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Modeling the Mechanical Response of Tetragonal Lysozyme Crystals

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Abstract

In this paper we investigate the mechanical response of tetragonal lysozyme crystals based on existing experimental data and a continuum-based crystal plasticity model. Compression analyses along different crystallographic directions reveal that the mechanical response of lysozyme crystals is highly anisotropic and orientation dependent. While the response is purely elastic along the [110] direction, it is elasto-plastic along the [100] and [2 12] directions. The yield stress and critical resolved shear stress are observed to be sensitive to temperature and the amount of intracrystalline water. An increase in temperature and the amount of intracrystalline water molecules leads to a decrease in the critical resolved shear stress of the slip systems and makes the crystal softer. The analysis presented in this paper may be applied to the study of other protein crystal systems as well as their optimal design for biotechnological applications.

1. Introduction

Proteins crystals are highly ordered three-dimensional structures, in which the protein molecules bind to each other with specific intermolecular interactions. In the past, the main goal of protein crystallization was to explore the structure of protein molecules using X-ray and electron microscopy¹. Recently, protein crystals have emerged as promising bionanoporous materials for different applications including highly selective biocatalysis, biosensing, bioseparation, vaccine formulation, and drug delivery^{1–4}.

Over the past decade, significant research and development efforts have been focused on engineering protein crystals, efficacy testing, model development, and production and characterization^{7–10}. Despite these successes, many challenges associated with the characterization of protein crystals, such as their stability, remain. The environmental working conditions require the protein crystals to be both chemically and mechanically stable. The chemical stability of protein crystals has been the subject of intense research^{11–14} but our understanding of the mechanical stability of protein crystals under different conditions still remains largely obscure. However, the structure, behavior, and mechanical properties of protein crystals play important roles in their performance and life cycle¹⁵.

In this work we introduce a strategy for modeling the mechanical response of protein crystals with tetragonal lysozyme as a model. We apply different procedures to explore the orientation dependent mechanical stability of this crystal under external forces and different conditions of temperature and amounts of intracrystalline water.

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Lysozyme is an enzyme which is found in egg white, tear, saliva, mucus, and other body fluids. Its main role is to damage bacterial cell walls and prevent infection. Lysozyme can be easily crystallized and its well-known and stable structure makes it a good choice for our study. Some of the mechanical properties of this crystal have been investigated using micro hardness measurements and sound velocity under different temperatures and amounts of intracrystalline water^{16–19}. It has been observed that temperature and amount of intracrystalline water have significant effects on the elastic and plastic properties of tetragonal lysozyme crystals. At lower temperatures and water content, the crystal is more brittle while it is more ductile at higher temperature and humidity. Dislocations have been found to be the main source of plastic deformation in this crystal¹⁷.

In previous works the plastic behavior of lysozyme crystals was explored using microindentation on specific molecular planes^{16–19}. Although these investigations revealed the plastic deformation mechanisms, they do not provide enough information about the complex anisotropic mechanical behavior. In this work, we use the existing experimental data with a combination of analytical and computational methods to explore the anisotropic mechanical response of lysozyme crystals.

2. Analysis and methods

In this work we have used an experimentally validated micromechanical model developed in²⁰, to model the behavior of tetragonal lysozyme single crystals (figure 1) under uniaxial loading. These crystals belong to the $P4_32_12$ space group with lattice constants of $a=b=7.91\text{nm}$, $c=3.79\text{nm}$, and $Z=8$ ¹⁷. The Young's modulus is reported to be sensitive to both temperature and humidity^{16, 18–19}. It has been reported that the Young's modulus decreases with increasing the temperature¹⁸ according to the following relationship:

$$\Delta E = -C_T E_0 \Delta T \quad (1)$$

where ΔE and ΔT are increments in the Young's modulus and temperature, respectively, E_0 is the young modulus at 300K and the temperature coefficient of Young modulus, C_T , for lysozyme crystals is $2 \times 10^{-3} \text{K}^{-1}$ ¹⁸. The Young's modulus increase with increase in the amount of the intracrystalline water molecules^{16,19} according to the relationship:

$$\Delta E = C_w E_0 \Delta t \quad (2)$$

where Δt is the evaporation time and C_w is the humidity coefficient whose value depends on environmental parameters such as temperature. Using the experimental data in¹⁹ for natural evaporation of water from lysozyme crystal surface at room temperature, C_w was calculated as $0.0396 (1/\text{min})$.

