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Lysozyme crystal nucleation in solution layers

F.V. Hodzhaoglu, L.N. Stanoeva, C.N. Nanev*

Rostislaw Kaischew Institute of Physical Chemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl.11, 1113 Sofia, Bulgaria

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By using the classical approach of *separation in time* of nucleation and growth stages, protein crystal nucleation was investigated in thin protein solution layers confined between two glass plates of custom made quasi two-dimensional all-glass cells. Solution layer thickness was varied from 0.05 down to 0.01, 0.0065 and 0.002 cm. Two commercial samples of hen-egg-white lysozyme, HEWL, Seikagaku 6 times crystallized and Sigma 3 times crystallized, were used as model proteins. The number of HEWL crystal nuclei decreased with diminishing solution layer thickness but the crystal nuclei reduction was considerably lesser than proportional to solution layer diminish. Heterogeneous (on-glass) protein crystal nucleation was separated from bulk one in 0.05 cm solution layers, the corresponding nucleation rates being measured separately. Up to 80% of the crystal nuclei were formed heterogeneously, on the glass, from 0.05 cm protein solution layers of Seikagaku HEWL. On the contrary, only 10 to 13% of the nuclei were observed on glass under the same conditions in Sigma solution; bulk nucleated crystals represented the main crystal fraction in this case. A plausible explanation of the experimental results was suggested. It is that the bulk crystal nucleation occurs on rests of source biomaterial that are always present in the protein solutions. Moreover, they may be even more active nucleants than the glass.

Key words: protein crystal nucleation, thin protein solution layers, heterogeneous *vs.* homogeneous nucleation, crystal nucleants of biological origin.

INTRODUCTION

The big success of the Human Genome Project recently stimulates protein crystallography. Deeper understanding of the proteins bio-function requires knowledge of their exact molecular structure. X-ray diffraction is still the most frequently used technique for protein structure determination, sufficiently large and high quality protein crystals being needed. Nowadays it is generally anticipated that protein crystallization is the rate-determining step in the protein crystallography.

Spontaneous crystallization is usually practiced with proteins. Crystal nucleation is its first stage. Precise control over the rate of protein crystal nucleation is worth achieving because it fixes the number and determines the quality and final size of the crystals. Therefore we need to better understand all peculiarities of the crystal nucleation process with proteins.

The tendency to constantly decrease solution volumes is a general trend in protein crystallization. Using drop techniques this leads to capillary pressure effects, especially with the tiniest droplets. For instance, the capillary pressure, P_c of 1μL droplet of protein solution in air is about 100 Pa, while that of 1nL droplet is ten times higher. Therefore such droplets evaporate very fast. To avoid evaporation, and its influence on the protein crystal nucleation and growth, oil is used [1]. Nevertheless, the effect of the capillary pressure remains. Besides, the surface to volume ratio is increased.

To shed light on these issues, series of experiments on the protein crystal nucleation were carried out by decreasing one dimension of the liquid phase, namely its thickness. Quasi two-dimensional protein solution layers of different thickness were confined between two glass plates of custom made all-glass cells. Separation in time of the nucleation and growth stages, i.e. the *classical double pulse approach*, was applied. To obtain additional information the amounts of substrate (on-glass) and bulk protein crystal fractions were measured separately in 0.05 cm cells. Crystal number-densities *vs*. nucleation time dependences were plotted and the stationary nucleation rates of HEWL crystals were measured. The results suggest the presence of bulk nucleants and stress on their role in protein crystal nucleation process. The impact of other factors on the protein crystal nucleation like natural convections, crystal

* To whom all correspondence should be sent.
E-mail: nanev@ipc.bas.bg;
Phone: (359-2) 8719306; Fax: (359-2) 971 26 88.

sedimentation, capillary pressure, etc. was considered as well.

Two commercial samples of hen-egg-white lysozyme, HEWL, Seikagaku and Sigma, were used in the investigation. The reason behind this approach is to establish also the role of the admixtures [2] that are present in every protein solution. In fact, a noticeable difference in the crystallization behavior of the two HEWL samples was observed.

EXPERIMENTAL

Experimental set-up

The investigations were performed with quasi-two-dimensional all-glass cells. For cell construction a pair of optical glass plates in disk form was welded in exactly parallel position. The HEWL crystals (Fig. 1) were nucleated and grown in thin solution layers confined in the gaps between the plates. The gaps were varied in a series of cells, from 0.05 down to 0.01, 0.0065 and 0.002 cm. These custom-made cells have small inside volumes. The cells allow excellent microscopic observation and easy cleaning.

