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Poly(ethylene) Glycol Monomethyl Ethers – an Alternative to Poly(ethylene) Glycols in Protein Crystallization

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Abstract

Poly(ethylene) glycol monomethyl ethers (Peg-mmes) are a series of methyl substituted poly(ethylene) glycols that have been used with some success in the crystallization of a number of hydrophobic proteins. Crystallization of a lipase from *Humicola lanuginosa* complexed with the C12 substrate analogue from Peg-mme 5000, an endoglucanase 1 and a 59 kDa fragment of human topoisomerase IIα crystallized from Peg-mme are described. The use of Peg-mme for improving the quality of crystals previously grown from normal poly(ethylene) glycol 8000 is also described. We suggest that these modified Peg-mmes should be regularly used in screening for crystallization.

Introduction

Poly(ethylene) glycols have been used extensively in protein crystallization to great effect. We would like to draw the attention of workers in this field to a series of poly(ethylene) monomethyl ethers that we have found to be very useful, especially for the crystallizations of proteins with more hydrophobic-like nature or precipitating rapidly with the use of conventional precipitants.

Materials and methods

Poly(ethylene) glycol monomethyl ethers (Peg-mmes) [$CH_3(OCH_2CH_2)_nOH$] are available in a series of molecular weights 350, 550, 750, 2000, 5000 from a number of chemical suppliers (Aldrich, Sigma, Fluka). The chemical formulae of the Peg-mmes show similarity with the general formula of the Brij detergents – $R(OCH_2CH_2)_nOH$, where R represents an aliphatic chain (from $C_{12}H_{35}$ to $C_{18}H_{35}$) and n varies from 4 to 20. In general, Peg-mmes were used much the same as the more familiar poly(ethylene) glycols [usually as 50%(w/v) stock solutions]. The lower molecular weight Peg-mmes are liquids, Peg-mme 750 is waxy solid, the highest molecular forms are powders. The proteins were crystallized by the vapour-diffusion hanging-drop method and the microbatch method as well. All trials were performed at 291 and 277 K. The resulting crystals were mounted in capillaries and their X-ray diffraction quality was preliminary tested using a Rigaku R-AXIS IIC image-plate detector on a Rigaku rotating-anode RU-200 X-ray generator with a Cu target and an Ni filter (operated at 50 kV and 100 mA).

Results and discussion

The first successful use of Peg-mme was for the crystallization of a complex of lipase from *Humicola lanuginosa* (Hll) with a 12-carbon substrate analogue *n*-dodecyl phosphonate ethyl ester (Brzozowski, 1993). Although a lipase complexed with a C6 substrate analogue – *n*-hexylphosphonate ethyl ester, had been successfully crystallized (Brzozowski *et al.*, 1991; Derewenda, Brzozowski, Lawson & Derewenda, 1992), the C12–Hll complex would not crystallize under any of the conditions examined, including the use of a number of detergents. It was thought that the methyl-substituted Pegs may behave in a more detergent-like manner. Crystals were successfully grown using Peg-mme 5000 [17%(w/v) in 50 m*M* Tris–HCl buffer pH 8.0]. The initial crystals were thin irregularly shaped plates, bearing close resemblance to the crystals of the lipase from *Candida antarctica* which had been grown from high-concentration detergent solution (A. M. Brzozowski, unpublished results). It was noticed, however, that over a period of 3–4 weeks the crystals began to change morphology eventually forming pseudotetragonal orthorhombic prisms, the largest of which were 0.4 × 0.4 × 0.25 mm. These crystals have been successfully used for structural determination. A similar result occurred in the crystalization of a complex of Hll with a C8 substrate analogue; the crystals again gradually changed morphology from poor thin irregular plates to much more useful orthorhombic rods.

This observation may indicate that the gentle detergent nature of the Peg-mmes allows the protein

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greater flexibility in crystal packing, choosing the form of greatest stability over time.

