Phase transformations in crystals composed of macromolecules

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The dehydration of a protein crystal is considered. It is shown that increase in the degree of dehydration at first leads to hydrostatic compression of the crystal, because of the surface tension. This compression may be accompanied by conformational changes. Subsequently, the increase in the dehydration leads to penetration of the surface water film into the crystal and then to internal boiling that develops at room temperature. In this case, the hydrostatic pressure ceases to depend on the degree of dehydration. The dependence of the saturated vapor pressure (for vapor in equilibrium with the protein crystal) on the degree of dehydration of the crystal is analyzed.

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Some unusual effects are considered in the present paper that arise in crystals composed of macromolecules and are connected with the presence of water in the intermolecular space. Similar phenomena, also connected with the presence of water within the material can be observed and, indeed have been observed, in porous glasses. A well-studied example of such crystals is the protein crystal. The structure of the protein crystal is described by a crystalline lattice at the sites of which are located globular molecules having characteristic dimensions ~ 50–100 Å and molecular weights of ~ 10^4–10^6. The interstitial space of such a crystal turns out to be filled with water. This latter circumstance has significance in principle for the structure and properties of the protein crystal. The point is that the spatial organization of the polypeptide chain forming the protein globule is due in significant measure to the presence of the aqueous medium: the protein globule becomes self-organized in such a way that the links of the polypeptide chain containing polar radicals (the hydrophilic parts of the chain) are located on the outer surface of the globule, while the links containing the nonpolar radicals (hydrophobic parts) are contained inside the globule. Thus the outer surface of the protein globule can be regarded as wetted.

Investigations carried out on crystals of myoglobin have shown that the dehydration of a protein crystal leads first to a decrease in the parameters of the crystal lattice, and then to a serious disruption of its regularity, which mainifests itself in the spreading and weakening of the x-ray reflections.[1,2] An attempt is made in the present paper to consider phenomena accompanying the process of dehydration of the crystal.

1. SURFACE TENSION IN THE DEHYDRATION OF A PROTEIN CRYSTAL

If we neglect the effect of surface tension of the water, then the dehydration of the protein crystal should lead to the result that the part of the protein molecules located near the surface of the crystal should be "raised" above the water level. This, however, does not occur because of the surface tension of the water. Since the protein globules are wettable, their "rise" above the level of the water should lead to a curving of the water surface and consequently to the appearance of a Laplacian pressure that tends to "drown" the protein globule. This pressure leads to an elastic protein-crystal deformation obeying Hooke's law:

$$p = K\varepsilon_{ll}, \tag{1}$$

where K is the hydrostatic compression modulus, p is the pressure due to the surface film and ε_{ll} is the trace of the strain tensor that describes the relative change of the volume of the crystal. Its value can be uniquely connected with the degree of dehydration $\Delta V_w/V_w$ of the crystal, which represents the ratio of the volume of the removed water to the volume V_w of water occupying the interstitial space of the initial unhydrated crystal. In order that all the protein globules of the crystal stay in the aqueous medium when the degree of dehydration is $\Delta V_w/V_w$, it is necessary to subject the "protein" lattice to hydrostatic compression. In this case the relative change in volume of the protein lattice should be equal to the degree of dehydration

$$\varepsilon_{ll} = \Delta V_w/V_w. \tag{2}$$

It follows from the expression (1) that the strain (2) requires application of a hydrostatic pressure equal to

$$p = K\Delta V_w/V_w. \tag{3}$$

The hydrostatic pressure (3) should be balanced by the surface (Laplacian) tension, created by the surface film of the water which is curved by the "protruding" protein globules. The latter means that

$$p = p_L \equiv \alpha\left(\frac{1}{r_1(S)} + \frac{1}{r_2(S)}\right), \tag{4}$$

where p_L is the Laplacian pressure, $r_1(S)$ and $r_2(S)$ are the principal radii of curvature of the surface of the water film at an arbitrary point of the surface S, α is the coefficient of surface tension of the water, and p is the elastic stress of the hydrostatic compressions and is determined by the expression (3). It follows from (3) and

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(4) that

$$p_l=\alpha\left(\frac{1}{r_1(S)}+\frac{1}{r_2(S)}\right)=K\frac{\Delta V_w}{V_w}, \qquad (5)$$

i. e., the sum of the reciprocals of the principal radii of curvature is a constant quantity for any point of the water surface and is directly proportional to the degree of dehydration $\Delta V_w/V_w$.