Lysozyme crystals exhibit elastic-plastic response under external loading¹⁶. The plastic deformation mechanism is known to be due to crystallographic slip [17]. The crystal has two sets of slip systems¹⁸: a primary $\{110\}\langle 001\rangle$ system and a secondary $\{110\}\langle 110\rangle$ system. Some of the important parameters corresponding to these slip systems are shown in table 1. In this table, “dry” and “wet” conditions correspond to 42% and 98% relative humidity, respectively.

A major parameter appearing in our model is the critical resolved shear stress (CRSS) of the slip systems which is defined as the minimum shear stress to active a slip system. In order to determine the CRSS for different environmental conditions of temperature and humidity, we have simulated microindentation experiments of lysozyme single crystals. During these

tests, a diamond indenter is forced to penetrate the specimen under loading. The hardness (H_V) of the specimen is expressed as $H_V = P/A$, where P is the maximum load applied during indentation and A is the projected contact area of the indenter. In the Vicker's microhardness measurement technique, the indenter is a pyramid and the hardness is expressed as $H_V = 1.854 (P/d^2)$, where d is the diagonal of the indenter (figure 2).

In our simulation of Vicker's microindentation experiments, the geometry of the Vicker's indenter was chosen according to the procedure proposed in ^{21–22}. To avoid singularity and to reduce computational ambiguity, the Vicker's pyramid was modeled as an equivalent cone (figure 2). Since only the projected area of the indenter enters into the computation of the hardness, the equivalent radius of the cone at an indentation depth of h is computed as $r_{eq} = a / \sqrt{\pi}$, where a is the side length of the Vickers pyramid corresponding to the same depth (figure 2). This ensures that the cross-sectional areas A and B of the pyramid and conical indenters at the same penetration depth are equal.

In order to account for anisotropy, a cylindrical finite element model of a lysozyme crystal was developed (figure 3) which was fixed at the bottom. The contact between the indenter and the specimen was assumed to be frictionless. The model was then indented on the (110) plane to obtain the force and tip displacement curves at different temperatures and amounts of intracrystalline water. The simulations were conducted at 6 different temperatures between 278K and 307K and at different natural evaporation times between 0 and 100min at room temperature. The CRSS values for the different slip sets were chosen such that the Vickers hardnesses obtained from the simulations match published experimental values.

After obtaining the CRSS values, uniaxial compression of the lysozyme crystals was carried out along different crystallographic directions to explore the anisotropic mechanical rigidity of the tetragonal lysozyme crystal. The results are discussed in the next section.

3. Results and discussion

Figure 4 shows the results of the microindentation tests on a lysozyme single crystal on the (110) molecular plane in the wet condition at 298 K and 307 K and in the dry condition at room temperature. It is clear that lysozyme becomes softer with increasing temperature and water content. Both temperature and intracrystalline water affect the elastic and plastic deformation regimes. In the following subsections, we explore the effects of temperature and intracrystalline water on the mechanical response of lysozyme crystals.

3.1. The effect of temperature on yield stress

Figure 5 shows a plot of the hardness and yield stress for a lysozyme crystal indented on its (110) molecular plane in the temperature range of 278–307 K. The CRSS is computed from hardnesses values extracted from¹⁸ as described in section 2. The numerical compression tests are then performed to compute the yield stresses. The yield stress decreases with increasing temperature. However, it is less temperature sensitive at temperatures below room temperature. This may be explained based on the understanding that the deformation mechanism of lysozyme crystals is predominantly elastic below room temperature but is elastic-plastic at higher temperatures¹⁸. Since the plastic deformation, which is due to the dislocation activation, is more temperature sensitive than the elastic deformation, the slope of the yield stress curve increases at higher temperatures. This will be discussed in more detail in the following sections.

3.2. The effect of intracrystalline water on yield stress

The amount of intercrystalline water molecules, which is a function of the evaporation time, has a significant effect on the mechanical properties of protein crystals. The hardness decreases with increasing water content. Figure 6 shows the effect of the evaporation time on the yield stress. The hardness values are extracted from¹⁹ and used to compute the CRSS. With evaporation, the number of water molecules in the lattice decreases and this increases the yield stress. Three different regimes of interest may be identified in this graph. For the first 20 minutes of evaporation time, (stage1) the yield stress remains almost constant at about 5.8 MPa. Between 20 and 40 minutes of evaporation (stage2) the yield stress increases to about 45MPa and beyond that (stage3) the yield stress continues to increase but with a different slope. Based on our microindentation analysis we hypothesize that this may be related to the activation of the slip systems in the three different regimes. In stage1 both slip system sets (table 1) are easily activated while in stage2 the effect of the secondary slip systems fades gradually and it is no longer active in stage3.

