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On the elementary processes of protein crystallization: Bond selection mechanism

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ABSTRACT

The paper explores the application of bond selection mechanism (BSM) in protein crystal growth; previously, BSM was employed to explain the slow rate of protein crystal nucleation, equilibrium crystal shape and energy barrier in nucleus formation (C.N. Nanev, Prog. Cryst. Growth Charact. Mater. 59 (2013) 133–169). Now, the elementary growth processes are considered from BSM perspective and the crystal growth shape is tackled, the latter resulting from a strong directional kinetic anisotropy in step advancement rates in different crystallographic directions. The most significant surface patterns of growing protein crystals, such as two-dimensional nuclei and growth spiral shapes observed by atomic force microscopy (AFM), are also considered. The activation barrier associated with entering of a protein molecule into the kink site is evaluated and the start of the kinetic roughening is established. Crystal lattice bond energies are estimated (being well above the thermal energy, k_BT) from the supersaturation dependence of 2D- into 1D-nuclei transformation.

1. Introduction

Having a long development history (since 1840), protein crystallization has turned into a mature science branch in its own right [1]. Simultaneously, inorganic (small molecule) crystals have shaped the core of crystallization studies mainly due to the extensive application of such crystals, thus giving rise to a sophisticated theory. However, the theory has failed to account for some specifics of protein crystallization, the single exception being the recent notion of multi- (or two-step) nucleation mechanism [2,3].

Although some earlier studies posit complete similarity between crystallization of small inorganic and large bio-molecules, it is protein crystallization kinetics that has been emphasized as being significantly different. Firstly, proteins are much more reluctant to crystallization than the small molecules. Furthermore, the proteins require much higher supersaturation to achieve crystal nucleation, at least many hundred percents. In contrast, only a few supersaturation percents are usually required for small molecule crystals to nucleate. Nonetheless, protein crystal nucleation is significantly slower than one of the small inorganic molecules.

The reasonable question is: why are these distinctions? The answer is simple: there is a fundamental difference between small inorganic and large protein molecules. Small molecules possess spherical interaction fields with constant interaction potential, and in supersaturated media every hit between them, independently of their spatial orientation, has the potential to contribute to a crystal bond formation. In contrast, the surfaces of the protein molecules are highly heterogeneous and patchy. Thus, it is safe to suggest that the differences between the small molecules and the proteins are manifested macroscopically through the difficulties for crystallization in the latter case.

The so-called bond selection mechanism (BSM) has been suggested [4] in an attempt to describe the most important features of the extremely complex molecular-kinetic mechanism of protein crystal nucleation. BSM is devised from phenomenological point of view, by piecing together experimental data for slow protein crystallization (that is observed despite the use of extraordinarily high supersaturations) and X-ray diffraction data for protein crystal lattice contacts. Imposing a severe steric restriction to crystal lattice bond formation, BSM provides rationale for the well-known reluctance of proteins to crystallize (partial dehydration of the lattice contact interface might be mentioned additionally). Protein crystal nucleation is substantially decelerated by the bond selection mechanism.

BSM approach, coupled with the so-called mean work of separation, MWS method of Stranski and Kaischew, has allowed determination of equilibrium crystal shape and energy barrier for nucleus formation [4]. It is logical to assume that BSM should act

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during protein crystal growth as well. Repeated selection of the most appropriate bonds contributes to formation of stable protein crystal lattices; we might speculate that any violation of the BSM rule results in crystal lattice defect formation. The present paper deals with the application of BSM approach to explain step kinetics, kinetic roughening, two-dimensional nucleation and spiral growth of protein crystals.

2. Experimental observations hinting to BSM hypothesis

A logical assumption about protein intra-molecular interactions in the bulk would be that they do not participate in protein crystal lattice binding. The reason is that the bulk intra-molecular interactions are concealed under the amino-acid residues situated at the molecular surface. Therefore, only the surface structure of a protein molecule dictates its ability to bind to partners during protein crystallization. For any person working with/on proteins, this is a fundamental postulate; for protein crystallization in particular it is supported by our observations. In evidence comes the entirely analogous crystallization behavior of apo- and holo-ferritin, observed to occur due to the same molecule surface structure, regardless of the dramatically different molecule core; it is worth recalling that apoferritin is an empty shell, while a mineral core is present in the holoferritin. Nevertheless, when forming under the same conditions, the crystals of both proteins have exactly the same shape, Fig. 1; the crystals differ only in their color, apoferritin crystals being yellowish, while the holoferritin's – reddish-brown [5]. Moreover, nearly identical step kinetic coefficients for apo- and holoferritin were measured [6].

