Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The dual role of CHAPS in the crystallization of stromelysin-3 catalytic domain

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Received 26 June 2002
Accepted 30 September 2002

CHAPS {3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate} is a non-denaturing detergent widely used for protein solubilization and stabilization. CHAPS was used to avoid protein aggregation during concentration of the recombinant stromelysin-3 (ST3) catalytic domain and was required to stabilize the protein, allowing its crystallization. The crystal structure of the complex between the ST3 catalytic domain and a phosphinic inhibitor shows two CHAPS molecules binding to ST3 in two different orientations. One CHAPS molecule is masking a hydrophobic surface of the protein, thus avoiding protein aggregation. This detergent molecule is also involved in packing interactions. The other detergent molecule is located in a pocket formed by the N- and C-terminal parts of the ST3 and stabilizes a loop that normally binds a Ca atom.

1. Introduction

CHAPS {3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate} is a non-denaturing detergent employed for protein solubilization, stabilization (Matuo *et al.*, 1985, 1991, 1988; Poetsch *et al.*, 1999) and crystallization (Khazanovich *et al.*, 1996; Chen *et al.*, 2000; Garman *et al.*, 2000; Bogin *et al.*, 2002; Itou *et al.*, 2002).

We used CHAPS to avoid aggregation of the recombinant stromelysin-3 (ST3 or MMP-11) catalytic domain during the concentration step prior to the crystallization trials (Kannan *et al.*, 1999; Gall *et al.*, 2001). This detergent proved to be essential for both protein stability and crystallization. The crystal structure of the ST3 catalytic domain complexed with a phosphinic inhibitor at 2.6 Å resolution shows two CHAPS molecules binding to ST3 in two different orientations (Gall *et al.*, 2001). In this paper, we provide a detailed analysis of CHAPS structural and functional character-

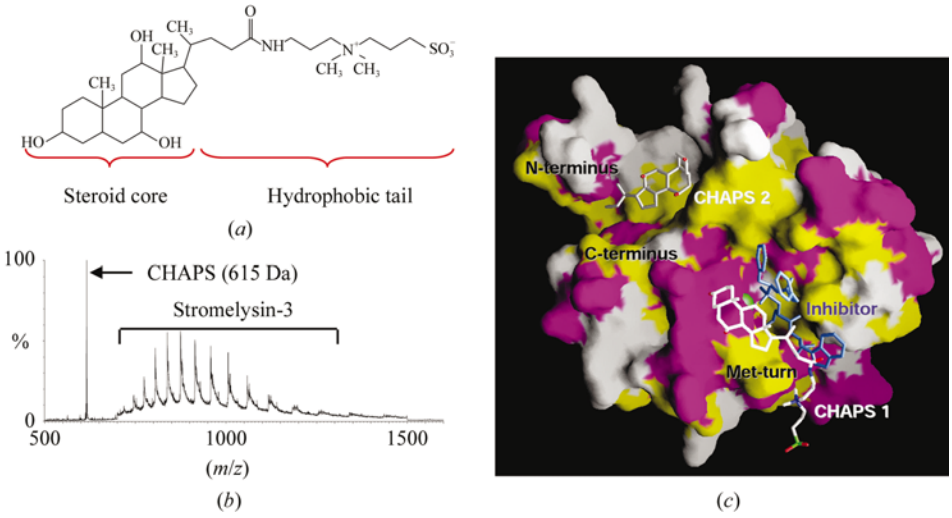

Figure 1
The two CHAPS molecules bound to the ST3 catalytic domain. (*a*) General formula of CHAPS {3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate}. (*b*) Analysis of the crystal content by mass spectrometry under denaturing conditions. The crystals contain the entire recombinant protein (20 098 kDa) and CHAPS molecules (615 Da). (*c*) Representation of the ST3 molecular surface according to the polarity of the residues (hydrophobic residues are coloured yellow and polar residues are coloured magenta). The catalytic zinc (green sphere) is lying on the surface of the active site and the inhibitor is coloured blue. Two CHAPS molecules are bound to ST3. One detergent molecule (CHAPS 1) is located near the Met-turn, with the hydrophilic side of the steroid core directed towards the ST3 polar surface and the detergent tail masking a hydrophobic patch of ST3. The second CHAPS molecule (CHAPS 2) is located in a hydrophobic pocket formed by the N- and C-terminal parts of the protein and interacts through the hydrophobic side of its steroid core. Figure created with *GRASP* (Nicholls *et al.*, 1991).

istics that explain the dual role of the detergent molecule.

