Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Use of super-water-repellent fractal surfaces in the crystallization of macromolecules

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Received 4 January 1999
Accepted 9 March 1999

The effect of fractal surfaces on protein crystallization has been investigated. A super-water-repellent fractal surface consisting of alkylketene dimers was formed on microscope cover slips. Crystallization experiments with hen egg-white lysozyme using the sitting-drop vapour-diffusion method have been undertaken on the fractal and conventionally siliconized surfaces. The size of the crystals obtained on the fractal surface is significantly larger than on the conventionally siliconized surface. The number of crystals on the fractal surface is markedly smaller than on the conventionally siliconized surface. The results clearly indicate that the fractal surface is very effective and promising in the crystallization of macromolecules using the sitting-drop vapour-diffusion method.

1. Introduction

The sitting-drop variant of the vapour-diffusion method (Ducruix & Giegé, 1992) is widely applied in the crystallization of macromolecules. The sitting-drop vapour-diffusion method is superior to the hanging-drop method when it is necessary to increase the volume of the drops in order to obtain crystals of suitable sizes for diffraction experiments.

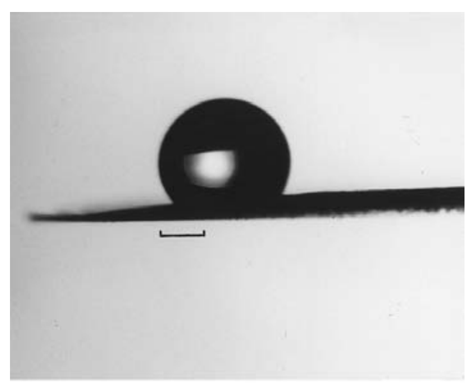
Microscope cover slips are normally used to seat drops. To ensure proper drop formation and prevent spreading, the cover slips must be carefully coated with non-wetting silicon (McPherson, 1982, 1985). Sitting drops inevitably tend to deform and spread over the conventionally silicon-coated surface owing to the inherent geometry. Deformation and spread of the drops are caused by wettability of the surface of the cover slips.

The wettability of the surface is governed by two factors: chemical and geometrical factors. Onda *et al.* (1996) paid attention to the geometrical structure of solid surfaces and found the excellent effectiveness of fractal surfaces on the wettability. The fractal surface is a kind of rough surface, the structure of which is characterized by self-similarity and a non-integer dimension (Mandelbrot, 1982). Shibuichi *et al.* (1996) have demonstrated that the fractal surface formed using alkylketene dimers shows a super water repellency with a contact angle of up to 174° for water droplets.

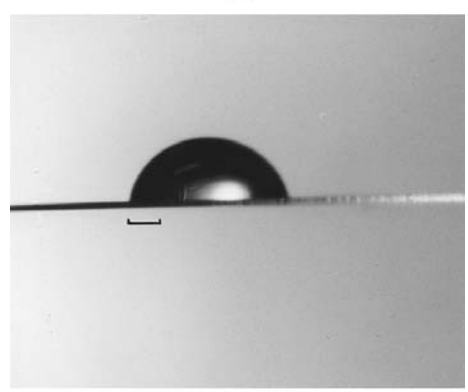
The super-water-repellent fractal surface is expected to contribute to ensuring proper drop formation and preventing spreading. To investigate the effect of the fractal surface on crystallization of proteins in the sitting-drop geometry, the present study has been undertaken.

2. Methods

Stearoylketene dimer (SKD), which is one of the alkylketene dimers, spontaneously forms a fractal structure in its surfaces on solidification from the melt. We synthesized SKD according



(*a*)



(*b*)

Figure 1
Shapes of protein drops at the beginning of crystallization. The scale corresponds to 1 mm in all photographs. (*a*) Drops on the SKD fractal surface. (*b*) Drops on the conventionally siliconized surface.

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to the methods described by Shibuichi *et al.* (1996). The synthetic procedures are summarized as follows. Stearoyl chloride dissolved in toluene was added dropwise to a toluene solution of triethylamine. The by-product triethylamine hydrochloride was filtered off. Crude SKD was obtained by evaporating the solution *in vacuo*. The crude SKD was purified using a silica-gel column. Precautions were taken to dry glassware and organic solvents to avoid hydrolysis of the SKD.

