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Analysis of slip systems in protein crystals with triclinic form using the phenomenological macro-bond method

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Slip systems in triclinic hen egg-white lysozyme (Tri-HEWL) crystals, which is one of typical protein crystals, were identified by the indentation method. Eleven kinds of the slip systems are clearly observed. These slip systems are determined by the surface energy estimation using phenomenological macro-bond methods. Based on the analysis of the slip systems, it can be suggested that the active slip systems are controlled by not the Burgers vector but the slip plane with lower surface energy for the crystals composed of the complex macromolecules and lower crystallographic symmetry such as Tri-HEWL crystals. This mechanism to decide active slip systems is unique compared with the conventional crystals such as FCC metals and covalent materials. It is expected to establish the more comprehensive dislocation theory including the macromolecular crystals.

Introduction

It is well-known that plastic behavior of many crystals is controlled by dislocation mechanisms [1,2]. In order to understand the behaviors of ductile crystals, the best method is the compression and/or tension tests using an Instron-type machine. However, concerning the brittle or fragile molecular crystals, it is quite hard to perform them. Thus, an indentation test is quite reasonable to investigate the plastic behavior of molecular crystals. Until now, there are interesting studies on the mechanical features of molecular crystals such as organic crystals by using an indentation technique [3–8]. A balance between the various intermolecular interactions in the crystal structure plays a key role in the mechanical properties. Moreover, the mechanical properties of protein crystals composed of huge protein molecules with complex shapes and containing a lot of water have been also investigated by the indentation method [9–15].

Recently, we confirmed that the stress-induced dislocations with large Burgers vectors such as b = 8.56, 9.39, and 9.96 nm in dislocation-free glucose isomerase (GI) crystals by the indentation have been directly observed in-situ by synchrotron X-ray topography [16]. This direct observation of these dislocations indicates the important evidence that the plastic deformation even in protein crystals with the huge protein molecules is due to the dislocation mechanism. However, the in-situ observation of stress-induced dislocations by synchrotron X-ray topography is quite difficult because the dislocation-free protein crystals are required, which is quite hard to grow the crystals except GI crystals. Therefore, to understand the deformation mechanisms of common protein crystals, especially the slip mechanisms, the analysis of the slip traces by the micro-indentation test is important.

For tetragonal and orthorhombic hen egg-white lysozyme (HEWL) crystals, slip systems associated with several kinds of slip planes and Burgers vectors have been characterized [13–15]. The characteristics of slip planes on both HEWL crystals appear several of slip traces on the indentation planes. According to the conventional dislocation theory, the possible slip planes are defined as the most closely packed molecules planes with large lattice spacing [2]. Interestingly, however, it is difficult to explain the active slip systems by the conventional dislocation theory because various slip systems have been simultaneously appeared in these protein crystals. This means that the major slip system cannot be determined unambiguously in protein crystals compared with the case of the simple metals and inorganic crystals.

In typical simple crystals such as Cu, Ag and Au of FCC metals, and Si and Ge of covalent materials, the Burgers vector is defined by the shortest lattice constant because of the minimum dislocation energy [2]. In addition, the possible slip planes correspond to the most closely packed molecules planes with large lattice spacing [2]. However, these rules are broken in protein crystals. In tetragonal and orthorhombic HEWL crystals, not only Burgers vectors are not always the smallest lattice constants but also the slip planes are not closed packed planes with larger lattice spacing. Especially, the slip planes strongly depend on the surface energy of each plane in HEWL

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crystals, which is calculated by the phenomenological models rather than the closed packed planes [13–15].

One of the phenomenological models of the molecular interaction for protein crystals, macro-bond model is much useful for estimation of the surface energy. The macro-bond contact is defined when one atom pair which has a distance less than 0.4 nm exists between molecules including water molecules [17]. It has been successfully employed to explain the morphologies of HEWL crystals with tetragonal (Tet-), orthorhombic (O-) and monoclinic except triclinic [17]. Moreover, the slip systems of Tet- and O-HEWL crystals have been successfully explained by the surface energy estimated by the macro-bond method [13–15]. In order to comprehensively understand the mechanical properties, the surface energy for Tri-HEWL crystals has been desired.

In this paper, the estimation of the surface energy and the analysis of the active slip systems of Tri-HEWL crystals are carried out. The slip traces are clearly observed around the indentation marks. Surprisingly, corresponding slip systems are identified as eleven kinds. This behavior is quite unique compared with the simple metals, inorganic and organic crystals [1,2,18–21], which is explained by the conventional dislocation theory. Based on the analysis of slip systems of Tri-HEWL crystals, it can be suggested that the active slip systems could be mainly controlled by not the Burgers vectors but the slip planes with lower surface energy for the crystals composed of the complex macromolecules and lower crystallographic symmetry.