2. Material and methods

The recombinant protein used for the determination of the crystal structure was cloned into a pET-3b plasmid, expressed in *Escherichia coli* BL21 (DE3 pLysS) cells and purified from inclusion bodies (Kannan *et al.*, 1999). After renaturation, the protein solution was concentrated in the presence of 0.3%(*w*/*v*) CHAPS {3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate} (Fig. 1*a*) to avoid protein aggregation. In the presence of CHAPS, the protein is monodisperse and active as tested by an indirect colorimetric assay (data not shown; Kannan *et al.*, 1999). The phosphinic inhibitor was added to the protein solution during the last step of concentration (inhibitor:protein ratio of 2.2:1).

Crystals (20 × 20 × 50 µm) were obtained at 277 K under the following conditions: 5 µl hanging drops containing 5 mg ml^{-1} protein, 0.25 m*M* inhibitor, 50 m*M* MES pH 5.5 and 500 m*M* ammonium sulfate (AS) pH 5.5 were equilibrated against 500 µl of 50 m*M* MES pH 5.5, 500 m*M* AS pH 5.5 and 1% trifluoroethanol as previously described (Gall *et al.*, 2001). These crystals diffracted to 2.6 Å and diffraction data were collected at the ESRF (European Synchrotron Radiation Facility, Grenoble, France). The crystal structure was solved by molecular replacement using human collagenase-1 as a starting model. The asymmetric unit contains six ST3 molecules, forming large solvent channels. Good electron density was observed for two CHAPS molecules. Analysis of dissolved crystals by mass spectrometry under denaturing conditions confirms the presence of the detergent in the crystals (Potier *et al.*, 2000) (Fig. 1*b*).

3. Results and discussion

CHAPS {3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate} (Fig. 1*a*), a non-denaturing detergent, was used to avoid aggregation of the recombinant stromelysin-3 catalytic domain during concentration (Kannan *et al.*, 1999).

In order to determine the optimal CHAPS concentration, different amounts of CHAPS were tested and the protein recovery during concentration was recorded (Table 1).

During refinement, electron density corresponding to the presence of two CHAPS molecules bound to the ST3 catalytic domain was detected. Their presence was confirmed by analysis of dissolved crystals by mass spectrometry under denaturing conditions (Potier *et al.*, 2000) (Fig. 1*b*). One molecule (CHAPS 1) is located near the Met-turn and the other (CHAPS 2) is near the N-terminal part of the enzyme (Fig. 1*c*; Gall *et al.*, 2001).

The binding of these two detergent molecules does not disturb the inhibitor-binding mode: if we compare our crystal

