Acta Cryst. (1998). D**54**, 276–278

Crystallization of the α-hemolysin heptamer solubilized in decyldimethyl- and decyldiethylphosphine oxide

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(*Received 30 December 1996; accepted 23 July 1997*)

Abstract

Crystals of the α-hemolysin heptamer, a transmembrane pore-forming toxin from *Staphylococcus aureus*, have been grown using the nonionic detergents *n*-decyldimethyl- and *n*-decyldiethylphosphine oxide, phosphorus homologs of the ionic amine oxide detergents. Five crystal forms were obtained, one of which diffracted X-rays to 3 Å resolution. This crystal form displayed elements of pseudo *mm* symmetry in screened precession photographs yet it was triclinic with unit-cell dimensions a = 173, b = 173, c = 102 Å, α = 92.6, β = 94.8, γ = 90.2°.

1. Introduction

In general, membrane protein crystallization requires an aqueous solution of micelles that will (i) stabilize the protein in a folded native and biologically active conformation and (ii) facilitate the growth of a well ordered three-dimensional lattice (Michel, 1990). Interactions between the protein and the detergent micelle and monomers are of primary importance for maintaining the stability of the protein while protein–protein, protein–detergent and detergent–detergent interactions participate in nuclei formation and lattice propagation in type II crystals (Michel, 1983). Thus, a detergent must satisfy a host of criteria for efficacy in membrane-protein crystallization. To date, only a small group of detergents and phospholipids are employed in membrane protein crystallization, such as *n*-octyl-β-D-glucoside (Michel & Oesterhelt, 1980; Papiz *et al.*, 1989; Picot *et al.*, 1994), *n*-decyl- (Tsukihara *et al.*, 1995) and *n*-dodecyl-β-D-maltoside (Iwata *et al.*, 1995), 6-O-(*N*-heptylcarbamoyl)-methyl-α-D-glucoside (Lee *et al.*, 1995), decanoyl-*N*-methylglucamide (Yu *et al.*, 1996), *n*-octyl tetraethyleneglycol (Kreusch *et al.*, 1991), *n*-dodecyl octaethyleneglycol (Shinzawa-Itoh *et al.*, 1995), *N,N*-dimethyldodecylamine-*N*-oxide (Michel, 1982), sucrose monolaurate (Kawamoto *et al.*, 1994), and micelle-forming phospholipids (Eiselé & Rosenbusch, 1989; Yu *et al.*, 1996) that include dipentanoyl-, dihexanoyl-, diheptanoyl- and monotetradecanoyl phosphocholine (Huang *et al.*, 1997; Song & Gouaux, 1997). Detergents and phospholipids that have proven successful for membrane protein crystallization are nondenaturing, usually chemically homogeneous and typically form small uniformly sized micelles.

Decyldimethyl- and decyldiethylphosphine oxide (C_{10}DMPO and C_{10}DEPO, respectively) (Fig. 1) have favorable properties for use in membrane protein crystallization and here we describe their application to the crystallization of the α-hemolysin heptamer (αHL). These phosphine oxide detergents are stable in typical aqueous solutions to oxidation, reduction and hydrolysis, they do not contain asymmetric centers that might undergo racemization, and they can be prepared on a large scale. C_{10}DMPO and C_{10}DEPO have critical micelle concentrations (c.m.c.'s) in the m*M* range and form relatively small micelles which exhibit a low degree of polydispersity. The monomers and micelles are readily soluble in aqueous solution (Herrmann *et al.*, 1966; Lunkenheimer *et al.*, 1987; Table 1). Furthermore, the solubility properties of phosphine oxide detergents are not pH dependent over the range of most crystallization screens (pH 4.0–9.0), in contrast to the pH-dependent solubility of the amine oxide detergents, which results from the titration of the amine oxide moiety (pK_a = 4.9; Herrmann, 1964). Thus, the design of crystallization screens is not further complicated by large changes in detergent solubility as a function of pH.