The following protocol was used. Initially the cells were purified with a hot 5:1 sulfuric to nitric acid mixture, and than flushed by bi-distilled water (till it reached neutral pH). It turned out that this procedure was very important because it insured complete wetting, and filling of the whole cell. (Evidently, the precondition for a regular flow, and avoiding air bubble formation, is the complete wetting within the entire cell.)

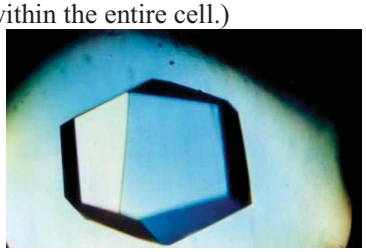


Fig. 1. Hen-egg-white lysozyme crystal, about 0.4 mm in size.

After drying the cell was loaded with HEWL solution that is metastable at room temperature, 20ºC. The solution metastability was proven in preliminary experiments – no crystals appeared at 20ºC for a month or two. (Indeed, the existing crystals grew under these conditions.) Two commercial samples of HEWL, Seikagaku 6x crystallized and Sigma 3x crystallized (approx. 95% protein), were used without additional purification of the products. Aqueous solutions of 40 mg/ml protein at pH = 4.5 (50 mM acetate buffer) and 0,43M NaCl as precipitant found application. At 20ºC the dimensionless super-saturation ($\triangle\mu = \ln(c/c_e)$) is approximately 1.0. The equilibrium state, when $\triangle\mu = 0$, is at 26ºC.

In the present study we performed series of parallel experiments on crystal nucleation and growth of HEWL using the whole set of crystallization cells. Besides the solution layer thickness however, other factors like natural convections, crystal sedimentation, capillary pressure, etc., may influence the protein crystal nucleation process. To shed light on these issues experiments with thicker, 0.05 cm quasi-2D-glass cells were performed initially. Taking advantage of a sharp-focus microscope, separation of substrate from the bulk crystal nucleation was achieved. We were able to distinguish between HEWL crystals growing on the upper glass plate, those in the solution bulk and the crystals on the bottom cell plate; simply, the microscope was focused on those particular levels. Note that this experimental approach was impossible with cells thinner than 0.05 cm because the crystals grown in thinner cells usually touch simultaneously both glass plates, and it was not clear where they were born.

The reasoning is simple. Focusing initially the microscope on the upper glass plate of a 0.05 cm glass cell we observed HEWL crystals growing there; evidently, those were truly heterogeneously nucleated crystals. The reason for this conclusion was that since the nuclei stuck to the glass plate strongly enough [3], they grew afterwards remaining on the same places. Thus, we counted the crystals on the upper glass plate separately and this was our *benchmark for the substrate type of heterogeneous nucleation*. Although several HEWL crystals were observed sometimes also in solution bulk, most of the crystals were found on the cell bottom. Therefore it was logical to assume that also sediment crystals were found on this particular place (because the number density of the crystals, which were nucleated truly heterogeneously onto the bottom glass plate, should not exceed this one observed for the upper glass plate).

Measurement of HEWL crystal nucleation rates

The classical approach [4] which enables measurement of the nucleation rates experimentally, without ever actually seeing the nuclei themselves, was applied. This classical technique is very simple and reliable. However its proper fulfillment requires some experimental skills, e.g. see [5–7]. It requires strict separation in time of the nucleation and growth stages.

Practically, the crystallization experiment is divided into two periods (Fig. 2). During the nucleation period the crystals do only form. This is possible because crystal nucleation demands considerably higher supersaturation as compared to that which is sufficient for the subsequent crystal growth. By keeping the nucleation period relatively short (minutes), the nuclei do not have enough time to grow and to exhaust the overall supersaturation. Indeed, some of them which appeared very first could grow a little bit. Keeping the nucleation time sufficiently short, however, these clusters remained so small that they did not consume an appreciable amount of the protein; thus, they were unable to decrease substantially the overall protein concentration.

Note that excessively long nucleation times violate the principle of separation of the nucleation and growth stages. In such cases the nuclei that are born very first may deplete the local supersaturation, and even turn it to metastable condition. Thus, nucleus formation could be hindered in some zones around such crystals. In contrast, during short or moderate nucleation times the eventually appearing nucleation excluded zones will be very few and small and can not appreciably decrease the volume in which crystal nucleation takes place further. Also not too high supersaturations were used because otherwise it would be impossible to quantify the very large (sometimes countless) number of the grown crystals.