The equilibrium shape of the surface film is determined from the condition of minimum surface energy

$$\oint \alpha\, dS=\iint dx\, dy\, \alpha\left[1+\left(\frac{\partial z}{\partial x}\right)^2+\left(\frac{\partial z}{\partial y}\right)^2\right]^{1/2} \qquad (6)$$

with the additional condition of conservation of the volume

$$\iint dx\, dy\, z(x, y)=\text{const}, \qquad (7)$$

where x and y are the coordinates along the surface of the crystal, z is the coordinate in the direction perpendicular to the surface of the crystal, $z=z(x, y)$ describes the shape of the water surface, which forms menisci between the protein globules. The corresponding variational procedure yields the partial differential equation

$$\frac{\partial}{\partial x}\frac{\partial f(u,v)}{\partial u}+\frac{\partial}{\partial y}\frac{\partial f(u,v)}{\partial v}=p_L, \qquad (8)$$

$$f(u, v)=\alpha\sqrt{1+u^2+v^2},\quad u=\partial z/\partial x,\quad v=\partial z/\partial y;$$

here p_L is an undetermined Lagrangian multiplier, which is equal to the Laplacian pressure. In the case considered here, Eq. (8) is subject to rather complicated boundary conditions and can scarcely be solved exactly.

It follows from Eq. (5) that as the degree of dehydration increases, the radii of curvature of the water film decrease. This latter circumstance allows the film to offset the increasing elastic stress of the hydrostatic compression of the protein crystal. However, mechanisms exist which limit the possibility of unbounded cancellation of the elastic stresses. Beginning with some critical pressure $p_0=K(\Delta V_w V_w)_0$ (or, equivalently, with some critical degree of dehydration $(\Delta V_w/V_w)_0$) the surface film of water is not in a state to withstand the pressure which the compressed protein lattice exerts on it. The film loses its stability and the protein globules of the surface layer of the crystal break through the film and land outside the aqueous medium. This takes place at the instant when the reduced radius of curvature $\bar{r}$:

$$\frac{2}{\bar{r}}=\frac{1}{r_1(S)}+\frac{1}{r_2(S)},$$

reaches its minimum possible (critical) value.

In the case considered here, the problem of the determination of the critical radius of curvature and, consequently, the critical pressure p_0, is extraordinarily complicated,[1] since it requires the solution of Eq. (8) under complicated boundary conditions. However, the critical pressure p_0 can be estimated if it is assumed that the very "broad" hole which connects the neighboring unit cells can be approximated by a circle of some radius r_0. In this case, the determination of the critical pressure p_0 reduces to the simple problem of the extrusion of a film with a surface tension coefficient α through a circular aperture of radius r_0. In this formulation of the problem, Eq. (8) can be solved exactly. It has two solutions, described by two parts of the spherical surface

$$x^2+y^2+z^2=2\alpha/p_L \qquad (9)$$

which are separated by a secant plane parallel to the plane of the circular opening. The location of the secant plane is such that intersection with the sphere is a circle of radius r_0 (Fig. 1). The solution that corresponds to the portion of the spherical surface having the lesser area also corresponds to minimum surface energy.

The film loses stability in the case when the radius of the spherical surface formed by the film becomes equal to r_0. The condition for this

$$2\alpha/p_0=r_0 \qquad (10)$$

determines the critical pressure

$$p_0=2\alpha/r_0. \qquad (11)$$

If we assume the numerical values $\alpha\approx 70$ dyn/cm and $r_0\approx 10^{-7}$ cm, then an estimate for p_0 gives $\approx 10^3$ kg/cm^2. It follows from the expression (1) that at $K\approx 10^{10}$ dyn/cm^2[4,5] the critical change of the relative volume of the crystal, which is equal to the relative degree of dehydration, is of the order of $\varepsilon^0_{ll}=(\Delta V_w/V_w)_0\approx 0.1$. It must be kept in mind that the radius r_0 which characterizes the dimensions of the very "broadest" aperture between the neighboring unit cells of the protein crystal is the solitary critical radius only for the case of an ideal crystal. In a real crystal, in which a large number of defects is usually present (vacancies, dislocations, pores, etc.), a hierarchy of critical radii $r_0<r_0^{(1)}<r_0^{(2)}\ldots$, is always present and their dimensions exceed r_0: For this reason the loss of stability of the water film will take place at lower degrees of dehydration than in the ideal crystal. In the process of dehydration, the water film should "pierce" through the "aperture" with the maximum dimension first, and then, as the degree of dehydration increases, it should pierce through apertures of smaller dimensions.

