Controlling the grystal growth. Why is poly(ethyleneglycol) the most effective precipitant for protein crystallization?

Jindřich Hašek

Institute of Biotechnology, Czech Academy of Sciences, Průmyslová 595, 25 250 Vestec

hasekjh@seznam.cz

The lecture introduces a special approach to controlled crystallization based on modification of protein-protein adhesion properties using additives modifying properties of adhesive patches on protein surface.

Stability of protein crystal depends on stability of the 3D network of identical **protein-protein interfaces (PPI)** ensuring the correct position and orientation of the molecules in highly concentrated solution. Details on the **PPI** can change with crystallization solution and water content in the crystal. However, an inspection of **protein crystal architecture (PCA)** can easily reveal a **set of adhesion modes** characteristic for each crystal form. The strength of adhesion mode depends on adhesion forces between pairs of mutually attractive **adhesive patches** on the opposite protein surfaces. We can identify a set of dominant **protein-protein adhesion modes (PPAM)** that is responsible for formation of the respective crystal polymorph.

Classical model of crystallization process supposes that only the dominant **PPAM** between naked protein molecules is responsible for correct stacking of protein molecules into the growing crystal. New dynamic concept of the **protein surface-shielding agents (PSSA)** introduces more complex crystallization model based on competition between the **protein-protein interactions** and the **PSSA-protein interactions.**

Principal advantage of this approach is that using selective **PSSA**, we can rationally suppress the original dominant adhesion mode and initiate the crystallization process according to the new dominant **PPAM**.

Protein molecules stacked into the crystal by contacts of adhesive patches are always in dynamic equilibrium with solution. The optimal solvent content in crystal, corresponding to the best diffraction ability, depends largely on the **protein crystal architecture (PCA)** and on the physico-chemical composition of the crystallization solution. With increasing osmotic pressure and higher water content, the crystals lose stability. With lower water content, the regular crystal lattice brakes. In both cases, the crystal loses its diffraction ability. Experience shows that there we are not able stabilize protein crystals with solvent content over 85 % or bellow 25 %

The concept of the selective **PSSA** provides rational approach to protein crystallization has important practical implications and allows us:

1. Rational change of crystallization solution allows us a design of more suitable polymorph for our purposes (e.g. polymorph with active site open for ligand soaking, polymorph with biological oligomerization, polymorph with the best diffraction accuracy of structure determination, etc.)
2. The weakening of the dominant $\mathbf{P_1P_1AM}$ and the strengthening the $\mathbf{P_1P_2AM}$ allows us to grow the required co-crystals of several proteins (multicomponent crystals).

PEG (poly(ethyleneglycol) has, in addition to its precipitating ability, also a large scale of special adhesive modes to protein surface. One can easily regulate the PEG activity on various patches on protein surface almost of all types – binding to positively charged residues, binding to hydrogen donor or acceptor clefts, hydrophilic and also hydrophobic areas. The review of more than 3000 PEG-protein

interactions published in [2,3] shows the way how one can skip between different PEG-protein adhesion modes and thus change the preferences of dominant adhesion modes driving the crystallization process. It explains why PEG is so convenient tool for a control of crystal growth.

Practical part shows the examples clarifying activity of PEG on the protein surface.

A. Protein-protein interfaces **(PPAM)** of (1) biological relevance, (2) crystal oligomerization, (3) crystal contacts [4]

B. PSSA-protein interfaces (1) blocking the crystal contacts, (2) build in the crystal lattice [2], etc.

C. Review of PEG-protein interfaces (1) in presence of different salts, (2) build in the crystal lattice [3], etc.

1. Hašek J. *Protein surface shielding agents in protein crystallization.* J. Synchr. Radiation (2011) **18**, 50-52.

2. Hašek J. *Poly(ethylene glycol) interactions with proteins.* Z. Kristallogr. (2006) **23**, 613-619.

3. Hašek J. , Labský J., Skálová T., Kolenko P., Dohnálek J., Dušková J., Štěpánková A., Kovaĺ T., *Polymer structure database and protein-polymer interactions.* Z. Kristallogr. (2011) **28**, 475-480.

4. Skálová T., Bláha J., Harlos K., Dušková J., Kovaĺ T., Stránský J., Hašek J., Vaněk O., Dohnálek J., *Four crystal structures of human LLT1, a ligand of human NKR-P1, in varied glycosylation and oligomerization states,* Acta Cryst. (2015) **D71**, 578-591.

The project was supported by BIOCEV CZ.1.05/1.1.00/02.0109 from ERDF, RVO 86652036, and Czech Science Foundation 15-15181S.