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The Optical Activity of Lysozyme Crystals

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Abstract

The components of the gyration tensor of the enzyme lysozyme were measured by using the HAUP method: $g_{11} = -0.90 \times 10^{-5}$ and $g_{33} = 1.05 \times 10^{-5}$ at 303.4 K and a wavelength of 4880 Å. The optical rotatory powers along the *a* and *c* axes in the same conditions were calculated: $\rho_a = -21.3$ and $\rho_c = -24.8^\circ$ cm^{-1}. The optically active property of lysozyme is strange in that, although it contains a considerable quantity of α-helices (about 30%), the rotatory powers are unexpectedly small in magnitude, one order of magnitude less than those of quartz and with very large anisotropy. A conceptual consideration of this phenomenon is given. In order to assess the difference between the structures in both crystalline and solution states, the chirality index *r* was calculated to be 0.16. This value indicates that the structural change of lysozyme from the solution into the crystalline state is expressed by an increase of 19% in optical activity. From the NMR results [Smith *et al.* (1993), *J. Mol. Biol.* **229**, 930–944], it is anticipated that the *r* value reflects the increased constraint in atomic motion in the side chains of exposed amino acid residues in the crystalline state.

1. Introduction

Biological functions of proteins are mainly displayed in liquid solutions. Accordingly, it is necessary to reveal structures of proteins in solution to understand their biological activity. In reality, crystal structures of proteins have been analyzed accurately using X-ray diffraction. The structures of protein molecules in solution have been exclusively analysed by nuclear magnetic resonance (NMR) through distance geometry like nuclear Overhauser enhancement (NOE) restraints and torsion-angle restraints. Structures deduced by the two methods have been compared for various proteins (Wüthrich, 1986). These are similar in the main backbone of polypeptide chains. However, in solution there is good evidence that the conformation found by X-ray analysis is in dynamic equilibrium with a slightly different structure involving localized folding (Blundell & Johnson, 1976). New methods need to be developed for discriminating accurately the differences between structures of proteins in the two states.

Lysozyme was the first enzyme whose crystal 7shy;structure was accurately determined by X-ray diffraction (Blake *et al.*, 1962, 1965). Subsequent X-ray refinements confirmed the validity of the original analysis (Aschaffenburg *et al.*, 1980; Artymiuk & Blake, 1981; Mason *et al.*, 1984; Strynadka & James, 1991). It was also the first protein to be studied by NMR (McDonald & Phillips, 1967; Campbell *et al.*, 1973). In this sense, this protein is the most appropriate example for elucidating the differences between structural features of proteins in the two states. According to X-ray analysis of lysozyme, it has a globular fold and the structure can be divided into two domains; one domain (I) is composed of residues 1 to 35 and 85 to 129, the other (II) contains residues 36 to 84 (Blake *et al.*, 1965; Aschaffenburg *et al.*, 1980; Strynadka & James, 1991). Specific attention should be directed to the following fact: the I domain contains four α-helices, *A*, *B*, *C* and *D*, and a short 3_{10} helix, while the II domain contains a deformed 3_{10} helix, but the total content of these helices was regarded to be relatively small (about 30%) (Blake *et al.*, 1962, 1965; Phillips, 1966) compared with other proteins, *e.g.* myoglobin (about 77%) (Kendrew *et al.*, 1958).

Optical activity (OA) takes place through the constraint of paths along which outer electrons can migrate by the applied electric field of light. By measuring OA, effects of helical paths traced by electrons can be surveyed. In this sense, OA can be a useful tool for looking at the crystal structure at a different viewpoint from X-ray diffraction. Theoretical relations between OA and the crystal structure have been studied by many workers (Drude, 1902; Oseen, 1915; Gray, 1916; Born, 1922; Kuhn, 1929; Kirkwood, 1937; Ramachandran, 1951; Glazer & Stadnicka, 1986). In particular, Glazer's group (Glazer & Stadnicka, 1986; Devarajan & Glazer, 1986) examined the relation between OA and the atomic arrangement of some inorganic crystals. However, for organic crystals this problem belongs to an almost unknown field owing to the extreme lack of data on OA. It was expected that mutually complementary studies of X-ray and OA methods would advance our

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knowledge of the relation between the structure and function of proteins. If the OA method is skillfully applied to a protein crystal, it might provide us with otherwise unobtainable information on the structure of the protein. Moffitt & Yang (1956) have given an empirical rule by which the helix content can be evaluated by measuring the OA of protein solutions. However, to our knowledge, the OA has not been successfully measured for any protein crystal to date.

Measurements of OA along any arbitrary directions of solids remained unfeasible for about 170 years from its discovery (Arago, 1811), because the OA of solids are overwhelmed by the coexisting birefringences, which are usually more than 10^3 times larger than them (Nye, 1985). We developed a new optical apparatus called HAUP (Kobayashi & Uesu, 1983), which enabled us to measure simultaneously OA, birefringence and the rotation of the indicatrix of any crystals, even those belonging to the monoclinic and triclinic systems. An important condition by which the HAUP method became successful was the thorough elimination of systematic error of the simplest optical devices contained in the measuring system, *viz* a polarizer and an analyzer. This means that the measurements of the OA of protein crystals forbid insertion of any other optical devices and any solid materials into the light beam. For instance, the glass capillary tubes, which are usually used in X-ray analysis for specimen containers, wholly nullify the OA measurements of proteins. However, protein crystals immediately lose their crystallinity when they are exposed directly to the vacuum. Thus, the measurements of the OA of proteins give the HAUP technique an additional severe difficulty.

Recently, the structure of lysozyme in solution was solved in detail by Smith *et al.* (1993) by using the two-dimensional ^{1}H NMR method and was compared with the crystal structure. It seems timely to commence the OA study of proteins using lysozyme crystals in order to obtain new information on the structural differences in the two states of the protein. This paper reports measurements of all the components of the gyration tensor of a lysozyme crystal.

2. Specimens and optical nature

Single crystals of hen egg-white lysozyme chloride were prepared by the sitting-drop method of Alderton *et al.* (1945). An aqueous solution of lysozyme chloride with a concentration of 3.5 mmol l^{-1} was mixed with an equal volume of the buffer solution of CH_3COONa (0.1 mol l^{-1}) and NaCl (1 mol l^{-1}) at pH = 4.5. A drop (about 0.2 ml) of the mixed solution placed on a glass plate was subjected to the sitting-drop method. A {110} form was predominantly developed on the growing crystals. However, this habit was changed to the {101} form by a slight addition (0.2 mmol l^{-1}) of $MnCl_2$ to the original solution.