Being nanosized particles, the crystal nuclei are not visible under light microscope, like the so-called *image centers* in the photographic plates. In order to make them visible, after the expected nucleation onset the supersaturation is rapidly lowered below the threshold, which is necessary for crystal nucleation (Fig. 2). Being now in the so-called metastable zone, the system is unable to produce further nuclei. During this second (growth) stage only the existing nuclei grow to microscopically visible crystals. (Picturesquely speaking, the invisible nuclei are "developed", like the image centers in the photographic plates.) Purposely, the growth stage is set as long as necessary, usually several days. Finally, the number density of crystals, **n** is determined and by plotting **n** *vs* the nucleation time, **t** the stationary nucleation rate **I** is obtained from the linear part of the curve. Historically the classical principle of separation in time of the nucleation and growth stages has been applied in studying crystallization of glasses, formation of droplets, electrochemical nucleation of metal crystals, etc. Protein crystal nucleation has been studied as well [5-7].

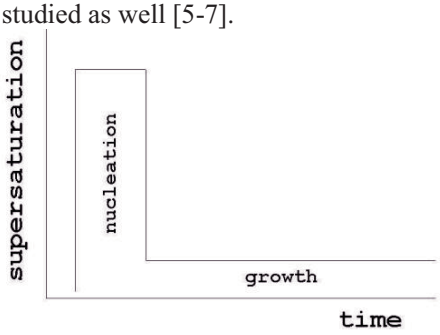


Fig. 2. Schematic representation of the classical principle of separation in time of the nucleation and growth stages.

For creating relatively fast supersaturation changes, thermal jumps were used in the present study because lysozyme exhibits strong temperature dependent solubility, perhaps the strongest for a protein. Although the classical technique is sometimes also called "double-pulse method", a term that comes historically from the electrochemical nucleation of metal crystals, it is obvious that with any temperature change, both cooling and heating, the system requires certain time to respond to that change. In order to shorten this period we chose water cooling for our samples because the thermal conductivity of water is more than 20 times higher than that of the air (and the glass possesses a relatively good thermal conductivity, a little bit higher than that of water).

Lysozyme exhibits normal temperature-dependent solubility, i.e. it decreases with temperature drop. Therefore the sufficiently high supersaturation that is necessary for crystal nucleation was established by sudden temperature decrease. To evoke crystal nucleation we set the temperature of our samples at 10°C because HEWL solubility at this temperature is known, 5 mg/ml [8]. At this conditions the dimensionless supersaturation ($\triangle\mu = \ln (c/c_e)$) is approximately 2.1. Another important benefit of our experimental setup was used. The imposing of rapid temperature shift was achieved using water baths. We simply immersed the whole cell with the protein solution directly in water of temperature 10°C. In fact, we measured with our quasi-2D-glass cells that water cooling was 2 to 3 times faster than air cooling (Fig. 3); purposely a thermoprobe was inserted directly in the cell. The solution in the cell was tempered from metastability temperature of 20°C to 10°C for about 15 s (Fig. 3). Keeping in mind this fact, and in order to diminish the experimental error, we chose nucleation time of minimum 5 min

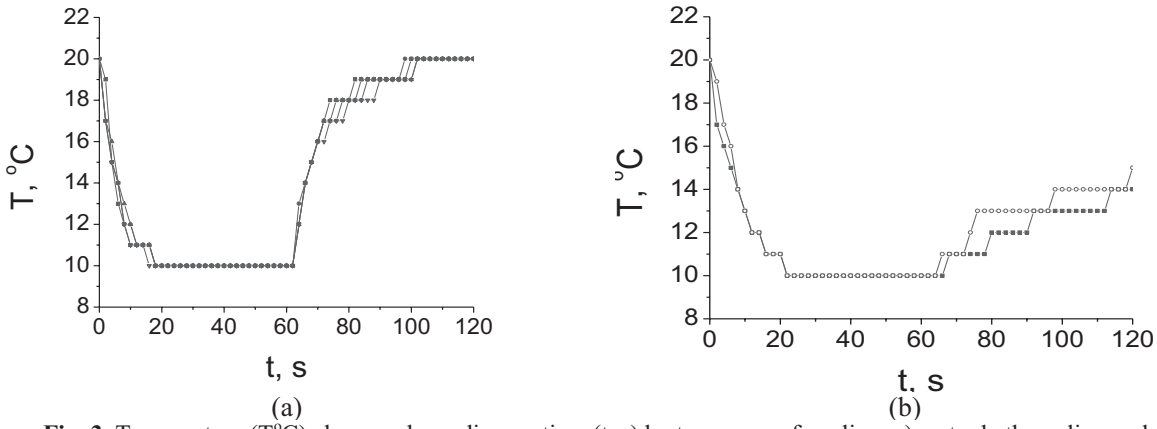


Fig. 3. Temperature (T°C) changes depending on time (t, s) by two ways of cooling: a) water bath cooling and reheating; b) water bath cooling and air reheating. (Data of several measurements are superimposed.)