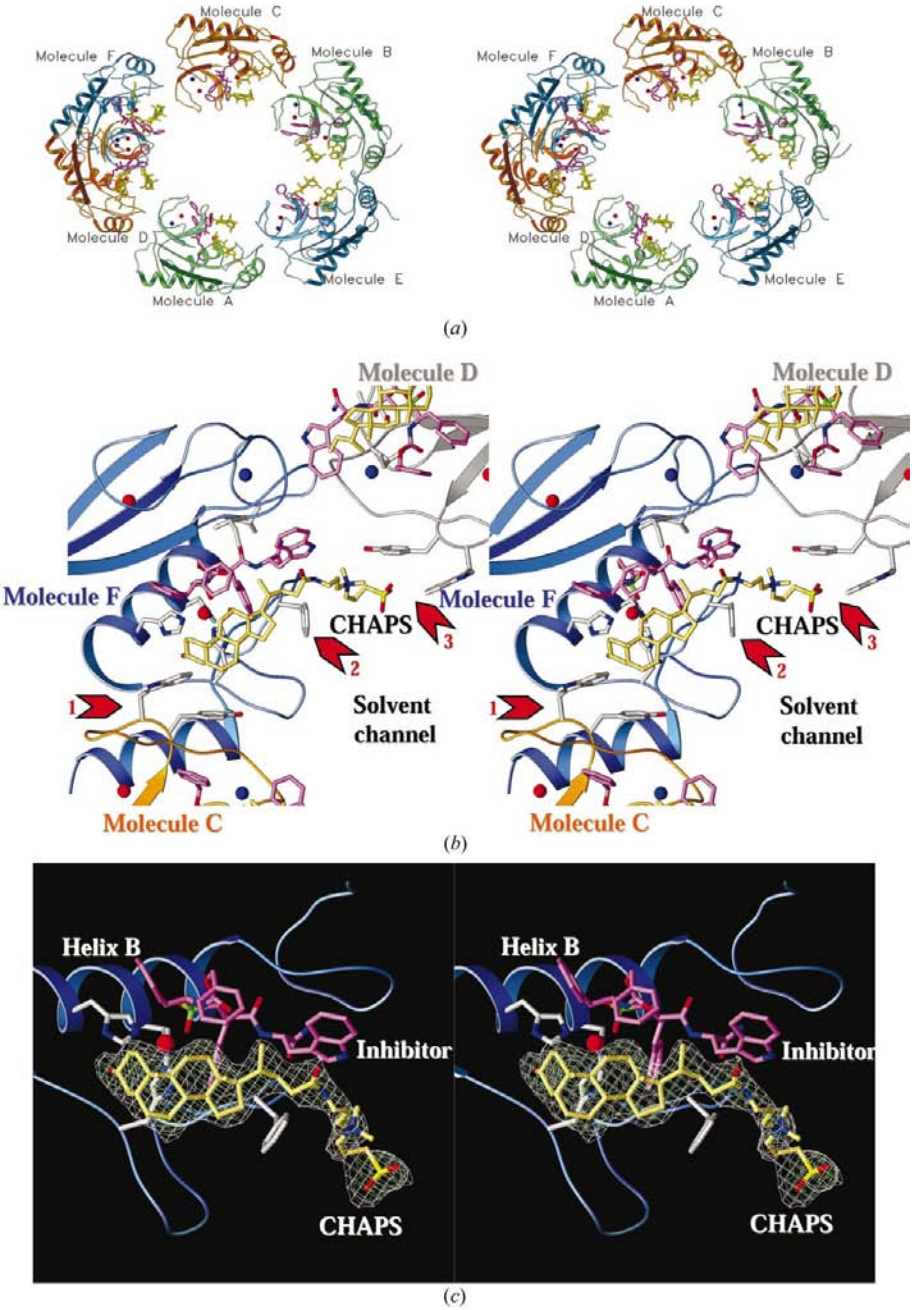

Figure 2
The CHAPS molecule located near the Met-turn. (*a*) The six independent molecules of ST3 are arranged to form an imperfect sixfold axis: molecules *B* and *E* are at a distance of 15 Å. They form a solvent channel with a diameter of 55 Å. The CHAPS molecules are represented in yellow, the phosphinic inhibitor in pink, the Zn atoms as red spheres and the Ca atoms as blue spheres. (*b*) Packing interactions between the CHAPS molecule and molecules *F*, *D* and *C*. The pile-up interactions between the inhibitor P_1 group (Phe) bound to molecule *F*, the CHAPS steroid core and Tyr166 and Trp167 of molecule *C* are labelled '1'. The packing interactions involving the inhibitor P_2' group (Trp) bound to molecule *F*, the detergent tail and Phe240 of molecule F are labelled '2'. The pile-up interactions between Tyr166 and Trp167 of molecule *D* and the end of the CHAPS tail are labelled '3'. (*c*) Final electron-density map ($2F_{obs} - F_{calc}$) contoured at 1.0σ (white density) and at 2.0σ (dashed green density) around the entire CHAPS molecule. Figures created with *SETOR* (Evans, 1993).

structure with the structures of other MMPs, the zinc-binding motif ($H^{219}EXXHXGXXH^{229}$) superimposes perfectly (r.m.s. fit of 0.209 Å compared with the collagenase-1 zinc-binding motif; Table 2). Two detergent molecules are present in each of the six complexes in the asymmetric unit (Fig. 2*a*).

The r.m.s. fit was calculated by superimposing the zinc-binding motif ($H^{219}EXHXXGXXH^{229}$) of different MMPs with the crystal structure of collagenase-1 (PDB code 1hfc), stromelysin-3 (PDB code 1hv5), gelatinase A (PDB code 1qib), stromelysin-1 (PDB code 1sln), matrilysin (PDB code 1mmp), collagenase-2 (PDB code 1mmb), collagenase-3 (PDB code 830c) and MT1-MMP (PDB code 1bqq).

