB acts as a promoter of specific intermolecular interactions that lead to stronger crystal contacts. Our data suggest the use of CAPB as an additive to improve the crystal quality without changing the already established crystallization conditions. To expand our experience, projects using CAPB for more tightly packed crystals and as an additive in primary screening protocols are still in progress.

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