Measurements of the lattice constants of a specimen were made by using a Weissenberg camera at 300 K. The results were: $a = b = 79.2\,(3)$ Å [79.1 Å] and $c = 37.8\,(2)$ Å [37.9 Å]. They are in good agreement with those [in brackets] reported by Blake *et al.* (1965). Conoscopic microscope images at the extinction and orthogonal positions of a (101) specimen are represented in Figs. 1(*a*) and (*b*), respectively. These figures confirmed that the specimen was optically uniaxial with an optic axis along the *c* axis. The data enabled us to identify our crystals as lysozyme chloride with point group D_4 as reported by the above authors. By using a quartz wedge, it could be determined from the (101) specimen that the *a* and *c* axes corresponded to the optical X' and Z' axes, in agreement with a previous report (Jones, 1946).

3. Measurements of optical properties

The HAUP method (Kobayashi & Uesu, 1983) was used to determine all the components of the gyration tensor and the birefringence. From the symmetry of the crystal, there exist two components in the gyration tensor, $g_{11} = g_{22}$ and g_{33}, and a birefringence, Δn. HAUP measurements provide us with the following three quantities (Kobayashi & Uesu, 1983; Kobayashi *et al.*, 1986):

$$\Delta = (2\pi/\lambda)\Delta n d, \tag{1}$$

$$B(0) = (\gamma - 2k)\sin\Delta + 2\delta\Upsilon\cos^2(\Delta/2), \tag{2}$$

or

$$B(0)/\sin\Delta = (\gamma - 2k) + \delta\Upsilon\cot(\Delta/2), \tag{3}$$

and

$$\theta_0 = -\tfrac{1}{2}(p+q)\cot(\Delta/2) - \tfrac{1}{2}\delta\Upsilon + \Psi, \tag{4}$$

where $\gamma = p - q$, p and q being the systematic errors due to parasitic ellipticities of the polarizer and analyzer. d is the thickness of the specimen, k the ellipticity of the eigenpolarization of light travelling in the crystal, Ψ the rotation angle of the indicatrix (vanishing in the present case) and $\delta\Upsilon$ the error of the deflecting angle of the analyzer from the crossed Nicols position. As has already been mentioned, any unknown errors should be strictly excluded from the optical system. Light should impinge directly on the specimen uncovered by any solid materials.

In order to keep the crystallinity of the thin specimens on a plate holder of the HAUP system, we constructed a special humidifier made of the steel cylinder and metal funnel exit (3 mm diameter). It produced a water vapour flow of the humidity of 100% around 305 K. The vapour blew on a surface of the specimen which was attached to the sample holder by the mother liquor as shown in Fig. 2. The temperature of specimens could be maintained within an accuracy of ±0.1 K around this temperature. We tested changes of the three parameters, Δ, $B(0)$ and

θ_0, of a (110) specimen with the change of temperature T. They all began to diverge from a constant value from 306.5 K as can be seen in Figs. 3(*a*), (*b*) and (*c*), indicating that the specimen began to be denatured above this temperature. On the other hand, the three parameters could be kept constant at 303.6 K for about 2 h, a run of the stable measurement being able to be performed within this duration. For the determination of the systematic errors, the reference crystal method (Kobayashi *et al.*, 1988) was used, although Moxon & Renshaw (1990) proposed another method. We used $LiNbO_3$ (point group C_{3v}) as the reference crystal. In calculating the systematic error p by this method, k in equations (2) and (3) was taken to vanish, since the crystal is optically inactive. However, a recent paper by Simon *et al.* (1996) reported that a centrosymmetric crystal (MgF_2) manifested non-zero k probably due to surface treatments, *e.g.* polishing of the specimen. However, we have already proved that such an error did not occur in our preparation of samples (Asahi, Takahashi & Kobayashi, 1997). As the crystallinity of lysozyme was found to be affected by slight elevation of T, the three parameters of lysozyme specimens were measured at a constant T of 303.4 K but at different wavelengths λ issuing from an Ar laser.

The first step of the present experiment was to examine whether the systematic errors of the optical system were altered appreciably by the small change of λ or not. The three parameters of an $LiNbO_3$ specimen with an area of 2.645×1.642 mm and thickness of 0.121 mm were measured at $\lambda = 4579$, 4880 and 5145 Å.

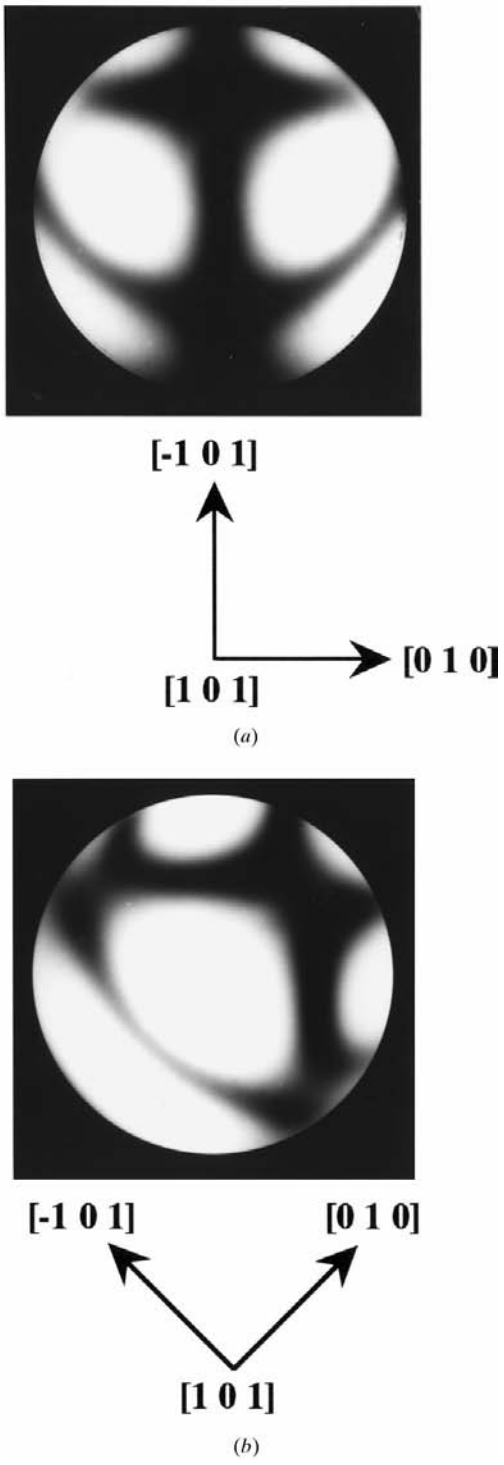

Fig. 1. Conoscopic figures observed on a (101) plate of lysozyme: (*a*) at an extinction position; (*b*) at a diagonal position.