Experimental Methods

Crystallization of Tri-HEWL

Three times crystallized HEWL (Wako Pure Chemical Industries, Ltd.) was used without further purification. All other chemicals used for preparing solutions were of reagent grade. Tri-HEWL crystals ($P1$, a = 2.73 nm, b = 3.20 nm, c = 3.43 nm, α = 88.53º, β = 108.57º, γ = 111.85º, Z = 1) were grown by a liquid-liquid interfacial precipitation method. The elementary crystal structure and unit cell are shown in Figure 1 (a). The crystallization solution containing 7.5 mg/mL HEWL, 15 mg/mL $NaNO_3$ and 0.05 M sodium acetate buffer solution at pH 4.5 was prepared. Fluorinert liquid with a high density of 1,880 kg/m^3 (FC-43, 3M) was poured into a bottle. Then, the crystallization solution was gently added into the bottle to form a liquid-liquid interface. The bottle was placed at 278 K for 5 hours first and kept at 296-298 K for 6 weeks. As shown in Figure 1 (b), the crystals up to 1.5 mm were grown at the interface. Almost all the crystals were bounded by the habit planes of (100), (010), (001), and $(10\bar{3})$, although it reported that these crystals have those of (100), (010) and (001) in previous research [22,23].

Measurements of Microindentation hardness

The Vickers hardness, H_v, was measured by using a micro-hardness testing machine (HM-221, Mitutoyo Co.) with a diamond indenter. The measurement was conducted at 296-298 K in air with controlled relative humidity 60-80%. The humidity was controlled using humidity control agents (DRY WET, Toshin Chemicals Co.), as reported previously [15].

The indentation was conducted on (100) and (010) habit planes of Tri-HEWL crystals. It is not suitable to indent in normal to (001) and $(10\bar{3})$ habit planes since they are not to be parallel as shown in Figure 1. The indenter, a load of 4.9 mN (0.5 g weight) was pulled down to the crystal plane at a velocity of 0.01 mm s^{-1}. The contact period of the indenter with the plane was 5 s, which is hold time at a maximum load. The indentation marks were observed by using an optical microscope. The H_v was determined with equation: $1.854(Fd^{-2})$, where F (N) and d (mm) are the load and the average length of diagonal of the indentation mark, respectively. Just after the crystals were transferred from solution to air, the crystal plane was covered with solution droplet. The clear indentation marks are confirmed after a few minutes with the evaporation of water on the surface, which that time when the first indentation mark is observed is defined as t = 0, as reported previously [14,15]. In this research, the hardness was measured under higher humidity since the crystal surface was strongly cracked under lower humidity less than 60% RH.

Results and discussion

Figure 2 shows typical behavior of Vickers hardness on (010) habit plane of Tri-HEWL crystals at 296 K exposed to air with approximately 76% RH. The hardness depends on the exposure time. That is, the hardness increases with increasing exposure time and shows that three stages. It should be noted that the exposure of the crystal to air can lead to the easily evaporation of the intra-crystalline water. These behaviors were reported previously in Tet- and O-HEWL crystals [14,15]. First stage is called the incubation stage in which the magnitude of the hardness keeps a constant value even during evaporation of the intra-crystalline water as shown in Figure 3 (a). This shows that in this stage, the indentation plane is still kept in wet condition. While the water on the surface is easily evaporated, the interior water in the crystal can also flow to the surface. Next, the magnitude of the hardness drastically increases with increasing exposure time. This stage is called the transition stage and the indentation plane is getting dry. Finally, the hardness reaches the maximum and keeps almost constant. This is called the saturation stage. In this stage, the indented plane is highly dried due to the evaporation of intra-crystalline water.

Indentation tests were also carried out on the other habit plane of (100) in Tri-HEWL crystals. The behavior of the hardness with evaporation exhibited the same three stages on the (100) indented plane such as the incubation, transition and saturated ones.

Figure 3 (b) shows that the incubation stage is clearly observed on (100) plane as same as (010) plane as seen in Figure 3 (a). The hardness in the incubation stage on (010) and (100) habit

planes is 25.2 ± 2.91 and 27.7 ± 5.23 MPa, respectively. It should be noted that the hardness in the incubation stage is independent of relative humidity. The hardness with the range between 25.2 and 27.7 MPa is considered to be intrinsic hardness of Tri-HEWL crystals. In the previous report, the hardness of O-HEWL crystals also depends on the habit planes [14]. Moreover, the hardness of Tri-HEWL is about three times as high as 6-10 MPa of O-HEWL crystals and is also about twice as high as 16 MPa of Tet-HEWL crystals [14,15]. It could be considered that the hardness values in the HEWL crystals depend on characteristic of each plane such as the anisotropy associated with the protein molecules.