(i.e. 20 times longer than thermal transition time), but maximum of 120 min.

After the chosen nucleation time elapsed the supersaturation was sharply decreased to metastability level, the cell was re-heated to 20°C for about 15 s (Fig. 3 a). Under this condition the existing super-critically sized clusters grew to visible sizes, were counted, and the numbers plotted depending on the nucleation time, etc. Special attention was paid in our investigation to the optimization of the second (growth) "pulse" because, evidently, it was not sufficient only to prevent the appearance of new nuclei; it was also necessary to avoid any nuclei loss due to dissolution. Therefore, to properly choose the growth temperature, the growth of HEWL crystals was scrutinized in preliminary experiments, performed at a series of temperatures. While secondary nucleation was observed at 18°C we found that at 22°C the crystals were dissolved. Therefore, during the second (growth) "pulse" the cell with the protein solution was re-heated to 20°C and this temperature was maintained, at least overnight, but preferably for several days, till one week, till the crystals grew to microscopically visible sizes.

Having the advantage of being a direct method, the thermal variant of the classical approach also shows some drawbacks. It is reasonable to think that during the very beginning of the first stage (cooling) the nucleation rate *progressively* increases, till it reaches the stationary rate; and *vice versa*, it is quieting down relatively slowly during re-heating to 20°C. (Thus, although transient, some additional and uncontrolled nucleation takes place for 15 s, during the reheating to 20°C.) The theoretical analysis of these effects is not simple. Fortunately, they should be small, oppositely directed, and it is logical to assume that they nearly cancel each other. Because an inherent scatter of the experimental data is typical due to the stochastic character of the crystal nucleation process the inaccuracy should be below the measurement accuracy. Therefore we repeated the measurements many times and averaged the results.

RESULTS AND DISCUSSION

Applying a typical for the protein crystal nucleation supersaturation c/c_e = 800% (c and c_e being the actual and equilibrium protein concentrations, respectively) to a solution of 40 mg/ml HEWL at 10°C, a quantitative study was performed on protein crystal nucleation; the thermal variant of the classical double-pulse method was utilized. Investigations were carried out with quasi-two-dimensional glass cells by varying solution layer thickness δ, 0.05, 0.01, 0.0065 and 0.002 cm; identical solutions were loaded in all cells. It was already mentioned that in cells thinner than 0.05 cm the HEWL crystals usually touched both glass plates, so that it was not clear where they arose. Therefore the total (on glass substrate plus bulk) number **N** of the HEWL crystals nucleated and grown **per unit visible cell area** was plotted *vs*. the nucleation time **t**, Fig. 4 a, b. Linear dependences of different slopes were observed initially, and then plateaus appeared. A definite time-lag was observed only with Seikagaku HEWL but not with Sigma HEWL (Fig. 4 a, b).

Note the constantly decreasing slopes of the linear parts of the curves in Fig. 4 with decreasing layer thickness from 0.05 cm downwards, and especially the abrupt drop of $\mathbf{N}/cm^{2*}$ *vs*. **t** with Seikagaku solution below 0.05 cm layer thickness, Fig. 4 b. The decreases are power functions for Sigma HEWL, while the dependence is almost linear with Seikagaku HEWL (Fig. 5).