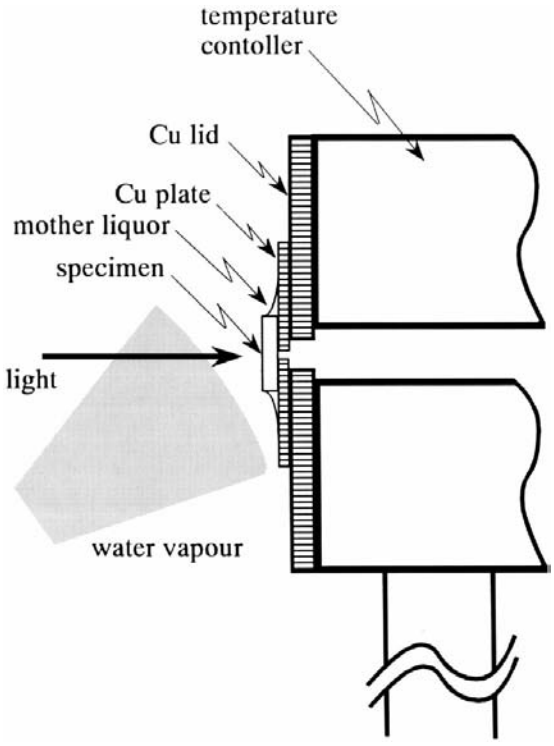

Fig. 2. Schematic representation of the experimental environment of a lysozyme specimen in the HAUP system.

Then, relations $B(0)/\sin\Delta$ *vs* $\cot(\Delta/2)$ and θ_0 *vs* $\cot(\Delta/2)$ for each wavelength were obtained as depicted in Figs. 4 and 5, respectively. The strict linearity held in both figures at every wavelength. $\delta\Upsilon$ and γ were obtained from the derivatives and intercepts of the straight lines in Fig. 4. $p+q$ was obtained from the derivatives in Fig. 5. The systematic errors thus obtained are shown in Table 1. It can be seen from the table that the systematic errors scarcely change within these changes of wavelength. Then the systematic errors in the

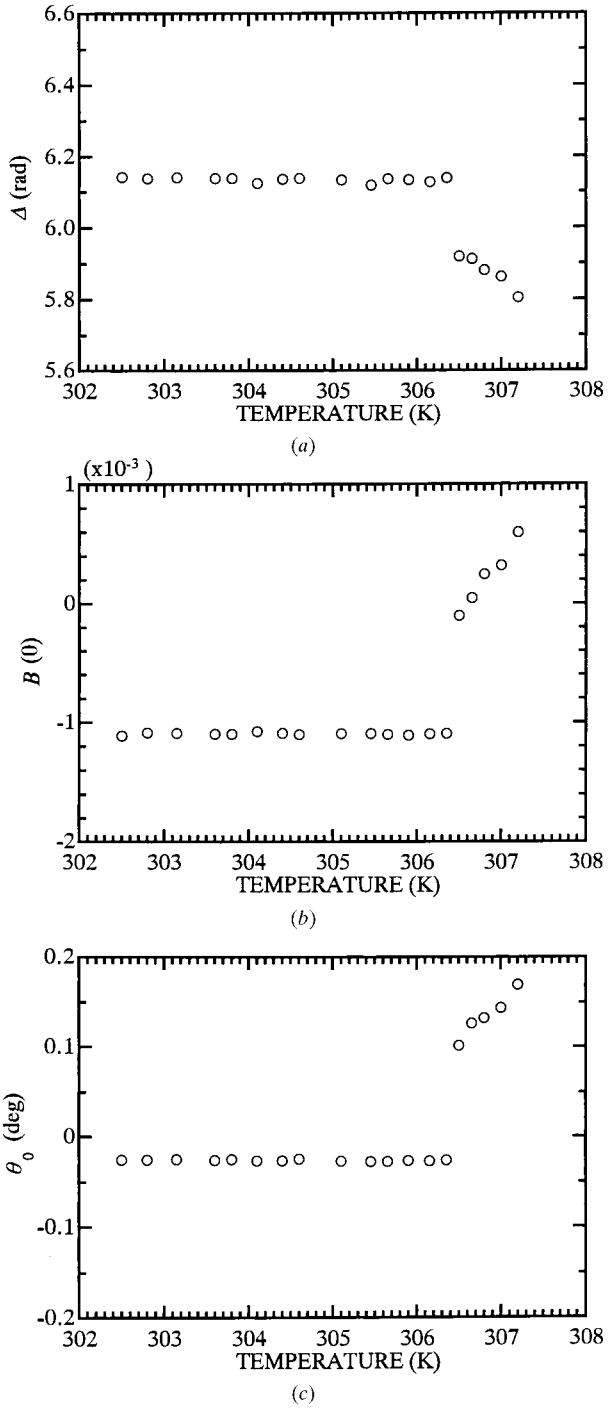

Fig. 3. Changes of (*a*) Δ, (*b*) $B(0)$ and (*c*) θ_0 of the (110) plane of lysozyme with respect to temperature.