3.3. Temperature dependence of the critical resolved shear stress (CRSS)

Based on our microindentation simulations, we plot the CRSS for the two different slip systems of the lysozyme crystal at different temperatures in figure 7. This is in agreement with the experimental observation that the self-energy of dislocations for the $\{110\}<001>$ slip system is larger than that for the $\{110\}<110>$ slip system¹⁸. The self energy of dislocations is a function of both the shear modulus, G , and Burger's vector, b , and is proportional to Gb^2 . As in equation1, the Young's modulus of the lysozyme crystal decreases with increasing temperature and so does the shear modulus, which is related to the Young's modulus according to the relationship $E = 2G(1 + \nu)$, where ν is the Poisson's ratio. Thus the self energy of the dislocations in lysozyme crystal decreases with increasing temperature which eases the nucleation of dislocations thereby decreasing the CRSS. However, it is reported in¹⁸ that the effect of temperature on the hardness of lysozyme crystals is more significant than its effect on the elastic constants. Therefore, we may conclude that the effect of temperature on the CRSS must also be related to the dislocation mechanisms.

As mentioned before, plastic flow in lysozyme crystals occurs by creation and motion of dislocations. It is recognized that thermal fluctuations provide the energy to carry the dislocations over the lattice potential barriers^{23–24}. Hence, dislocations at higher temperatures have a higher probability of overcoming lattice potential barriers due to higher thermal fluctuations. This explains why the CRSS decreases with increasing temperature for both slip systems, as shown in figure7. At higher temperatures, the CRSS of the two slip systems are smaller and closer to each other and therefore, both the slip systems can be easily activated. At lower temperatures, the CRSS of the $\{110\}<001>$ slip system is much less than the CRSS of the $\{110\}<110>$ slip system, and can therefore be more easily activated. This difference in slip activation behavior is the cause of the anisotropic deformation characteristics at different temperatures.

As discussed before, at temperatures below room temperature, the deformation in lysozyme crystals is primarily elastic while at higher temperatures it is elastic-plastic. Therefore, at lower temperatures the temperature variation of the yield stress in figure 5 is primarily due to the temperature dependence of the elastic constant and hence the CRSS. However, at higher temperatures, both the elastic constant and dislocation mechanisms are affected by temperature which results in a higher drop in yield stress with increasing temperature.

3.4. Effect of intracrystalline water on CRSS

Protein molecules have a significant amount of intercrystalline water. The decrease in CRSS with increasing temperature (figure 7) may potentially be related to these water molecules. Two types of intracrystalline water may be present in the lattice: mobile water, which can easily traverse through the crystal and bounded water, which is bound to the molecules^{18–19}. Mobile water has a high diffusion coefficient at higher temperatures²⁵ and therefore has little interaction with dislocations. However, at lower temperatures it may interact with dislocations and thereby affect dislocation creation and motion in the lattice¹⁸.

As shown in figure 8, the CRSS for both slip system sets increases with evaporation time. This can be explained as follows.. It has been reported that a change in intracrystalline water changes both the lattice and elastic constants of protein crystals. A decrease in the amount of the intracrystalline water, as a result of evaporation, leads to an increase in elastic constants and a decrease in lattice parameters²⁶. This increases the self energy of the dislocations significantly and hinders their nucleation and activation, thereby increasing the CRSS of the slip systems.

Another parameter that may significantly affect the CRSS is the Peierls stress. The Peierls stress is the minimum force necessary to move a dislocation within an atomic/molecular plane in a crystal. It is an intrinsic property of a crystal and depends on temperature and impurity in the crystal. It has been observed that the Peierls stress of lysozyme crystals significantly increases with decreasing amounts of intracrystalline water¹⁸. However, a detailed understanding of the Peierls stress in protein crystals is yet to be achieved. This may be due to the difficulty of studying the interactions of adjacent molecular planes when the lattice sites are occupied by complex protein molecules.

In this study only the effects of the water molecules was investigated. In some applications, e.g., drug delivery, protein crystals contain drug molecules or other fluid molecules in the lattice. The presence of these molecules can have more complex effects on the mechanical properties which has to be studied separately.