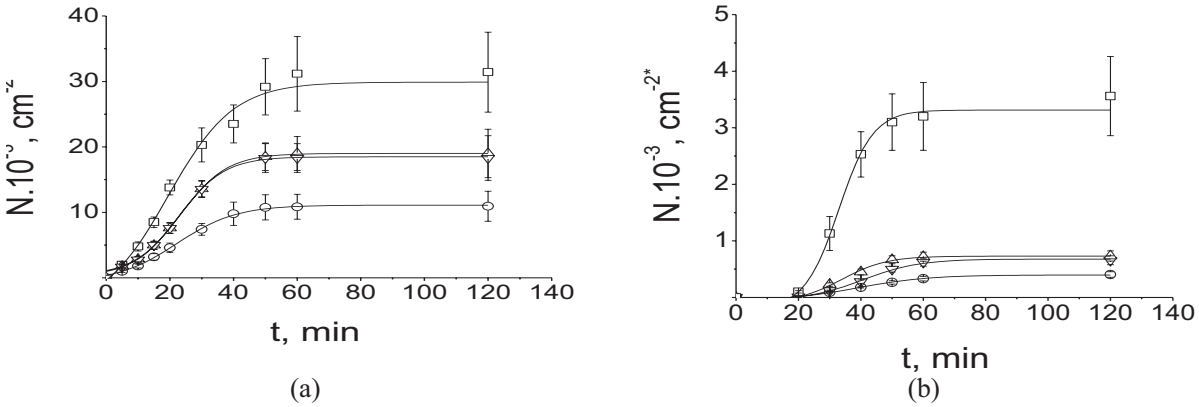


Fig. 4. Number densities **N**/cm^{2*} (the asterisk indicates that N is per unit visible cell area) of HEWL crystals *vs* nucleation time, t, min and solution layer thickness δ: □ - 0.05 cm, Δ - 0.01 cm, ∇ - 0.0065 cm, ○ - 0.002 cm; a – Sigma, b – Seikagaku (the sizes of the marks in Fig. 4 b that differ from □ reflect the error bar sizes).

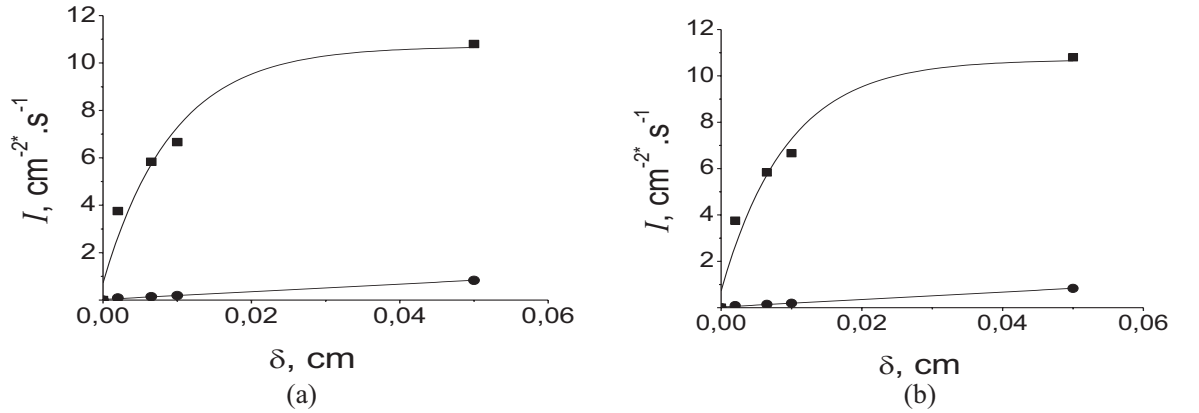


Fig. 5. a. Slopes (*I*) of the linear parts of the curves in Fig. 4 *vs*. solution layer thickness (δ); b. maximum crystal number densities, N_{plato}/cm^{2*} (the asterisk indicates that N_{plato} is per unit visible cell area) *vs*. solution layer thickness δ. ■ – Sigma, ● – Seikagaku.

The differences in the plateau levels of Sigma and Seikagaku substances in Fig. 4 also deserve attention. They rise systematically with the decrease in solution layer thickness. Changing the thickness from 0.05 cm downwards to 0.002 cm the plateau levels decreased about three times with Sigma HEWL, while the decrease with Seikagaku HEWL was more than 8 times. Note however that nucleants' amount should decrease proportionally to the decrease in solution volume, which is equal to the solution layer thickness, i.e. 25 times. Besides, comparing the data for the two sorts of HEWL one can see that, despite the same crystallization conditions, the total number densities for Sigma HEWL crystals at the plateau regions on Fig. 4 a are about (and more than) one order of magnitude larger compared to Seikagaku HEWL crystals in Fig. 4 b.

The results obtained during the investigation with decreasing solution layer thickness put questions about the possible reasons for these peculiarities. For instance, the solubility of two lysozyme sorts may slightly differ giving impact on supersaturation, despite the same crystallization conditions. Factors like natural convection, which was observed in the 0.05 cm cells and which was almost fully suppressed in the thinner ones, especially in the 0.002 cm cells should be considered. In contrast, crystal sedimentation which could be thought as another factor hardly plays any virtual role in the crystal nucleation process. The sedimentation velocity of HEWL crystals that are much larger than the nucleus was estimated from solution viscosity and density. It was found that 1 μm^3 crystals should sediment in the 0.05 cm cells for about 85 min; indeed, growing larger the crystals sediment sooner.