We thus arrive at the following picture of the dehydration of the crystal. Hydrostatic compression of the

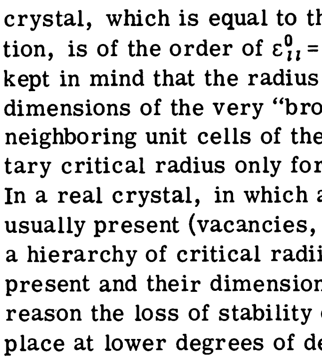

FIG. 1. Surface corresponding to two solutions of Eq. (10): the solid line describes the stable solution, the dashed line the unstable solution.

crystal takes place first. In principle, this can be accompanied by polymorphic transformations and conformations of the protein molecules.[2)] Next, when the critical pressure is reached, the protein globules located on the surface of the crystal pierce through the water film. Here the question arises as to whether other alternative mechanisms exist which allow cancellation of the increase in the Laplace pressure p_L upon increase in the degree of dehydration. One such mechanism is, apparently, boiling, i. e., internal evaporation of water in the volume of the protein crystal.

2. BOILING IN THE INTERIOR OF THE PROTEIN CRYSTAL

As is well known, boiling is defined as the process in which evaporation begins to occur in the interior of a liquid. This takes place at a temperature at which the pressure of the saturated vapor becomes equal to the atmospheric pressure. However, the situation is more complicated when we are dealing with a protein crystal. The fact here is that the formation of the vapor bubble crowds the water out to the surface of the crystal and, consequently decreases the elastic stress of the hydrostatic compression. This effect assures a decrease in the free energy of the system by an amount

$$\delta E_v = -pV_0^{H_2O}\delta m_{H_2O}, \tag{12}$$

where p is defined by expression (1), $V_0^{H_2O}$ is the volume of one mole of the water vapor, and δm_{H_2O} is the number of moles of vaporized water. The increase in the thermodynamic potential upon evaporation of δm_{H_2O} moles of water is equal to

$$\delta E_x = (p_{at} - p_{sat})V_0^{H_2O}\delta m_{H_2O}, \tag{13}$$

where p_{at} is the atmospheric pressure and p_{sat} is the saturated vapor pressure at the given temperature.

Finally, a contribution to the thermodynamic potential of the system is made, upon evaporation of water in the interior, by the surface tension on the liquid–vapor boundary. The value surface-tension energy of vapor bubbles of radius R, following evaporation of δm_{H_2O} moles of water, is

$$E_s = 3\alpha_1 V_0^{H_2O}\delta m_{H_2O}/R, \tag{14}$$

where α_1 is the surface tension coefficient on the vapor-water boundary. It follows from (12)–(14) that the total change in the thermodynamic potential of the system is equal to

$$\Delta\Omega = -pV_0^{H_2O}\delta m_{H_2O} + (p_{at} - p_{sat})V_0^{H_2O}\delta m_{H_2O} + 3\alpha_1 V_0^{H_2O}\delta m_{H_2O}/R. \tag{15}$$

Formation of the vapor bubbles of radius R becomes thermodynamically favored if $\Delta\Omega = 0$. The latter condition yields the equality

$$p = p_{at} - p_{sat} + 3\alpha_1/R \tag{16}$$

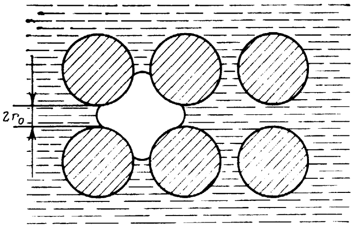

FIG. 2. Mechanism of propagation of vapor bubbles from one unit cell of the protein crystal to another.

or, using Eq. (1),

$$K(\Delta V_w/V_w) = p_{at} - p_{sat} + 3\alpha_1/R. \tag{17}$$

Since $3\alpha_1/R \gg p_{at} - p_{sat}$ at $R \approx 10^{-7}$ cm, Eq. (17) can be simplified by representing it in the form

$$K(\Delta V_w/V_w) = 3\alpha_1/R. \tag{18}$$

Since the vapor bubbles can be formed in an ideal lattice only inside the unit cells, the maximum possible radius of the bubble is the radius R_0 of the interstitial pore. Substituting $R = R_0$ in (18), we find that the spontaneous formation of vapor bubbles inside the unit cells of the protein crystal, i. e., boiling, becomes possible if the degree of dehydration $\Delta V_w/V_w$ reaches the critical value

$$(\Delta V_w/V_w)^0_{boil} = 3\alpha_1/KR_0. \tag{19}$$

Vapor bubbles will be formed in different unit cells, since they cannot coagulate up to dehydration degrees that assures the complete loss of stability of the water film relative to the Laplacian pressure. In fact, the propagation of the vapor bubble from one unit cell to the next requires the formation of a water surface with a curvature radius equal to the radius r_0 of the channel connecting the neighboring cells (Fig. 2). The Laplacian pressure in this situation should be equal to the critical pressure $p_0 = 2\alpha/r_0$ of stability of the water film, which exceeds the critical pressure of evaporization $p^0_{boil} = 3\alpha_1/R_0$ (we consider the case $2\alpha/r_0 > 3\alpha_1/R_0$).