Figure 4 shows the indentation marks formed on (010) planes at (a) incubation, (b) transition and (c) saturation stage. The lines around the indentation mark indicated by arrows are clearly observed in the incubation stage related to the wet condition. In general, the cracks after an indentation appear at the corner of the indentation mark, since the stress concentration appeared at the corner [1]. However, these lines are elongated from the not corner but the ridge of the indentation marks. As results, these lines are assigned as slip lines. The formation of slip lines depends on the multiplication and motion of dislocations in the crystals [2]. Thus, it has been suggested that the plastic deformation by the indentation in Tri-HEWL crystals mainly results from the dislocation mechanism. On the other hand, in the case of same loads (0.5 gf), no slip traces around the indentation mark are observed in the transition and saturation stages related to the dried condition. The decrease of the intermolecular distances in the saturation stage or dry condition may arise the increase of the bond strength [24,25]. It is difficult to observe the slip traces by the optical microscope due to the small deformation by the indentation with the low load. The indentations with 10 times higher loads (5.0 gf) were also carried out. As a result, the clear slip traces around the larger indentation marks were observed even in the saturation stage as shown in Figure 4 (d). This result suggests that the plastic deformation in all stages is controlled by slip deformation due to dislocations.

Let us consider the slip systems. The slip deformations occur when dislocations move on particular crystallographic planes, so-called slip planes which contain both dislocation line and the Burgers vector of dislocation. In typical simple FCC crystals such as Cu, Ag, and Au metals, the Burgers vector is defined by the shortest lattice constant because of the minimum dislocation energy [2]. The possible Burgers vectors ***b*** of dislocations in Tri-HEWL crystals are listed in Table 1. In addition, the possible slip planes are defined as the most closely packed molecules planes [2]. However, in protein crystals, it is difficult to estimate the molecular densities on each plane since they are composed of protein molecules with complicated shape.

In previous research, the slip planes for Tet- and O-HEWL crystals has been considered by using the surface energy, which is estimated by the energy of macro-bonds crossing a certain crystallographic plane [24,25]. The surface energy corresponding to the inter-planer bonds have been calculated taking into account of protein molecules with huge size and complex shape in a phenomenological macro-bond model [17]. Thus, it can be considered that slip planes correspond to the planes with lower surface energy. In order to characterize the slip systems, we estimate the surface energies of specific planes in Tri-HEWL crystals by the macro-bond model [17]. The surface energies γ_{vac} in vacuum state are given by

$$\gamma_{\mathrm{vac}} = \frac{\sum E_b}{2S(hkl)}$$

where $\sum E_b$ is the summation of macro-bond strengths crossing the crystal plane. $\sum E_b$ is calculated using E_b which is the strength of each macro-bond, as reported previously [17]. $S(hkl)$ is the area of the crystal plane in the unit-cell. Actually, protein crystals contain a lot of intra-crystalline water. The hydration of the crystal planes has to be taken into account for the estimation of the surface energies. The hydrated surface energies γ_{hyd} are given by

$$\gamma_{\mathrm{hyd}} = \frac{\sum \varepsilon_b}{2S(hkl)},$$

where

$$\varepsilon_b = \left[E_b{}^{1/2} - (2\gamma_W S_b)^{1/2}\right]^2.$$

Here, ε_b is the strength of each intermolecular bond in hydrated state. γ_W and S_b are the surface energy of water ($\gamma_W = 7.3\times10^{-3}$ $\mathrm{Jm^{-2}}$), and the contact area in each intermolecular bond, respectively [17]. The strength values of macro-bond for each position of molecule (*x, y, z*) in Tri-HEWL crystals are summarized in Table 2. The hydrated surface energies γ_{hyd} of Tri-HEWL crystals can be calculated as shown in Table 3.

As shown in Figure 5, slip traces are clearly observed in the incubation stage. Five kinds of slip traces around the indentation mark on (010) plane are observed to be parallel to [100], [001], [101], [$10\bar{1}$] and [301] directions, respectively. The measured and calculated values of the angle between slip traces are shown in Table 4. Here, we consider slip systems corresponding to [100] slip traces on (010) indented plane. From Table 3 and the Weiss' law of zones, the possible slip planes with [100] slip trace are (010), (001), (011) and/or ($0\bar{1}1$) planes. Note that the (010) plane should be exclusive because of the indented plane. According to the estimated surface energy as seen in Table 3, the (011) plane is suggested as the slip plane since the surface energy is lower ($\gamma_{\mathrm{hyd}} = 29.0\times10^{-3}$ $\mathrm{Jm^{-2}}$). Next, we assign the Burgers vector on the (011) slip plane. According to the geometry, the possible Burgers vectors on (011) are [100], [$01\bar{1}$], and [$11\bar{1}$]. From Table 1, the [100] (b = 2.73 nm) is the shortest lattice vector on (011) slip plane. However, the slip deformation along [100] cannot form the slip traces. Therefore, the second shorter vector of [$01\bar{1}$] is suggested as the Burgers vector. Thus, the (011)[$01\bar{1}$] system is suggested as the slip systems as shown in Figure 6. Similarly, all slip systems on (010) planes are characterized. As a result, the slip systems corresponding to the slip traces along [100], [001], [101], [$10\bar{1}$] and [301] are identified as (011)[$01\bar{1}$], (100)[010], ($10\bar{1}$)[010], (101)[010], and ($10\bar{3}$)[010], respectively. Moreover, in the saturates stage, the slip traces are also observed to be