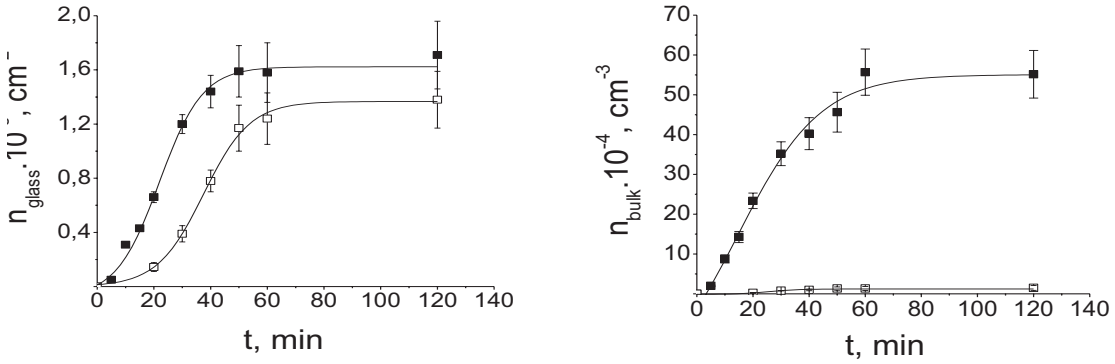
Still another factor may be the capillary pressure, P_c which has to act, especially in the thinnest two-dimensional glass cells. In fact it is known that hydrostatic pressures, up to 100 MPa augment HEWL solubility [9 – 14], thus decreasing the supersaturation. Moreover, the reason to consider the role of the capillary pressure was a peculiarity that was observed by filling those cells. In contrast to the 0.05 cm cell, inside the 0.002 cm cell the liquid was climbing up even when the latter was situated in a vertical position: we observed that the air-solution interface in the cell rose very quickly (note that two air-solution interfaces do exist at the cell inlets). The capillary pressure, P_c, which in the case under consideration is **negative**, was calculated by means of Young–Laplace equation:

$$P_c = \gamma\,(1/r_1 + 1/r_2) \qquad (1)$$

where γ is solution's surface tension, r_1 and r_2 being the two principal radii of curvature.

With $\gamma = (3{,}5\pm0{,}2).10^{-2}$ Nm^{-1}, measured with our working solution, and $r_1 = 0.001$ cm, $P_c \approx 3.5$ kPa was calculated for the 0.002 cm cell, while $P_c \approx$ 0.14 kPa was obtained for the 0.05 cm cell. Thus, it turns out that in the case under consideration P_c hardly plays any role because it is too small.

Still another factor was already mentioned, namely the presence of bulk nucleants. To shed light on the issue, the glass substrate HEWL crystal nucleation was distinguished from the bulk one in the same experiment, using 0.05 cm quasi-2D-glass cells (see above). Keeping the same supersaturation, $c/c_e = 800\%$ the number densities, **n**, of nucleated HEWL crystals *vs*. nucleation time, **t**, were plotted separately for glass substrate nucleation, Fig. 6 a ($\mathbf{n}_{glass}$ is the number density for the upper glass plate only, which is the same for the bottom plate) and bulk nucleation (Fig. 6 b, $\mathbf{n}_{bulk}$); 83 experiments were carried out for the plots in Fig. 6. Linear parts of different slopes were observed on the curves in Fig. 6 a and b; plateaus appeared in all four cases as well. A time-lag, which is most obvious for the bulk crystal nucleation of Seikagaku HEWL (Fig. 6 b) has to be emphasized as well.



(a) (b)

Fig. 6 a, b. Number densities, n *vs*. nucleation time, t of: (a) on-glass nucleated, and (b) bulk nucleated HEWL crystals. ■ – Sigma HEWL, □ – Seikagaku HEWL (the size of the mark □ in Fig. 6 b approximately coincides with the size of the error bars).

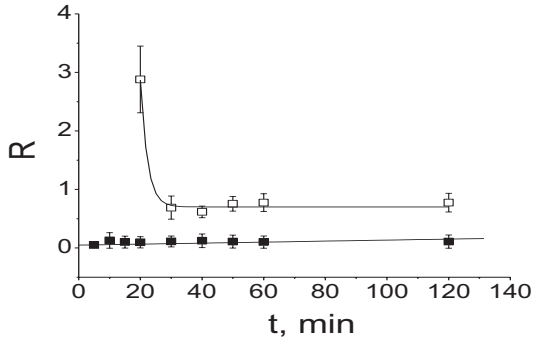


Fig. 6c. Ratio R *vs*. time, t; ■ – Sigma HEWL, □ – Seikagaku HEWL.