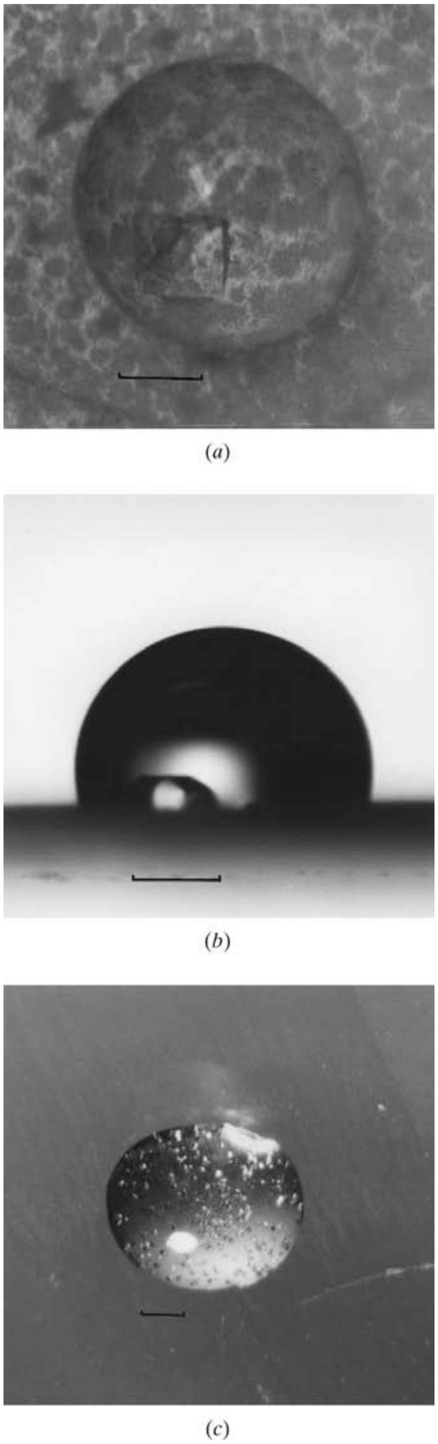

Figure 2
Crystals grown after two weeks. The scale corresponds to 1 mm in all photographs. (*a*) Top view of a drop on the fractal surface. One large crystal is seen at the lower left corner. (*b*) Side view of (*a*). (*c*) Top view of a drop on the conventionally siliconized surface.

The SKD was melted at 363 K and spread on dry clean cover slips kept at 363 K. The cover slips were cooled to room temperature on a cold metal plate. The SKD was air-dried at room temperature and allowed to solidify for one week. The water repellency of the SKD surface gradually improved. The contact angles for ultrapure water were estimated from photographs of the water droplets. Super-water-repellency, with a contact angle larger than 160°, was finally attained.

The super-water-repellency of the fractal surface was confirmed by washing the surface with ultrapure water. SKD-coated cover slips having a contact angle larger than 160° were used for crystallization. Cover slips which were washed with Sigmacote (Sigma) and dried for 4 h at 453 K were employed for control experiments. Commercially available hen egg-white (HEW) lysozyme (Sigma) was used for sitting-drop experiments. Q plates (Crystal Systems) were adopted to set up sitting drops. Crystallizing conditions are as follows. A 20 µl protein-precipitant drop consisting of 1% HEW lysozyme with 0.3 *M* NaCl in 100 m*M* sodium phosphate buffer at pH 6.0 was equilibrated with 1 ml reservoir solution consisting of 1.2 *M* NaCl in 100 m*M* sodium phosphate buffer at pH 6.0. The Q plates were stored at 283 (1) K. 230 drops were produced on the SKD and the conventionally siliconized surfaces.