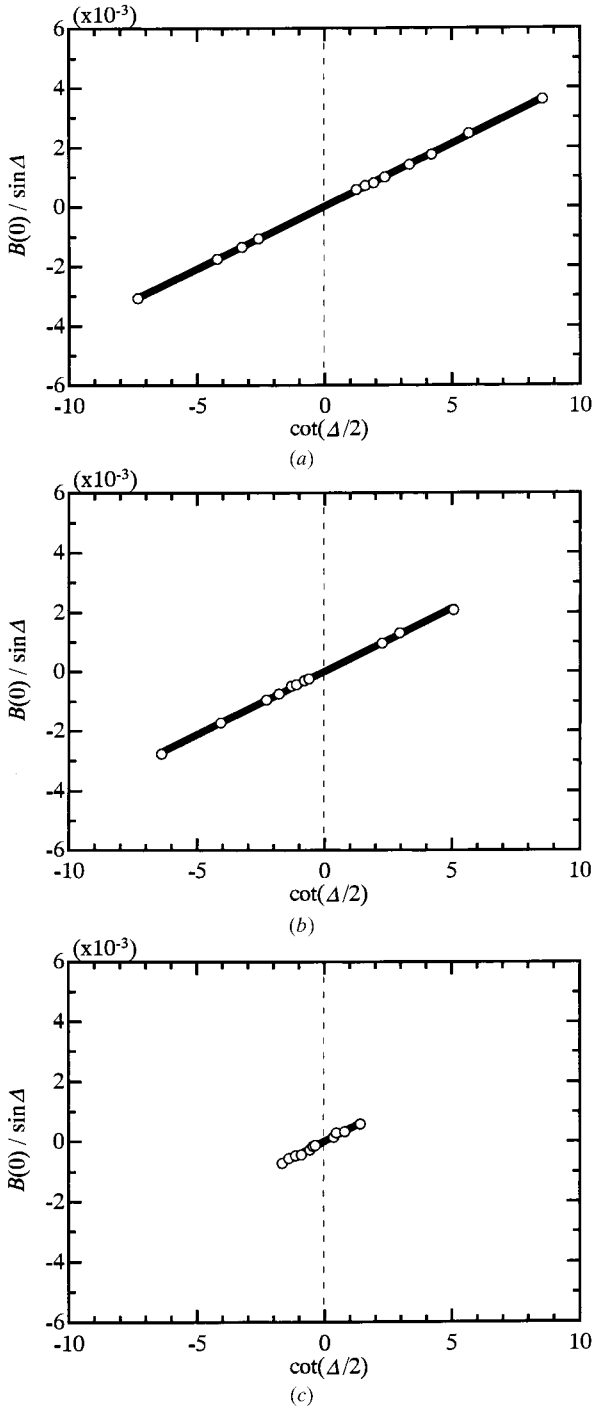

Fig. 4. Relations between $B(0)/\sin\Delta$ and $\cot(\Delta/2)$ of the (100) plane of $LiNbO_3$ at three wavelengths. (*a*) $\lambda = 5145$ Å, (*b*) $\lambda = 4880$ Å, (*c*) $\lambda = 4579$ Å.

present measurements were determined by taking mean values $\gamma = 0.3 \times 10^{-4}$, $\delta\Upsilon = 4.2 \times 10^{-4}$ and $p + q = -8.9 \times 10^{-4}$. As p is a common error in the $LiNbO_3$ and subsequent lysozyme experiments, it will be written as $\hat{p}$ in what follows (Kobayashi *et al.*, 1988).

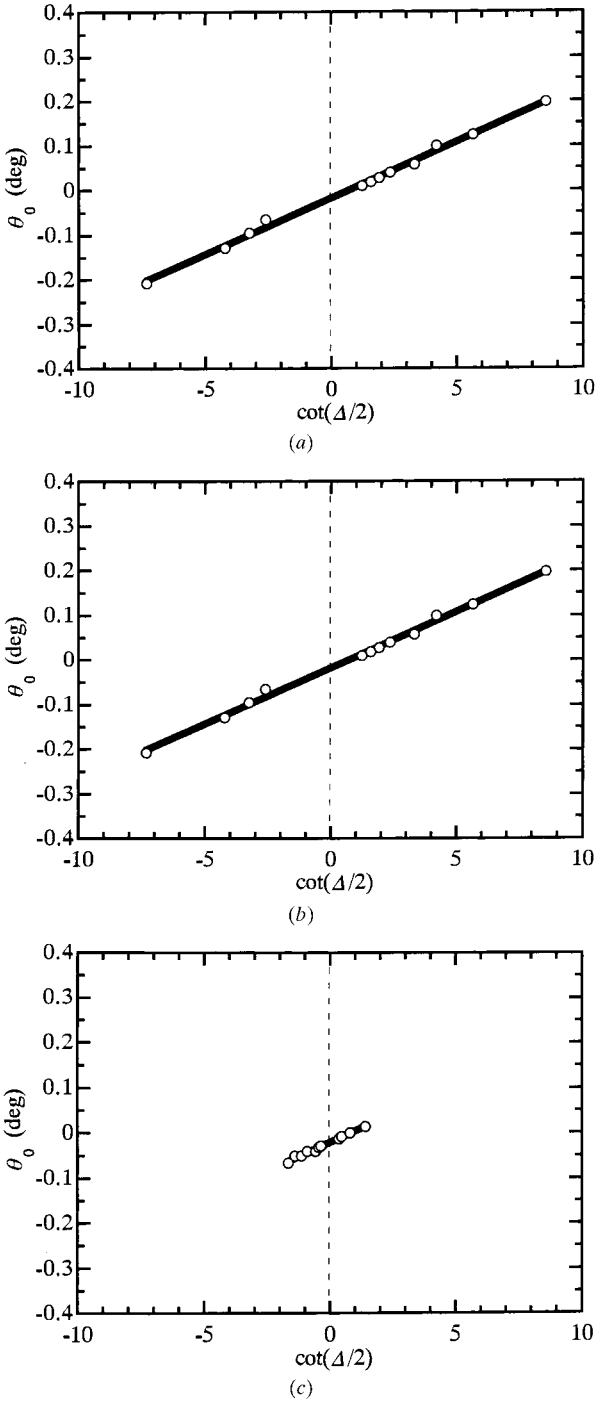

Fig. 5. Relations between θ_0 and $\cot(\Delta/2)$ of the (100) plane of $LiNbO_3$ at three wavelengths. (*a*) $\lambda = 5145$ Å, (*b*) $\lambda = 4880$ Å, (*c*) $\lambda = 4579$ Å.