The phenomenon of multiple crystal forms is not unknown, especially with crystals grown from Peg solutions. The enzyme endoglucanase I from *Humicola insolens* had been crystallized in a number of forms from Peg 8000 [15–20%(*w*/*v*) in Tris–HCl pH 8.0]. The most common form was a monoclinic plate, with the occasional appearance (sometimes in the same drop) of a hexagonal rod form. A third form, tetragonal bipyramids, was grown exclusively from ammonium sulfate (35–40% sat. in MES pH 5.4) (Davies *et al.*, 1992). When subsequent batches of protein would not crystallize under any of the conditions used previously, monomethyl ethers of Pegs were tried. Crystals were finally grown using Peg-mme 2000 [20–25%(*w*/*v*) in Tris–HCl pH 8.0]. This time the predominant form was hexagonal rods, the largest of which were 1.5 × 0.15 × 0.15 mm. The monoclinic form did not grow at all but the tetragonal form was found, often growing in the same drops as the hexagonal crystals. The hexagonal crystals are now being used for structural elucidation.

Crystals of a 59 kDa fragment of human topoisomerase IIα have been grown from Peg-mme 2000. This protein is rather hydrophobic and severe precipitation problems occurred with all the usual range of Pegs, from Peg 400 to Peg 8000, even at very low protein concentration (3 mg ml^{-1}). However, thin irregular-shaped plates were grown from 5 mg ml^{-1} solutions of the protein in 50 m*M* ADA, 2 m*M* DTT, 60 m*M* NaCl and 4 m*M* $MgCl_2$ using 4–9%(*w*/*v*) Peg-mme 2000. Unfortunately, the morphology of these crystals has not changed to a more useful form and at present they only diffract to 7 Å. Experiments are continuing to try and improve the quality of these crystals. This result illustrates another advantage of the Peg-mmes, their gentle precipitating properties make them more suitable for crystallizing less soluble proteins.

Other crystals that have recently been grown using Peg-mmes include a DNA-binding protein (from Peg-mme 750 solutions), a mutant of lactate dehydrogenase (from Peg-mme 2000) and a 120 kDa fragment of human topoisomerase IIα (from Peg-mme 2000) (all these are still in the early stages of development, it is envisaged that crystallization notes will be published in due course).

Another use for the Peg-mmes has been to improve the quality of crystals previously obtained from other precipitating agents, especially Peg 8000. The enzyme penicillin acylase from *E. coli* was crystallized using batch methods from 12%(*w*/*v*) Peg 8000 in 50 m*M* MOPS pH 7.2 (Hunt, Tolley, Ward, Hill & Dodson, 1990). The crystals were rectangular prisms which sometimes grew up to 1.0 × 0.5 × 0.2 mm. Unfortunately, crystals appeared somewhat sporadically, they frequently did not diffract, were not always stable in the X-ray beam and occurred with different cell dimensions from batch to batch. When Peg-mme 2000 [20–25%(*w*/*v*) in the same buffer] was used instead, the crystals were more stable, strongly diffracting and were completely isomorphous with the Peg 8000 crystals. Again, the Peg-mme produced crystals when conditions used previously failed, enabling the structural determination to proceed. Similarly, with the same enzyme from *Alcaligenes faecalis*, the use of Peg-mme 2000 instead of Peg 8000 enabled data to be collected from crystals which, when grown under any other conditions, were so thin as to make mounting in capillaries impossible. Crystals of phosphomannose isomerase were also improved by growing from Peg-mme 2000 rather than the Peg 8000 from which they were originally obtained (details to be published elsewhere).

All crystals mentioned above (except the human topoisomerase IIα crystals) diffracted up to minimum 3.0 Å on the R-AXIS-II C image-plate detector enabling successful crystal and molecular structure determinations.

Finally, on a purely practical note, it was found that in most instances Peg-mme 2000 could be used directly to replace Peg 8000 in crystallization experiments. Generally, the concentration of the Peg-mme required was higher, sometimes as much as double that previously found for the molecular-weight equivalent of the normal Peg. For this reason we suggest that trials with Peg-mmes are initially carried out over a broad concentration range. It is also worth noting that a 50%(*w*/*v*) solution of, for example, Peg-mme 2000 is considerably less viscous than the comparable Peg 8000 solution. This makes handling easier and Peg-mme 2000 may be a useful replacement for Peg 8000 in robotic or other automated systems where viscosity of solutions has always caused problems. It should be stressed that, in our experience, it has not been necessary to alter other parameters when replacing Pegs with Peg-mmes, only the concentration of the precipitant itself, and in all cases so far, crystals grown from the alternative precipitant have been isomorphous with those from the original precipitant. In conclusion, we suggest that the poly(ethylene) glycol monomethyl ethers are a useful addition to the precipitants presently used by those engaged in protein crystallization and should be included routinely in trials.

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