In the case of a real crystal, when there are vacancies, pores, and other defects in the lattice, the spontaneous formation of vapor bubbles (boiling) will occur at lower degrees of dehydration than $(\Delta V_w/V_w)^0_{boil}$, since the radii of the pores and vacancies exceed the interstitial radius R_0.

3. DEHYDRATION ISOTHERMS

We now consider the equilibrium between the water in the protein crystal and in the vapor (gas) phase. In the case considered by us, the water surface in the protein is not planar. Its radii of curvature are uniquely connected with the degree of dehydration by Eq. (5). As is well known at a given temperature the saturated vapor pressure above a curved surface differs from the corresponding pressure over a plane surface. If we assume that the gas phase can be described in the ideal-gas approximation, then the saturated vapor pressure over the curved water surface will be equal to

$$p_{\text{sat}}=\overset{0}{p}_{\text{sat}}\exp(-v_0 p_{\text{Л}}/kT), \tag{20}$$

where v_0 is the volume per water molecule in the water phase, k is Boltzmann's constant, T is the absolute temperature, and p^0_{sat} is the saturation pressure over the plane surface. Substituting (6) in (20), we obtain

$$p_{\text{sat}}=\overset{0}{p}_{\text{sat}}\exp\left(-\frac{v_0 K}{kT}\frac{\Delta V_{\text{в}}}{V_{\text{в}}}\right). \tag{21}$$

Equation (21) connects the equilibrium saturated-vapor pressure with the degree of dehydration of the protein crystal. It follows from (21) that to each saturated vapor pressure p_{sat} there corresponds an equilibrium degree of dehydration of the protein crystal, equal to

$$\frac{\Delta V_{\text{в}}}{V_{\text{в}}}=\frac{kT}{Kv_0}\ln\frac{\overset{0}{p}_{\text{sat}}}{p_{\text{sat}}}. \tag{22}$$

It should be kept in mind that the relation (22) describes the degree of dehydration of the crystal only so long as no conformational changes of the globule, polymorphic transformations, or boiling and loss of stability of the water film relative to the Laplace pressure take place. All the enumerated processes offset the change in the Laplace pressure and should lead to the appearance of plateaus on the dehydration curves.

1) In the one-dimensional case, a similar problem arises in the calculation of the critical stress necessary for the extrusion of a dislocation line between pinning points (theory of precipitation hardening).[3]

2) This effect has been observed in particular in crystals of gramicidine.[6,7]

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Gas-liquid phase diagram in a nonequilibrium electron-hole system in silicon

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We investigate the condensation of high-density excitons and nonequilibrium carriers, produced by intense optical pumping at low temperatures, into drops of electron-hole liquid. The phase diagram of the gas–liquid transition is plotted in coordinates n and T (density and temperature) on the basis of an analysis of the shapes of the recombination-radiation spectra and of the kinetics of their evolution in time. The principal thermodynamic characteristics are determined, as well as the temperature dependences of the chemical potential, of the density, and of the Fermi energies in the liquid phase. The critical parameters $n_c=(1.2\pm0.2)\times10^{18}$ cm^{-3} and $T_c=(28\pm2)$ K are obtained. It is shown that at an average density $n=(5\pm3)\times10^{6}$ cm^{-3} the dielectric exciton gas is transformed into a metallic electron-hole plasma.

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1. INTRODUCTION

At low temperatures, an exciton gas of sufficiently high density in semiconductors can become condensed into drops of an electron-hole liquid (EHL).[1] The region of existence of electron-hole drops (EHD) can be obtained from the $n-T$ phase diagram, in which the gas–liquid equilibrium curve is determined on the gas-phase side by the singularities of the kinetics of exciton condensation, and on the side of the liquid phase by the internal properties of the EHL. We point out here a singularity that should be possessed by the gas–liquid phase transition in a high-density system of excitons (or nonequilibrium carriers). It turns out that the density of the nonequilibrium $e-h$ pairs in the liquid is $n_c>n_{MI}$, where n_{MI} is the density at which the "metallization" of the excitons takes place. Therefore at average densities $\bar{n}<n_{MI}$ a dielectric exciton gas condenses into EHL drops whereas at $n_c>\bar{n}>n_{MI}$ a gas of metallic electron-hole plasma (EHP) should be condensed. Thus, the gas–liquid coexistence curve (at $\bar{n}\sim n_{MI}$) has a region in which the exciton gas is transformed into a metallic EHP. What is still in question is whether this transformation is analogous in character to a first-order phase transition.