The first CHAPS molecule (CHAPS 1), located near the Met-turn, is bound to stromelysin-3 through hydrogen bonds and hydrophobic contacts (Fig. 2*b*). Surprisingly, the hydrophilic side of the CHAPS steroid core points towards the enzyme, two O atoms (O_2 and O_3) making hydrogen bonds with the main chain and the side chain of His229 and the third one (O_4) with a water molecule interacting with both the carbonyl group of the Pro239 of the Met-turn and one O atom of the inhibitor phosphinic group (Table 3). The hydrophobic side is exposed to the lumen of a solvent channel. Pile-up interactions are observed between the CHAPS steroid core, the P_1 group of the inhibitor bound to ST3 (molecule F) and Tyr166 and Trp167 of another ST3 molecule (molecule *C*) (labelled '1' in Fig. 2*b*). Electron density is clearly observed for the steroid core and the first four C atoms of the tail of CHAPS bound to five of the six ST3 molecules of the asymmetric unit (molecules *A*–*E*). The quality of the electron-density map allows the construction of one entire molecule located near the Met-turn of the sixth molecule (molecule *F*) (Fig. 2*c*). The tail of this detergent molecule winds round Phe240 and is involved in packing interactions between two ST3 molecules (*F* and *D*) and the inhibitor P_2' group (labelled '2' in Fig. 2*b*). The end of the CHAPS tail makes pile-up interactions with Tyr166 and Trp167 of ST3 (labelled '3' in Fig. 2*b*).

The second CHAPS molecule (CHAPS 2) is located in a pocket formed by the N- and the C-terminal parts of the enzyme (Fig. 1). No electron density appears for the flexible tail of this detergent molecule. The hydrophobic side of the CHAPS steroid core interacts with the ST3 surface by hydrophobic contacts with a conserved loop that normally coordinates a Ca atom in other MMPs (Fig. 3*a*: ST3 is represented in red) (Becker *et al.*, 1995; Bode *et al.*, 1994; Browner *et al.*, 1995; Fernandez-Catalan *et al.*, 1998). According to the sequence, this calcium-binding site is potentially present in stromelysin-3 (Fig. 3*b*), but the loop ligating this Ca atom, located between strands IV and V, is displaced by the CHAPS molecule (r.m.s. fit of 3.204 Å when compared with collagenase-1; Fig. 3*a*). The CHAPS molecule replaces the calcium in the ST3 structure and thus stabilizes a weak calcium-binding site.

Table 1
Effect of CHAPS on the protein concentration yield.

CHAPS (%)	Yield (protein recovery) (%)
0.0	31
0.1	32
0.2	38
0.3	47

Table 2
R.m.s. fit based on the MMP-1 coordinates.

MMP	R.m.s. fit (Å)
Stromelysin-3	0.209
Gelatinase A	0.151
Stromelysin-1	0.186
Matrilysin	0.162
Collagenase-2	0.122
Collagenase-3	0.113
MT1-MMP	0.219

Table 3
Interactions between the ST3 and the steroid core of the CHAPS molecule located near the Met-turn.

	Distance (Å)	Residue	Atom
CHAPS atom			
O1	3.0	His229	N
O2	3.1	His229	ND1
O3	2.5	H_2O	O
Water molecule			
H_2O	3.0	Pro239	O
H_2O	3.1	Inhibitor	O3