A periodical alternation of layered apo- and holo-ferritin crystallization has been carried out to strengthen the abovementioned concept [7], see Figs. 2 and 3. Crystals of each protein are used as substrates for a sequential crystallization in contiguity of the counterpart protein, showing a repeatable process. The overlaying crystal layer formed is uniform in thickness with no reentrant corners, Fig. 3. This gives grounds to claim that the crystals obtained are single-crystals composed of alternating apo- and holoferritin layers, rather than poly-crystals. It is worth noting that the same solution

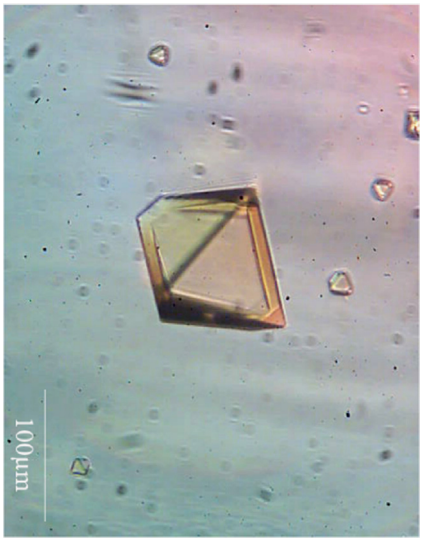

Fig. 2. A combined single crystal of apo- and holoferritin. Solutions conditions for the inner crystal are apo-ferritin 0.6 mg/ml, 1.6% (w/v) $CdSO_4$, 0.2 M/L buffer, pH=5.0, and for the outer crystal are holo-ferritin 1.04 mg/ml, 1.6% (w/v) $CdSO_4$, 0.2 M/L buffer, pH=5.0. The sharply outlined boundary between the crystal layers is due to a noticeable distinction of their refractive indices.

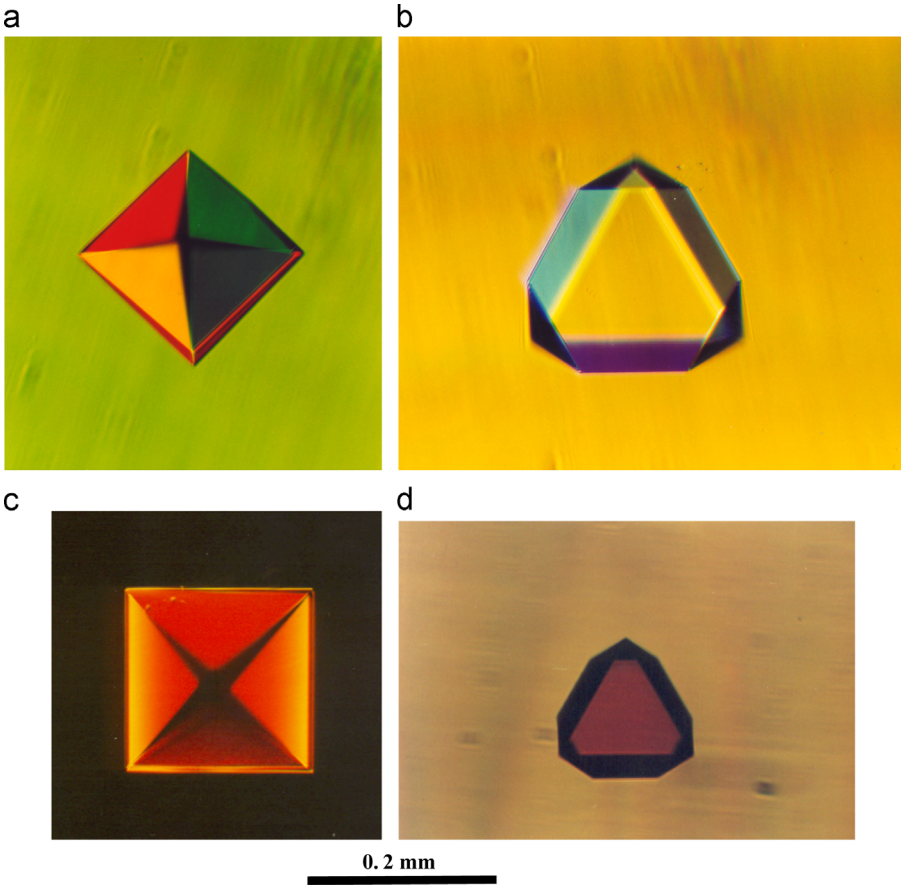

Fig. 1. Cubic, f.c.c. crystals of apoferritin (a, b) and holoferritin (c, d), lying on {100} (a, c) and on {111} (b, d) planes. (Interference contrast microscopy.)