Table 1. *Systematic errors of the HAUP system for $LiNbO_3$*

λ (Å)	γ ($\times 10^{-4}$)	$\delta\Upsilon$ ($\times 10^{-4}$)	$p+q$ ($\times 10^{-4}$)
5145	0.4 (1)	4.2 (1)	−8.9 (2)
4880	0.3 (1)	4.2 (1)	−9.0 (2)
4579	0.3 (1)	4.3 (1)	−8.8 (2)

The three parameters of the (110) specimen with an area of 0.760×0.201 mm and thickness of 0.199 mm were measured against λ as represented in Fig. 6. The relations $B(0)$ *vs* Δ and θ_0 *vs* $\cot(\Delta/2)$ are depicted in Figs. 7(*a*) and (*b*), respectively. From Fig. 7(*a*), $\delta\Upsilon$ was determined to be $-3.61\,(3) \times 10^{-4}$ from the interpolated value of $B(0)$ at $\Delta = 2\pi$. From the derivative of the straight line in Fig. 7(*b*), $\hat{p} + q = -2.24\,(3) \times 10^{-3}$. Therefore, $\gamma = \hat{p} - q = 1.38\,(2) \times 10^{-3}$. By using these systematic errors, gyration G_{110} $(= g_{11})$ and birefringence Δn_{110} $(\Delta n = n_c - n_a)$ were acquired as expressed in Figs. 8 and 9 by the open circles.

Analogous experiments were performed on the (101) specimen, 0.35×0.34 mm in area and 0.896 mm thick. The systematic errors in this case were $\gamma = 7.78\,(6) \times 10^{-4}$ and $\delta\Upsilon = 3.46\,(7) \times 10^{-4}$. As the wave normal of light is written as $s = (\cos 64.4^\circ, 0, \cos 25.6^\circ)$ from the lattice constants, the observed gyration reads $G_{101} = 0.187 g_{11} + 0.813 g_{33}$. g_{33} could be obtained by using the already determined values of g_{11}, as represented in Fig. 8 by solid circles. Also from the relation $\Delta n_{110} \simeq \Delta n(\sin^2 25.6^\circ)$, Δn were calculated as shown in Fig. 9 by the solid triangles. The excellent agreement of Δn values derived from the two specimens with the different crystal planes indicated the validity of the present experiment.

4. Discussion

The gyration-tensor components of lysozyme at $T = 303.4$ K and $\lambda = 4880$ Å are as follows: $g_{11} = -0.90 \times 10^{-5}$ and $g_{33} = 1.05 \times 10^{-5}$. Then the rotatory powers along the *a* and *c* axes are calculated as ρ_a (or ρ_1) $= -21.3^\circ$ cm^{-1} and ρ_c (or ρ_3) $= -24.8^\circ$ cm^{-1}. Here the signs of ρ accord with the chemist's convention (Caldwell & Eyring, 1971). These values are compared with those of various crystals in Table 2. It is seen that the rotatory powers of lysozyme are unexpectedly small, *e.g.* one order of magnitude smaller even than those of quartz (α-SiO_2). Moreover, the anisotropy is very large as can be seen by the gyration surface (Shubnikov, 1960) in Fig. 10, where the white part represents the positive sign and the black part the negative one. ρ_a and ρ_c are approximately the same in magnitude but opposite in sign. Accordingly, it follows that there is no rotatory power along the directions of the generators of a cone making an angle of 42.8° with the $a = b$ axes.

We have also measured the OA of solid poly-L-lactic acid (PLLA), a biopolymer, which is composed of two parallel helical conformations (Kobayashi, Asahi, Ichiki, Oikawa *et al.*, 1995) as shown partly in Fig. 11. It produces, as given in Table 2, a huge rotatory power of nearly $-10^{5\circ}$ cm^{-1} along the helical axis (c axis), but $10^{2\circ}$ cm^{-1} along the perpendicular direction (a axis). Therefore, it is surprising that lysozyme, which contains a considerable amount of helices, manifests such low values of OA. It seems that this is an important property of proteins. Needless to say, it is beyond our present knowledge to interpret properly the origin of this apparently strange phenomenon. However, it may be worthwhile for future solutions to investigate in a little more detail what this phenomenon means.

Referring to Ramachandran's theory (Ramachandran, 1951; Glazer & Stadnicka, 1986) and ours (Kobayashi, 1990), the rotatory power produced by a helix composed of a particular kind of atom can be approximately expressed as

$$\rho \propto C(l^2 tnN/u)(\alpha_r^2 - \alpha_t^2). \quad (5)$$

Here l designates the distance from the atom to the helical axis, u the pitch of the helix, α_r and α_t the

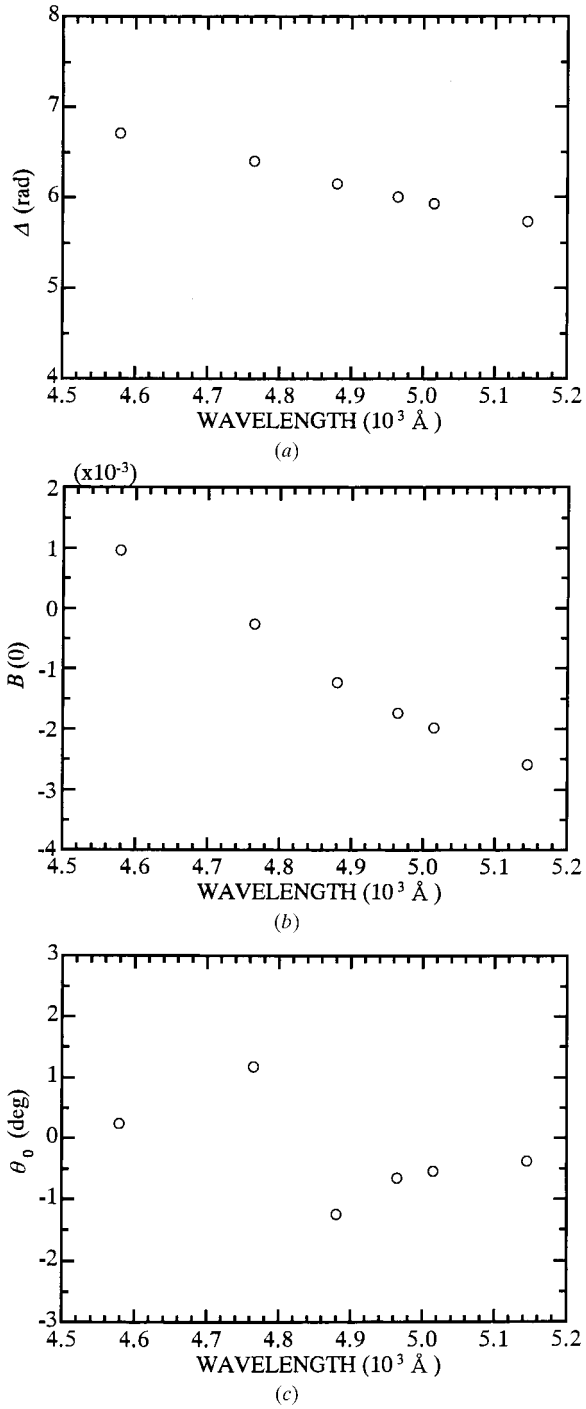

Fig. 6. Wavelength dependences of (*a*) Δ, (*b*) $B(0)$ and (*c*) θ_0 of the (110) plane of lysozyme.