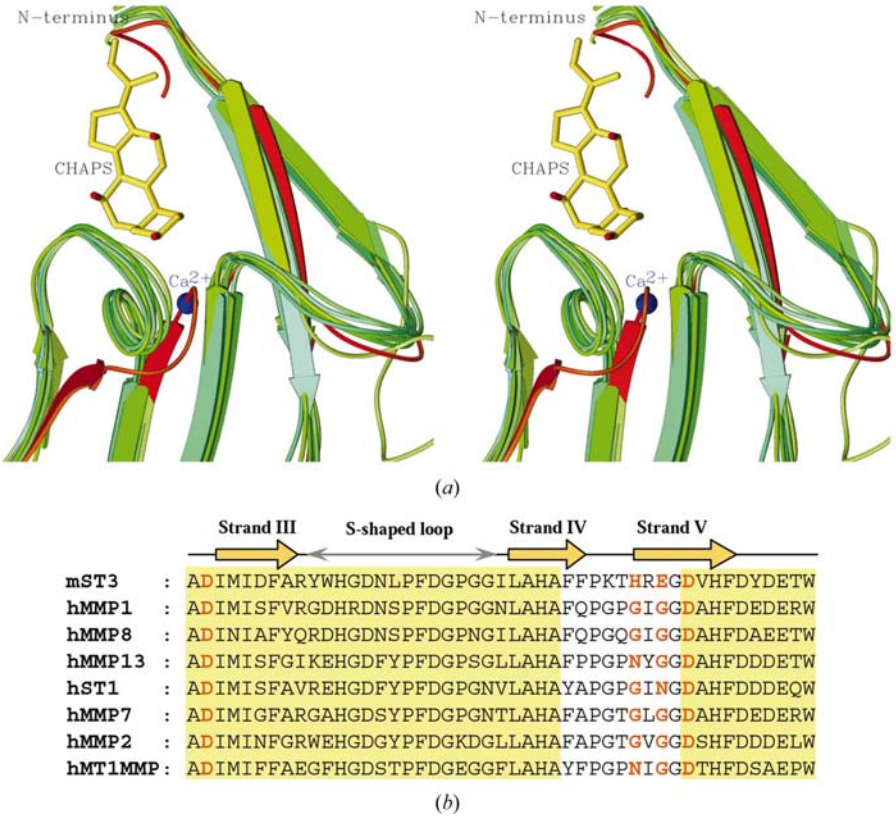

Figure 3
Superimposition of eight MMP catalytic domains in the loop sIV–sV. (*a*) The ribbon structures of eight different MMPs are superimposed in green (collagenase-1, collagenase-2, collagenase-3, MT1-MMP, matrilysin, stromelysin-1 and gelatinase A). The calcium present in these MMPs is represented as a blue sphere (Ca^{2+}). The ST3 backbone is represented in red and the CHAPS molecule, only found in our ST3 crystal structure, is coloured yellow with the O atoms in red. Owing to the presence of the CHAPS molecule, the loop connecting strand IV to strand V is displaced in the ST3 and cannot bind the Ca atom. Figure created with *SETOR* (Evans, 1993). (*b*) Structure-based alignment of eight MMP catalytic domains. The sequences of eight MMP catalytic domains have been aligned according to their three-dimensional structure with the program *MODELLER* (Sali & Blundell, 1993). The regions sharing the same structure are represented as yellow boxes. The residues ligating the calcium ion are coloured orange and are present in ST3.

4. Conclusions

In this work, we show the importance of CHAPS for protein stabilization and solubilization before crystallization and explain on a molecular level how this detergent stabilizes both ST3 and crystal-packing contacts. CHAPS (i) shields hydrophobic zones from non-specific aggregation, (ii) makes packing interactions and (iii) stabilizes disordered loops to reduce heterogeneity. In the crystal, the steroid cores have different orientations: one molecule has its hydrophobic side exposed to the solvent, whereas the other one has its hydrophobic side interacting with ST3. The role of the first CHAPS molecule that binds through hydrophobic contacts with Tyr and Trp resides and favours packing interactions is reminiscent of other observed cases (Bogin *et al.*, 2002; Klaholz & Moras, 2000; Itou *et al.*, 2002). The function of the second molecule, which occupies a calcium-binding site through hydrophobic contacts with a flexible loop involved in Ca coordination, is more surprising. Understanding the strength of this interaction and its specificity would justify further solution studies.

We thank Isabelle Stoll for excellent technical assistance, Edward Mitchell and Sean McSweeny (ESRF, Grenoble, France) for help during data collection and Noëlle Potier for mass-spectrometry analysis. This work was supported by funds from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Bristol-Myers Squibb Pharmaceutical Research Institute, the Association pour la Recherche sur le Cancer, the Ligue Nationale Française contre le Cancer and the BIOMED 2 (Contract BMH4CT96-0017) and BIOTECH 2 (Contract ERBBIO4CT96-0464) programs. ALG was partly supported by a fellowship from the CNRS and the Région Alsace (1997–2000) and from the Ligue Nationale Française contre le Cancer (2000–2001).

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