3.5. The anisotropic mechanical response of lysozyme crystals

To evaluate the mechanical response of lysozyme single crystals under different conditions, compression tests were performed along the different crystallographic directions for dry crystal and also for wet crystals at two different temperatures. Figure 9 shows the stress-strain curves for different orientations at three different conditions. The deformation behavior is observed to be highly anisotropic.

The wet crystals loaded along the [100] and $[\bar{2} 12]$ directions exhibit elastic-plastic response whereas compression along the [110] direction results in purely elastic response (figure 9a–b). The yield stress along $[\bar{2} 12]$ direction is lower compared to the [100] direction. When the wet crystals are loaded along the $[\bar{2} 12]$ direction, the (110)[001] slip system is activated first followed by the $(\bar{1}10)[001]$ slip system. Loading along the [100] direction activates both (110)[$\bar{1} 10$] and $(\bar{1} 10)[110]$ slip systems at the same time. The dry crystal shows purely elastic deformation along both [100] and [110] crystallographic directions (figure 9c) whereas its response is elastic-plastic along $[\bar{2} 12]$ direction. In this case also the (110)[001] slip system is activated first followed by the $(\bar{1}10)[001]$ slip system.

These analyses show that the yield stress in lysozyme crystal is highly orientation dependent. Hence, under external loading, lysozyme crystals exhibit higher mechanical strength along certain crystallographic directions compared to others.

5. Conclusion

The mechanical response of the tetragonal lysozyme crystals has been investigated based on a combination of indentation simulations, crystal plasticity modeling and existent experiments. The results of our investigation show that the mechanical strength of the lysozyme crystal is significantly sensitive to temperature and the amount of intracrystalline water molecules. The critical resolved shear stresses for all slip systems decreases with increasing temperature and the quantity of intracrystalline water molecules.

Further analysis of the deformation along different crystallographic directions shows that the deformation and the yield stress are highly anisotropic with certain directions exhibiting purely elastic response, whereas others exhibiting elasto-plastic behavior.

The analysis presented here may serve as a framework for the investigation of the mechanical response of various protein crystals. This may also be used to develop optimal design strategies for bionanoporous materials for future applications. However, further work is necessary, especially in experimental characterization of protein crystals, to develop sophisticated models for post yield behavior, hardening, damage, and softening under different environmental conditions. When the intra-crystalline spaces are filled by fluids other than water, or other complex molecules, specialized analysis may be necessary.

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Figure 1.

(a) A lysozyme molecule (image obtained from protein data bank) and (b) the tetragonal lysozyme crystal with its crystal coordinates and (110) slip plane

Figure 2. The Vicker's indenter, A, and its equivalent cone, B. For simulation purposes the indenter A was replaced by indenter B.

Figure 3. The finite element model of a Vicker's microindentation test used in our analysis ($R = 100\mu m$, $t = 120\mu m$, $r_{eq} = 55.86\mu m$, and $d = 20\mu m$)

Figure 4.

Force versus indentation depth curves corresponding to microindentation simulations under three different cases: dry condition, wet condition at 298K and wet condition at 307K

Figure 5. Yield stress and hardness at different temperatures along [110] crystallographic direction (the hardness values were extracted form [18] for comparison)

Figure 6. Yield stress and hardness at different states of hydration along the [110] crystallographic direction (the hardness values were extracted form [19] for comparison)

Figure 7.
The effect of temperature on critical resolved shear stresses

Figure 8.
The effect of the amount of intracrystalline water molecule on critical resolved shear stresses of slip systems in Lysozyme crystal

(a)

(b)

(c)

Figure 9.

Stress-strain response of a lysozyme crystal along different crystallographic directions; (a) in wet condition at room temperature; (b) in wet condition at 307K and (c) in dry condition at room temperature

Table 1

Slip systems and corresponding parameters in tetragonal lysozyme crystals

Slip system	Burgers vector, $b(nm)$	Young's modulus, $E(GPa)$	Self-energy(10^{-9} J/m)
(110)[001]	Wet: 3.79 Dry: 3.12	Wet: 1.918 Dry: 7.234	Wet: 10.0 Dry: 25.6
(1 $\bar{1}$ 0)[001]			
(110)[1 $\bar{1}$ 0]	Wet: 5.59 Dry: 5.22		Wet: 21.8 Dry: 71.9
(1 $\bar{1}$ 0)[110]			