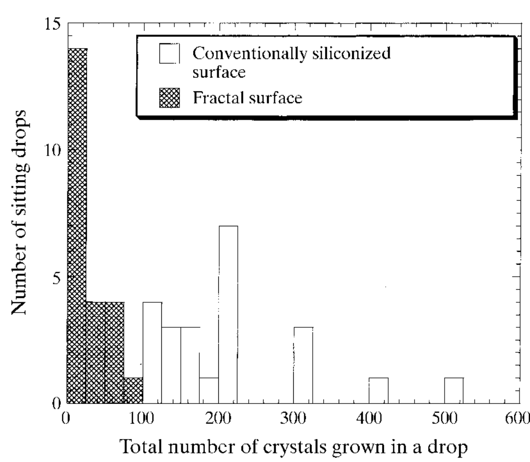

Figure 3
Comparison of the total number of crystals in sitting drops obtained on the fractal and conventionally siliconized surfaces.

3. Results

The shapes of the protein drops immediately after setting are shown in Fig. 1. Since the drops contain protein and salts, the contact angles of the drops placed on the SKD surface are less than those of pure water. Therefore, the shapes of the drops on the SKD surfaces are nearly spherical and those on the conventionally siliconized surfaces are almost hemispherical. The significant difference in shape suggests that the SKD surface is more water-repellent than the conventionally siliconized surface.

The size and number of protein crystals in the drops after two weeks were compared. Typical appearances of the drops are shown in Fig. 2. The size of crystals on the SKD surfaces is markedly larger than those on the conventionally siliconized surfaces. The number of crystals on the SKD surfaces is significantly smaller than on the conventionally siliconized surfaces. Distribution of the total number of crystals grown in the drops is compared in Fig. 3. The distribution definitely shows that the super-water-repellent fractal surface influences the number of crystals significantly.

The size of crystals in a drop is usually inversely proportional to the number of crystals, and this is also true in the present study. Although a few crystals which were obtained on the conventionally siliconized surfaces grew up to 0.4 mm in size, the sizes of the crystals were mostly smaller than 0.2 mm. On the other hand, the crystals obtained on the fractal surfaces ranged between 0.5 and 0.7 mm. The largest ones were nearly 1.0 mm in size. The sizes of the crystals in the drops on the conventionally siliconized surfaces varied widely, but those on the fractal surfaces were almost uniform. Moreover, the crystal growth on the fractal surface was delayed by 1–2 d compared with the growth on the conventionally siliconized surfaces.

The shapes of all of the drops became flattened after two weeks. The degree of flatness of the drops on the conventionally siliconized surfaces was more remarkable than that on the fractal surfaces. The shapes of the drops on the fractal surfaces changed from near spherical to hemispherical.

4. Discussion

One disadvantage of the sitting-drop method is that the drops inevitably tend to deform on the cover slips and increase the contact area between the drops and the cover slips. The large contact area usually promotes the adhesion of crystals and precipitate to the surface of the cover slips. The large contact area can also stimulate multinucleation, which gives a myriad of microcrystals. The contact area can be reduced by increasing the water-repellency of the surface of the cover slips. The water-repellency required for the sitting-drop method is more crucial than for the hanging-drop method.

The water-repellency achieved by the conventional silicon coating is determined by the chemical factor. The super-water-repellent surfaces formed by the SKD have fractal structures, and in this case the super-water-repellency is governed by the geometrical factor.

The size of crystals obtained on the fractal surfaces were three to four times larger than those obtained on the conventionally siliconized surfaces. On the other hand, the number of crystals grown on the fractal surfaces was nearly one-tenth that on the conventionally siliconized surfaces. The results clearly indicate that crystallization on the super-water-repellent surfaces significantly suppresses the multinucleation and increases the sizes of crystals. The present study unequivocally demonstrates that the fractal surface shows excellent effectiveness on the wettability of the protein solutions. The water-repellency of the fractal surfaces is significantly superior to that of the conventionally siliconized surfaces. The super-water-repellent fractal surface could be a promising alternative to the conventionally siliconized surface for the sitting-drop vapour-diffusion method.

We thank Drs S. Shibuichi and K. Tsujii for their helpful comments on preparation of the SKD.

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