Dynamic Theory of Protein Crystallization

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Thousands of laboratories around the world routinely use protein crystallization. However, the molecular order and corresponding diffraction quality of conventional organic molecules are much better. Speciality of protein crystallography is necessity of various additives in crystallization solution. There are experimentally proved additives to increase protein solubility, prevent its aggregation, control nucleation, modulate crystal habit, optimize buffer conditions, some additives modulate pH and ionic strength, some stabilize the protein, protect denaturation, other are necessary for freezing or for control of viscosity of solution. Many of these compounds play more roles in parallel. E.g. PEG is an efficient precipitant, also increases viscosity and also controls the way of deposition of protein molecules into the growing solid phase. Here, we discuss only the additives directly influencing regular stacking of protein molecules in the crystal called here **Crystallizing Agents (CA)**. By definition the CAs control amounts of miss-placed and miss-oriented molecules in the growing solid. Thus, they practically control the difference between the crystalline and amorphous state.

Practical protein crystallization is de facto a trial-and-error method. Many crystallization screens designed from some former experience are in use. Only few of the thousands of crystallization conditions tried are usually successful. Classical thermodynamics offers no explanation why the crystalline phase appears only in these few cases and why the presence of some CAs is usually necessary. Deep experimental studies (e.g. [1], [2]) indicate that very successful CAs are for example sodium malonate and poly(ethyleneglycol) (PEG).

Our task here is to clarify why such different compounds have very similar effects on correct ordering of protein molecules in crystal and to indicate the ways to even better CAs. Our dynamic theory of protein crystallization is based on the existence of **protein-CA adducts (PCA adducts)** in the molecularly overcrowded crystallization solution. The remains of PCA adducts can be often seen in the PDB [3]. Molecular movement in the molecularly dense environment of a saturated solution is quite complicated due to the presence of many intermolecular interactions. The effective CAs should have specific affinity to the protein surface. Therefore, most protein molecules in saturated solution form temporary (unstable) molecular PCA adducts with the CA molecules. These PCA adducts attach to the growing crystal surface instead of bare protein molecules. The crystallization solution is therefore a rather complex system that can hardly be considered as an ideal liquid. Movement of the PCA adducts in saturated solution leads to the desired unique orientation of the PCA adducts with respect to the direction of their movement to the surface of the growing solid state.

The orientation of PCA adducts before their deposition on the crystal surface is very important. Large surface of protein molecules have always many possible adhesion patches. If we do not block the redundant adhesion patches, the PCA adducts deposit in various orientations leading to an amorphous sediment instead to a regular crystal. The subsequent correction of wrongly deposited protein molecules is unlikely because the range of attractive intermolecular interactions able to do this is much shorter compared to the proteins size.

There is also additional effect. The PCA adducts have reduced number of possible adhesion modes compared to the naked protein, because the CAs block the access of the PCA adduct to the crystal surface in competitive adhesion modes. During the crystal growth, the CAs are mostly pressed out from the PCA adducts on the crystal surface and flow away. Thus, by the correct choice of CAs, one can affect the adhesion ability of PCA adducts approaching the crystal surface. This provides a tool for effective control of the crystallization process.

In conclusion, it can be said that the function of the CAs in the crystallization process is explained by a unique pre-orientation of protein molecules before their deposition on the surface of the growing crystal and by a clear preference of a single dominant adhesion mode by CAs protecting the protein surface.

[1] McPherson, A. (2001) *Comparison of salts for the crystallization of macromolecules*, Protein Science, 10418-10422.

[2] Kimber,M.S. et al, (2003) *Crystal screen optimization. Data mining crystallization databases: Knowledge-based approaches to optimize protein crystal screens*, Proteins, 51, 562-568.

[3] Hašek, J., (2011) *Principle of the unique adhesion mode in protein crystallization*, Acta Cryst. A67, C537;

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