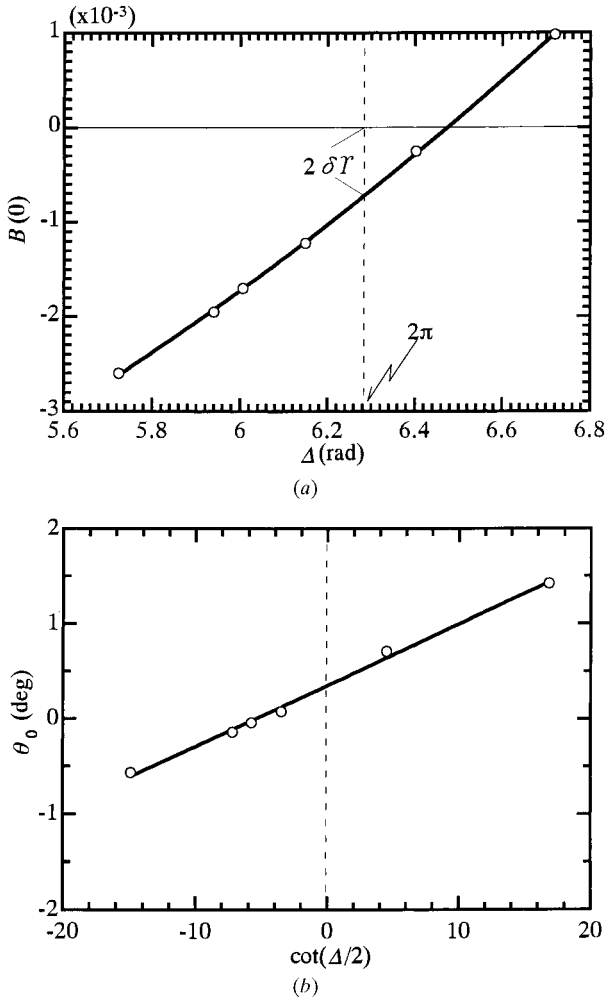

Fig. 7. Change of (*a*) $B(0)$ with respect to Δ and (*b*) the linear relation between θ_0 and cot(Δ/2) of the (110) plane of lysozyme.

Table 2. *Rotatory powers of various crystals*

Crystal	Class	λ (Å)	T (K)	ρ (° cm^{-1})	Reference
α-SiO_2	D_3	6328	293	$\rho_1 = -1.05 \times 10^2$ $\rho_3 = 2.51 \times 10^2$	(*a*)
$NaKC_4H_4O_6 \cdot 4H_2O$	D_2	6328	298	$\rho_1 = -9.6$	(*b*)
$BaMnF_4$	Incommensurate	6328	160	$\rho_1 = 5.06 \times 10$ $\rho_2 = -2.79 \times 10$ $\rho_3 = 1.22 \times 10$	(*c*)
Poly-L-lactic acid	D_2	5145	298	$\rho_1 = 1.4 \times 10^2$ $\rho_3 = -9.2 \times 10^4$	(*d*)
$(NH_4)_2SO_4$	C_{2v}	6328	200	$\rho_1 = 3.86 \times 10$	(*e*)
$Bi_2Sr_2CaCu_2O_8$		4880	20	$\rho_3 = 3.38 \times 10^2$	(*f*)
Glutamic acid	D_2	6328	293	$\rho_1 = -3.08 \times 10^2$ $\rho_2 = -9.78 \times 10^2$ $\rho_3 = 5.4 \times 10$	(*g*)
Aspartic acid	D_2	6328	293	$\rho_1 = 2.23 \times 10^3$ $\rho_2 = -1.86 \times 10^2$ $\rho_3 = -3.08 \times 10^2$	(*h*)
Lysozyme	D_4	4880	303.4	$\rho_1 = -2.13 \times 10$ $\rho_3 = 2.48 \times 10$	This work

References: (*a*) Kobayashi *et al.* (1988); (*b*) Kobayashi *et al.* (1990); (*c*) Asahi *et al.* (1992); (*d*) Kobayashi, Asahi, Ichiki, Oikawa *et al.* (1995); (*e*) Asahi, Ikeda *et al.* (1996); (*f*) Kobayashi *et al.* (1996); (*g*) Asahi, Utsumi *et al.* (1996); (*h*) Asahi, Takahashi & Kobayashi (1997).

polarizability components of the atom directed perpendicular and tangential to the helical axis, respectively, t the number of turns of the helix contained in the unit thickness (1 mm) of the specimen, n the number of atoms contained in a pitch of the helix, N the number of these helices contained in a unit cell and C a constant. It is particularly important to note that the sign of the rotatory power is determined by the difference between α_r and α_t. It follows that the sense of a helix and of the rotation of polarized light coincide only when $\alpha_r < \alpha_t$. Glazer & Stadnicka (1986) predicted successfully the sign of rotatory powers of some inorganic crystals on this basis. However, the helices in proteins consists of amino acid residues, where assessment of the overall polarizability of each residue becomes extremely difficult. In order to approach this assessment, comprehensive analyses of optically active properties of the constituent amino acid crystals are indispensable. We have begun measurements of the OA of amino acid crystals (Asahi, Utsumi *et al.*, 1996; Asahi, Takahashi & Kobayashi, 1997).