parallel to [10$\bar{1}$] as seen in Figure 4 (d). It means that the same slip systems can be active in all stages during the dehydration. The schematic figure of the typical slip systems on (010) plane is shown in Figure 6.

Similar indentation tests were carried out on (100) plane. Six kinds of slip traces parallel to [010], [001], [011], [01$\bar{1}$], [0$\bar{1}$2] and [0$\bar{2}$1] directions are observed as shown in Figure 7. The measured and calculated values of the angle between slip traces are shown in Table 5. As a result, slip systems on (100) plane are identified as (10$\bar{1}$)[101], (010)[100], (0$\bar{1}$1)[100], (011)[100], (021)[100] and (012)[100]. The schematic figure of the typical slip systems on (100) plane is drawn in Figure 8.

The all observed slip systems of Tri-HEWL crystals are listed in Table 6. The eleven kinds of slip systems are characterized such as (011)[01$\bar{1}$], (100)[010], (10$\bar{1}$)[010], (101)[010], (10$\bar{3}$)[010], (10$\bar{1}$)[101], (010)[100], (0$\bar{1}$1)[100], (011)[100], (021)[100] and (012)[100]. The numbers of slip traces and the slip trace directions which appeared on (010) and (100) indented planes are also shown in Table 6. The more numbers of the slip traces on the (010) indented plane correspond to the lower surface energies in order. On the other hand, the numbers of slip traces on the (100) indented plane are not always consistent with the surface energy. This is due to that the estimation of the surface energy using the macro-bond method is a rough phenomenological approximation. However, to our knowledge, this approach based on the surface energy is reasonable as a primary approximation, since the cohesive energy of a protein crystal could not be evaluated yet. Actually, the calculated surface energies have been successfully employed to explain the crystal morphologies of HEWL crystals so far [17].

On (010) indented plane, the numbers of [101] slip traces are about 41% of total numbers of slip traces. In addition, those numbers of [010] slip traces are about 35% of total numbers of slip traces on (100) indented plane. The common slip plane corresponding to these slip traces is (10$\bar{1}$) on both [101] and [010] slip traces although the Burgers vectors are different. Therefore, it is considered that the basic slip plane in Tri-HEWL crystals is the (10$\bar{1}$). It seems that the slip systems in Tri-HEWL crystals are unambiguously controlled by the slip planes rather than the Burgers vectors. As the deformation mechanism, this is unique compared with the conventional dislocation theory. In general, the active slip systems are decided by the shortest lattice vector on the closed packed plane as slip planes in order to minimize the dislocation energy for the crystals with simple atoms and/or molecules and higher crystallographic symmetry.

Recently, in organic crystals, it has been reported that a balance of the interactions between the molecules controls the mechanical property [3–7]. Moreover, various high index slip planes of the organic crystals with monoclinic form have been observed by the nanoindentation method [3]. It has been considered that the limited amount of available low-index planes requires higher index ones to deform the non-cubic organic crystals [3]. This trend is good agreement with the case of protein crystals with lower crystal symmetry. Until now, various slip systems have been identified in O-HEWL crystals [14]. In the case of GI crystals, not only the shortest Burgers vector, but also the longer ones have been observed, which is mentioned in the introduction section, by synchrotron X-ray topography [16]. In addition, eleven kinds of slip systems were successfully characterized in Tri-HEWL crystals. It seems that the slip deformation with various slip systems easily occurs in protein crystals. This might be due to the almost similar magnitude of the surface energy of slip planes. It can be suggested that for the crystals composed of the complex macromolecules and lower crystallographic symmetry such as Tri-HEWL crystals, the active slip systems could be mainly controlled by the slip plane.

Conclusions

We have observed Vickers indentation hardness and slip systems for different habit planes of Tri-HEWL crystals containing intra-crystalline water in wet condition. Eleven kinds of the slip systems are determined due to the surface energy estimated by the phenomenological macro-bond method. Based on the analysis of the slip systems, it is suggested that the active slip systems are controlled by not the Burgers vector but the slip plane for the crystals composed of the complex macromolecules and lower crystallographic symmetry.

Conflicts of interest

There are no conflicts to declare.

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