The relation between the substrate *vs*. bulk HEWL crystal nucleation can be established quantitatively from Fig. 6 a, b. For instance, the linear parts of the curves show that 65 to 72% (and 76 to 79% in the plateau regions) of the crystals nucleated of Seikagaku HEWL were born heterogeneously on glass substrate. In contrast, very small amount (only about 13%, by 10 min nucleation time, and 11%, by 120 min nucleation time) of Sigma HEWL nucleated crystals were found on the upper and bottom glass plates of the cell; bulk nucleated crystals represented the main crystal fraction (Fig. 6 b). It should be emphasized however that the comparison holds true for this

particular system only. The reason is that HEWL crystals arose on the glass support from a very thin (adjacent) solution volume, as compared to the much voluminous solution bulk. Despite this fact the comparison is instructive enough. It shows that the HEWL crystals nucleated on glass substrate prevailed strongly with Seikagaku solution while just the opposite effect was observed with Sigma HEWL, where the bulk nucleation prevailed. Note that this is a highly unexpected result because it is well known that heterogeneous crystal nucleation is easier [15].

Indeed, nucleation place does not determine explicitly the manner of nucleus formation. Although born in solution bulk some (or may be even all?) nuclei, especially in Sigma HEWL, may be formed on foreign particles that served as nucleants, for example other protein species, non-crystalline protein aggregates or traces of non-protein biomacromolecular impurities. Thus, the striking result seen in Fig. 6b may be explained in a sense that the nucleants in the bulk of Seikagaku's solution are less active or/and smaller in number than those in Sigma's one.

Although our SDS-PAGE didn't show any difference in the impurities' content of Sigma and Seikagaku HEWL it is known that foreign substances are always present in any protein solution, e.g. [16, 17] and may act as nucleants. Actually, Rosenberger has shown by SDS-PAGE the difference in impurity levels between Sigma and Seikagaku lysozymes. While in the two sorts proteins with molecular weight (MW) of about 25 kDa have been detected, the Sigma ones contain extra 40, 50 and 75 kDa proteins [18]. In fact, a simple estimation has shown that only the active nucleants should be below the detection limit of SDS-PAGE. The estimation was based on the (maximum) crystal number densities in the Sigma HEWL plateau region of Fig. 6b, and under the suggestion that all bulk crystals have nucleated on nucleants. Thus, we estimated that the total number of crystal nucleants in our SDS-PAGE trials should not exceed 30 active nucleant particles.

Also ratios, R, of the number of HEWL crystals nucleated on substrate (on both upper and bottom glass plates of the cell) *vs.* HEWL crystals nucleated in solution bulk are plotted in Fig. 6 c depending on the nucleation time for the same system. Fig. 6 c shows that while the Sigma R-value remains almost constant or increases very slowly with the nucleation time, the R-value of Seikagaku solution drops drastically (between 20 and 30 min nucleation time) and afterwards becomes also constant. An explanation may be that some active centers on glass are exhausted. (Indeed, glass may have also some spots that promote HEWL crystals nucleation but in such a case crystals had to arise constantly on the same place, which was not observed.) Another explanation may be that rests of source biomaterial may be adsorbed randomly on the glass and can serve as especially active nucleants. Last but not least the nucleants in Seikagaku solution may act more sluggish, i.e. they may be less active than those in Sigma solution.

Both heterogeneous and bulk crystal nucleation rates were measured separately (at the given supersaturation, $c/c_e = 800\%$) from the linear parts of the curves in Fig. 6a and Fig. 6b. The slopes of the linear parts in **n** *vs*. **t** plots in Fig. 6 a render $I_{heter} = 0.9$ nuclei.cm^{-2}s^{-1} for Sigma HEWL and $I_{heter} = 0.6$ nuclei.cm^{-2}s^{-1}, for Seikagaku HEWL. So, the heterogeneous on glass crystal nucleation rates of the two protein samples do not differ substantially. In contrast, the bulk nucleation rates calculated from Fig. 6b, $I_{bulk} = 167$ nuclei.cm^{-3}s^{-1} for the Sigma sample and $I_{bulk} = 4$ nuclei.cm^{-3}s^{-1} for the Seikagaku sample differ more than 40 times. A plausible explanation of the latter result, and the time-lag in Fig. 6b as well, may be given again with the presence of some foreign particles and their different activity as nucleants.