Lysozyme contains 129 amino acid residues (Jollés *et al.*, 1963; Canfield, 1963). It has also been disclosed in a recent NMR study (Smith *et al.*, 1993) that the I domain contains four α-helices and a short 3_{10} helix as was already found by X-ray diffraction. An α-helix is identified as a non-integral 18/5 helix (Pauling & Corey, 1951) and a 3_{10} helix with a threefold screw axis. On the other hand, PLLA includes two parallel 10/3 helical conformations along the c axis (Kobayashi, Asahi, Ichiki, Oikawa *et al.*, 1995). Let us make an approximate comparison of the rotatory powers of lysozyme and PLLA, assuming that lysozyme contains only a right-handed B helix (24–36 residues), which is almost parallel to the c axis. As shown in Fig. 11, backbones of the

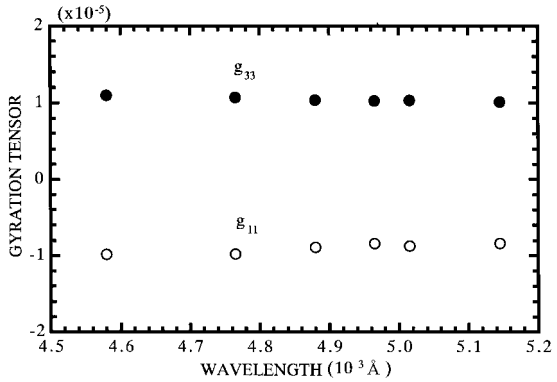

Fig. 8. Wavelength dependences of the components of the gyration tensor of lysozyme. Open circles represent g_{11}, solid circles g_{33}.

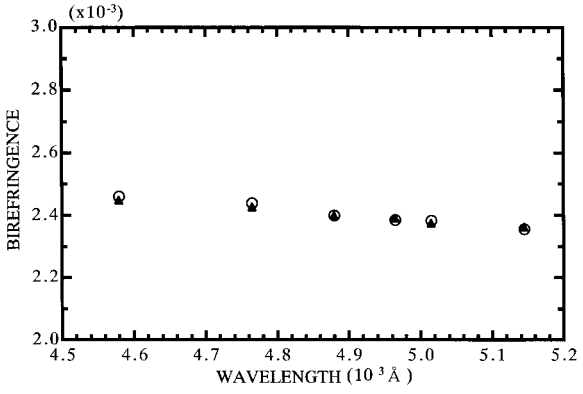

Fig. 9. Wavelength dependences of the birefringence of lysozyme. Open circles designate the values obtained from the (110) plane and solid triangles those from the (101) plane of lysozyme.

Table 3. *Parameters in equation (5) for lysozyme and poly-L-lactic acid*

	Lysozyme	Poly-L-lactic acid
u (Å)	5.4	9.6
l (Å)	1.74 ($O_{carbonyl}$)	1.24 (O_{ester}), 0.94 ($O_{carbonyl}$)
t (mm^{-1})	9.52×10^5	1.04×10^6
n	3.6	3.3
N	8	2

helices of the two crystals are similar except an N atom in lysozyme is replaced by an O atom in PLLA. The parameters in equation (5) except the polarizabilities are compared in Table 3. Then it can be estimated by using the table that the rotatory power of the lysozyme crystal containing only the *B* helix should be approximately equal to or a little larger than that of PLLA provided the polarizability of the O atom predominates over those of other atoms. In reality, however, ρ_c of PLLA is 10^4 times larger than that of lysozyme. This is the most remarkable feature of the OA of lysozyme. Furthermore, the complicated relation between OA and the structure in the lysozyme crystal can be found in other facts; for instance, 21, 57 and 77 residues are found in the left-handed helical region in the Ramachandran plot (Blake *et al.*, 1967). Thus, the accurate interpretation of lysozyme should await deeper and more systematic studies.

An ensemble of the superimposed 16 solution structures deduced from NMR data by Smith *et al.* (1993) was found to be in good agreement with the structures derived by using X-rays (Blake *et al.*, 1965) in the main-chain folding. Moreover, the five helices in the I domain were also well defined by the NMR method. However, it is important to notice that the NMR method disclosed considerable disorder in the calculated structures for the side chains of exposed residues on the protein surface. Furthermore, the 3_{10} helix in the II domain could not be found in the folding of the solution structures. Thus, results of previous experiments can be summarized in that, while the overall structures deduced by the two methods are in good agreement, there remain characteristically different parts found on examining them in detail. Besides, structural differences have not been

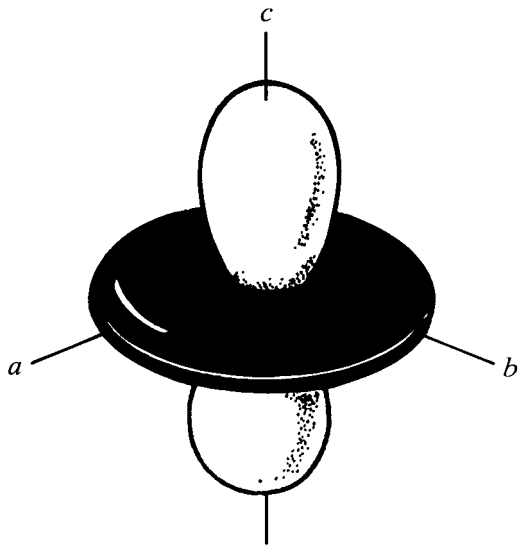

Fig. 10. Gyration surface of lysozyme.

