Protein crystallization with alternative polymer precipitants

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Introduction

Use of polymer-based precipitants is widely spread in the current protein crystallization screens. In spite of that, the variety of the used polymeric compounds is limited. Our effort was focused on widening the spectrum of available polymer precipitants with potential applications in crystallization of complexes.

PolyA and Poly B crystallization screens

As a result of polymer testing, eight polymers were selected on the basis of their solubility and other properties at varying pH and salt concentrations. After preliminary testing on proteins, new crystallization screens PolyA and PolyB based on these eight polymers were formulated. Each screen uses 4 polymers and contains 36 conditions, altogether 2 screens x 4 polymers x 24 salt & pH = 192 crystallization conditions in PolyA and PolyB. The screens were tested in the frame of SPINE2-Complexes project and some good quality protein crystals have been obtained. The work was published (Skálová et al., J. Appl. Cryst., 2010, 43, 737-742).

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Survey of the tested 16 polymers. Precipitants chosen for the protein crystallization screens PolyA and PolyB are in the first part of the table. Stock solution concentration is shown as used in the reported trials and for PolyA and PolyB preparation.

Crystals obtained with PolyA and PolyB

Conclusions

Eight of the sixteen selected polymers were subjected to complex experimental testing which led to the formulation of two novel protein crystallization screens PolyA and PolyB. This work extends the pool of available polymeric protein precipitants and confirms applicability of polyethylene glycol dimethyl ethers, di[poly(ethylene glycol)] adipate, poly(ethylene glycol-ran-propylene glycol), poly(acrylic acid), sodium salt, and poly(ethylene glycol) methyl ether methacrylate. The tests showed significance of polymer type for results of a crystallization trial and confirmed the importance of variation of the same crystallization condition by exchanging polymeric precipitants. PAA 2100 and PEG 12000 proved to be the most successful candidates for protein crystallization screening. Polymer DPA 800 led to new structural results for human CD69.

This research was supported by the European Commission (integrated project SPINE2-Complexes no. LSHG-CT-2006-031220), by GA AV CR (project IAA500500701), and by GA CR (project 305/07/1073).

Percentage of successful crystallization hits for each polymer in the screens PolyA and PolyB (ca. 500 experiments performed per polymer).

Structure of CD69 obtained using a novel polymer

Beautiful crystals of human CD69 with lengths up to 561.8 µm were obtained using hanging drop vapor diffusion method with 1 µl of protein and 1 µl of precipitant solution: 30 % (v/v) di[poly(ethylene glycol)] adipate and 0.1M imidazole, pH 6.6. The diffraction data were measured at ID14-1 BESSYII (Berlin) and processed up to 1.37 Å resolution. The structure was deposited under PDB code 3HUP and published (Kolenko et al., Acta Cryst. (2009), F65, 1258–1260). The high resolution structure of the protein yielded details about asymmetry of the dimer interface and binding of a sodium ion at the dimer interface.

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