Table 1. Heterogeneously on glass, n_{glass} being the crystal number density for the upper glass plate only, and bulk, n_{bulk} number densities of HEWL crystals, nucleated in 0.05 cm cell, when the former are nearly the same for both protein samples. (The small differences in the percentages of the on glass and bulk nucleated HEWL crystals as compared with the data extracted from Fig. 6 a, b are due to the fortuitous character of the nucleation process).

HEWL	$n_{glass}.10^{-3}$, cm^{-2}	$n_{bulk}.10^{-4}$, cm^{-3}
Sigma (3x)	1.47 1.59	54.36 55.66
Seikagaku (6x)	1.43 1.55	1.62 1.04

Interestingly, in separate experiments (some of those shown in the plateau region in Fig. 6 a), it was observed that the number densities n_{glass} of heterogeneously nucleated crystals were the same for both Sigma HEWL and Seikagaku HEWL, while the volume fraction was from 30 to 50 times smaller in the latter case, Table 1. This fact may be considered as a crucial (although indirect) evidence for the important role of the foreign nucleants in the process of protein crystal nucleation. Moreover, on the basis of the experimental results it is logical to assume that the most (and most active) nucleants

had been removed from Seikagaku HEWL as a result of its crystallization performed 6 times in contrast to those in the 3 times crystallized HEWL from Sigma.

CONCLUSION

It seems that the experiments performed with quasi-two-dimensional protein solution layers of different thickness put more questions than can be answered. Some light was shed by investigations on HEWL crystallization in 0.05 cm quasi-two dimensional cells. The similar substrate nucleation of two lysozyme sorts indicates that glass is poor a nucleant for crystallization of lysozyme and likely of other proteins. The comparison between bulk nucleated lysozyme crystals suggests that impurities in protein substances are a very significant source of heterogeneous nucleation centers. Evidently, the glass substrate is not as good nucleant for protein crystals as those impurity particles [16-18]. It is logical to assume that due to the similar nature the adhesion of the protein crystals to such particles should be stronger than to the glass. A stringent argument in favour of such explanation is the fact that horsehair [19] and human hair [20] induce crystal nucleation of some proteins. But due to the inherent fortuitous character of the nucleation process the data that are presently available do not allow a final conclusion. Moreover, the picture may be additionally dimmed due to the presence of differently active nucleants.

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ЗАРОДИШООБРАЗУВАНЕ НА ЛИЗОЗИМ В ТЪНКИ КВАЗИДВУМЕРНИ КРИСТАЛИЗАЦИОННИ СИСТЕМИ

Ф. В. Ходжаоглу, Л. Н. Станоева, Хр. Н. Нанев

Институт по физикохимия "Акад. Ростислав Каишев", Българска академия на науките, блок 11, 1113 София, България

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(Резюме)

Изследвана е кристализацията на моделния белтък лизозим в стъклени квазидвумерни клетки чрез прилагане на класическия двойно-импулсен метод на разделяне по време на етапите на зародишообразуване и кристален растеж. Дебелината на разтвора между стъклените плоскости беше точно задавана чрез използване на набор от работни клетки с разстояние между стъклените дискове от 0.05, 0.01, 0.0065 до 0.002 cm. За целите на изследването бяха използвани две различни белтъчни субстанции на кокоши яйчен лизозим (HEWL), по-скъпият белтък на фирмата Seikagaku (6х прекристализиран) и този на фирмата Sigma (3х прекристализиран). Експерименталните резултати показват, че броят на лизозимните кристални зародиши, образувани и нараснали в едни и същи изходни разтвори, но с различна дебелина, е непропорционален на дебелината на разтвора. Чрез оптично наблюдение на най-дебелите клетки (0,05 см) бяха измерени както скоростта на хетерогенно зараждане на белтъчните кристали върху стъклената подложка, така и скоростта на обемно зародишообразуване. При използвани едни и същи кристализационни параметри, двете белтъчни субстанции показаха различно кристализационно поведение. Приблизително 80% от кристалите на лизозим от Seikagaku 6х бяха образувани хетерогенно, докато дялът на същата кристална фракция при Sigma беше от 10 до 13%. При втората субстанция, броят на белтъчните кристали, образувани в обема, значително надвишаваше този на хетерогенните. Причина за това може да бъде различната степен на пречистване на използваните белтъци. Вероятно, кристалните лизозимни зародиши са се образували с участието на активни примесни частици, които винаги съпътстват и най-високо пречистените белтъци, но при Sigma 3х те са в значително по-голямо количество. Счита се, че чуждите биологични частици са по-активни нуклеанти в сравнение със стъклената подложка.