Table 1. *Surfactant properties of dimethyl- and diethyl decylphosphine oxides*

	C_{10}DMPO	C_{10}DEPO
M_r	218.31	246.33
C.m.c. (m*M*)	3.8–4.6†	~1.5‡
Micelle molecular weight	28600†	Not available

† Herrmann *et al.* (1966) ‡ Lunkenheimer *et al.* (1987)

Crystallization screens using C_{10}DMPO and C_{10}DEPO were applied to αHL from *Staphylococcus aureus*. αHL, a self-assembling pore-forming toxin (Bayley, 1995), is secreted as a water-soluble monomer of 33.2 kDa that creates heptameric transmembrane channels following oligomerization on a cell or synthetic bilayer surface (Song *et al.*, 1996). Crystallization of the detergent-solubilized heptamer under a wide range of solution conditions and in different crystal lattice environments is part of our effort to determine the conformational flexibility of the heptamer, the dependence of the structure on the detergent of crystallization, and the utility of new detergents for membrane-protein crystallization.

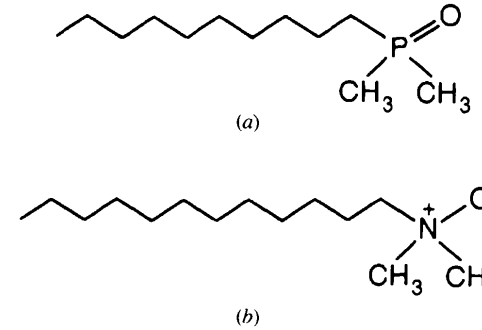

Fig. 1. (*a*) Decyldimethylphosphine oxide and (*b*) dodecyldimethylamine oxide.

2. Materials and methods

Batches of 300 glass cover slips (VWR Scientific) were treated with AquaSil (Pierce) prior to use in crystallization trials. Borosilicate glass capillaries (Charles Supper) were treated with dichlorodimethyl silane in methylene chloride. Purified phosphine oxide detergents were the generous gift of Dr Robert G. Laughlin of Proctor and Gamble and were used directly. Polyethylene glycols (PEG's) and the monomethyl ether (MME) variants were purchased from Sigma and Fluka. *n*-Octyl tetraethyleneglycol was obtained from Bachem. Expression, assembly and purification of αHL was carried out as previously described (Gouaux *et al.*, 1994; Song *et al.*, 1996), with the penultimate step involving size-exclusion chromatography in *n*-octyl tetraethyleneglycol (C8E4) (Song & Gouaux, 1997). The C8E4 was replaced with the phosphine oxide detergents by size-exclusion chromatography using a buffer composed of 150 m*M* NaCl, 10 m*M* sodium acetate, 9 m*M* C_{10}DMPO or C_{10}DEPO (pH 5.0) and a Superdex HR 200 (16/60, Pharmacia) size-exclusion column. Fractions containing the heptamer were pooled, concentrated to ~7 mg ml^{-1} and dialyzed against a solution of 10 m*M* sodium acetate, 9 m*M* C_{10}DMPO (pH 5.0) that was changed three times, allowing 8 h between changes. The protein concentration was reduced to 7 mg ml^{-1} using the acetate dialysis buffer immediately prior to crystallization. Crystallization screens consisted of full factorial and sparse sampling approaches (Weber, 1990; Song & Gouaux, 1997) and experiments were set up using the hanging-drop vapor-diffusion method. Drops were composed of one volume of protein solution combined with one volume of reservoir solution on a hydrophobic glass cover slip. Crystals were mounted in siliconized glass capillaries and were characterized by still screenless and screened precession photographs. X-rays were generated from a Rigaku RU-200 fitted with a copper anode and Ni filter.

3. Results and discussion

The phosphine oxide detergents, C_{10}DMPO and C_{10}DEPO, stabilized the αHL heptamer from dissociation more effectively than *n*-octyl tetraethylene glycol as judged by incubation at room temperature over time periods of months and analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and size-exclusion chromatography. In the case of αHL, phosphine oxide detergents with either dimethyl or diethyl head groups and a *n*-decyl alkyl moiety maintained the stability of the protein and are suitable in protein purification and crystallization trials. The stability of αHL in C_{10}DMPO and C_{10}DEPO may also result from, at least in part, the thermal stability of the heptamer, which resists dissociation by sodium dodecyl sulfate up to 338 K (McNiven *et al.*, 1972; Walker & Bayley, 1995).