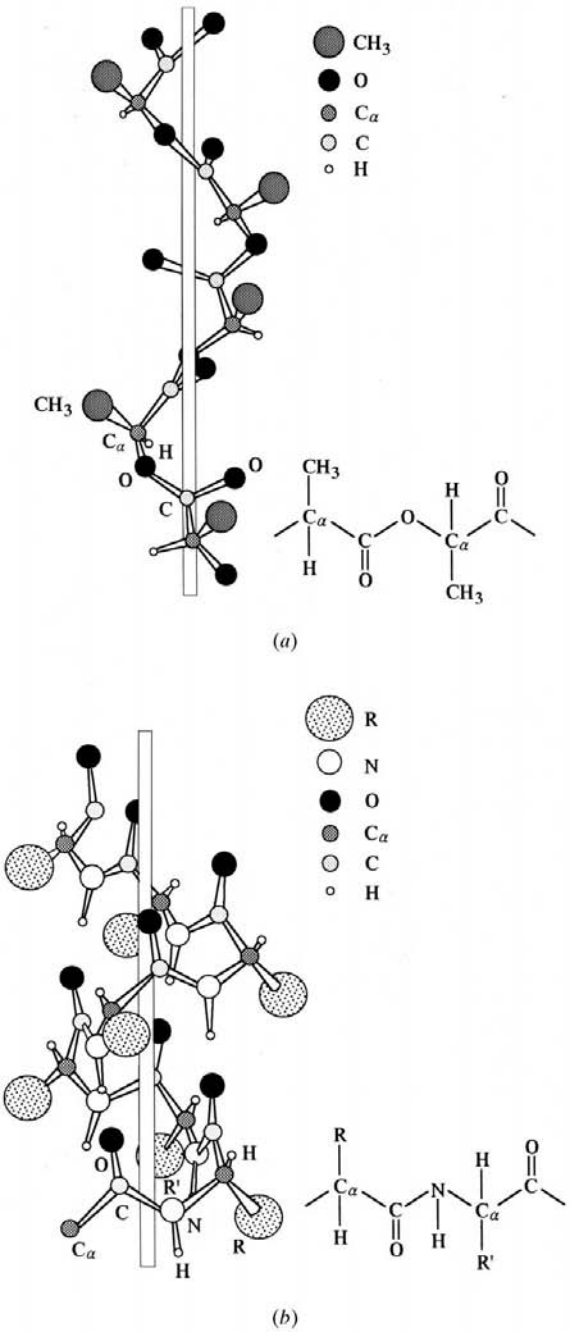

Fig. 11. Schematic representation of the helical conformations of (*a*) poly-L-lactic acid and (*b*) lysozyme.

Table 4. *Chirality index r of some crystals*

Crystal	*r*	Reference
Poly-L-lactic acid	0.999	(*a*)
Glutamic acid	0.998	(*b*)
Aspartic acid	0.994	(*c*)
Lysozyme	0.16	This work

References: (*a*) Kobayashi, Asahi, Ichiki, Oikawa *et al.* (1995); (*b*) Asahi, Utsumi *et al.* (1996); (*c*) Asahi, Takahashi & Kobayashi (1997).

elucidated on a sufficiently quantitative level. For instance, Smith *et al.* (1993) attributed the absence of the 3_{10} helix in the II domain of the solution structure partly to the insufficient NMR data.

It is significant to underline that OA increases in essence by increase of the severity of the restriction of the freedom of molecular orientation by forming a crystal lattice (Kauzmann & Eyring, 1941). In order to manifest a measure of this crystal-structure contribution to the OA of a crystal, we have already defined the chirality index *r* of the crystal (Asahi, Utsumi *et al.*, 1996; Asahi, Takahashi & Kobayashi, 1997) as

$$r = (\rho_C^0 - \rho_S^0)/\rho_C^0 = 1 - \rho_S^0/\rho_C^0. \quad (6)$$

Here, ρ_C^0 is the rotatory power per molecule in a crystalline state, while ρ_S^0 is that in solution. As a typical example, the OA of quartz disappears when it is dissolved in solutions. Therefore, its *r* equals 1. The oxo amide $C_{14}H_{19}NO_2$ is also one such material (Asahi, Nakamura *et al.*, 1997). When the structures are the same in both states, *r* should vanish. According to our limited calculations, a few amino acids and PLLA manifest an *r* of almost unity as shown in Table 4. However, crystals with *r* zero have not been found so far. It will be of particular interest to calculate *r* for lysozyme.

Calculations of *r* for a crystal require all the components of the gyration tensor. From them, a mean gyration tensor $\bar{g}$ of the assemblage composed of randomly orientated crystallites can be calculated according to the formula derived by us (Kobayashi, Asahi, Ichiki, Saito *et al.*, 1995). On the other hand, ρ_S^0 is easily calculated from the specific rotatory power of a solution. $\bar{g}$ of D_4 class is given by $\bar{g} = \frac{1}{3}(2g_{11} + g_{33})$, which is 2.5×10^{-6} in the case of lysozyme. Then the rotatory power of the assemblage of 1 ml becomes

$$\rho_C = (\pi/\lambda)(\bar{g}/\bar{n})(180/\pi) = -5.9^\circ\,\mathrm{cm}^{-1}\,\mathrm{ml}^{-1}. \quad (7)$$

The number of lysozyme molecules contained in this volume is $N_C = Z/a^2c = 3.37 \times 10^{19}\,\mathrm{ml}^{-1}$, where $Z = 8$ is the number of molecules in the unit cell (Palmer, 1947). Therefore,

$$\rho_C^0 = \rho_C/N_C = -1.75 \times 10^{-19\circ}\,\mathrm{cm}^{-1}. \quad (8)$$

On the other hand, the mean residue rotation $[R]$ of lysozyme in the glycine–HCl buffer solution was reported to be -69.2×10^2 (Hamaguchi & Sakai, 1965). As the number of residues contained in a molecule is 129, molecular rotation becomes $[M] = -89.3 \times 10^{2\circ}\,\mathrm{dm}^{-1}\,(\mathrm{mol}\,100\,\mathrm{ml}^{-1})^{-1}$. Therefore,

$$\rho_S^0 = -89.3 \times 10^3/N_A = -1.48 \times 10^{-19\circ}\,\mathrm{cm}^{-1}, \quad (9)$$

where N_A designates Avogadro's number. From equations (8) and (9), $r = 0.16$. It is compared with other crystals in Table 4. *r* for lysozyme exhibits a very remarkable contrast with those of amino acids and PLLA. It should be noted that this value of *r* is accurate, since the OA of lysozyme in both crystalline and solution states were accurately measured. Therefore, it indicates the important fact that there is a distinct change in the structures of crystalline and solution states of lysozyme in producing different OAs. Let us define that the ability of producing OA is changed by x% when lysozyme is transformed from the solution to the crystalline state. Then

$$x = [(\rho_C^0 - \rho_S^0)/\rho_S^0] \times 100 = [r/(1-r)] \times 100 = 19.0.$$

So, lysozyme in solution undergoes a structural change that can be evaluated as an increase of 19% in the ability of producing OA when it is transformed into the crystalline state. Judging from the report of Smith *et al.* (1993), it is very likely that the major part of this change is brought about by an increase of the constraints of atomic motion in the side chains of exposed residues. In addition, it may be possible that the 3_{10} helix in the II domain really disappears in the solution structure.

To summarize, by measuring all the components of the gyration tensor of lysozyme, the following two points are revealed: the optical activity of lysozyme is strange in producing unexpectedly small OA; the structural change of lysozyme from the solution to the crystalline state shows an increase of 19% in the ability of producing OA. The solution of the former problem would be too difficult to be given immediately on the basis of the present knowledge. The latter result establishes the existence of structural change of lysozyme in both solution and crystalline states quantitatively. Extensive studies of *r* values for other protein crystals will be interesting.

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