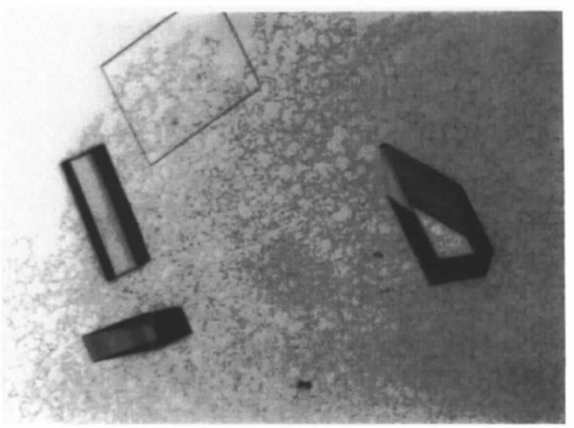

Fig. 2. Photomicrograph of a form 2 crystal, space group *P*1, grown using αHL solubilized in C_{10}DMPO. The largest crystal dimension is approximately 0.4 mm.

Crystallization screens yielded a number of distinct crystal morphologies using five reservoir solutions. Rhombohedra and plates were obtained with 5% PEG 5000 monomethyl ether (PEG 5000 MME; form 1); thick yet twinned rectangular plates were grown in the presence of 12.5% PEG 2000 MME, 0.4 *M* $MgCl_2$, and 0.05 *M* sodium citrate (pH 5.6, form 2); parallelpipeds were produced using 12% PEG 3350, 0.8 *M* $MgCl_2$ and 0.05 *M* citrate (pH 5.6, form 3); long thin bars formed in the presence of 8% PEG 3350, 0.3 *M* NaBr and 0.05 *M* sodium citrate (pH 5.6, form 4); and 10% PEG 3350, 0.8 *M* LiCl, 0.05 *M* sodium cacodylate (pH 6.5) or 12% PEG 3350, 1.5–2.0 *M* LiCl, and 0.05 *M* sodium cacodylate (pH 6.5) also produced plates (form 5). Growth of single large form 2 crystals (Fig. 2) was achieved by the inclusion of 1–2%(*w*/*v*) 1,2,3-heptanetriol (high melting isomer; Michel, 1983) in the protein solution prior to combining the protein solution with the reservoir solution.

The most well ordered crystals obtained from the initial screening diffracted to 3 Å resolution (form 2), were triclinic, and had unit-cell dimensions of a = 173, b = 173, c = 102 Å, α = 92.6, β = 94.8, γ = 90.2°. An exhaustive search of diffraction space using still and precession photographs did not provide evidence for the presence of a lattice with higher symmetry. Although elements of *mm* symmetry were displayed in screened precession photographs of the *hk*0 zone, a closer inspection revealed that there were many violations of the pseudo *mm* symmetry. Dissolved crystals of the αHL heptamer did not give reliable quantitative amino-acid analysis results (M. R. Hobaugh and J. E. Gouaux, unpublished results) and thus determination of the number of molecules per asymmetric unit, Z_a, by measuring the number of moles of protein per crystal volume, was not possible (Kwong *et al.*, 1994). Nonetheless, an estimate of Z_a was derived from the size and shape of a heptamer, which is mushroom shaped and approximately 100 Å tall and up to 100 Å in diameter (Song *et al.*, 1996), and from consideration of reasonable crystal-density values. Packing considerations indicated that more than five heptamers per unit cell were unlikely. V_m values for two, three, four and five heptamers per unit cell were 6.5, 4.4, 3.3 and 2.6 Å^3 Da^{-1}, respectively. Since the crystals were somewhat sensitive to mechanical manipulation and diffracted to moderate resolution, there were probably three or four heptamers in the unit cell.

In conclusion, we have found that two members of a family of phosphine oxide detergents, $DMC_{10}PO$ and $DEC_{10}PO$, maintained the solubility and stability of the αHL heptamer and produced crystals that diffracted X-rays to Bragg spacings of 3 Å. C_{10}DMPO and C_{10}DEPO, as well as other phosphine oxide detergents, may prove useful for the crystallization of membrane proteins in general and thermostable all-β membrane proteins, such as αHL, in particular.

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