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Preliminary X-ray data for some new crystalline forms of β-lactoglobulin and hen egg-white lysozyme.*

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In the course of an investigation of the structure of protein crystals in progress in this Laboratory, a new crystalline form of β-lactoglobulin and three new forms of salts of egg-white lysozyme have been studied. Preliminary X-ray data for these crystals are listed in Table 1, together with pertinent data published by earlier workers which are included for comparison. Values for the density and water content of most of the new forms are also listed. Densities were determined by flotation in benzene–bromobenzene mixtures, special care being taken to avoid interaction between the crystal and the flotation medium. The water content was taken as the weight lost by the wet crystals when placed in an oven at 100 °C. for 20 minutes; it is so designated in the table. It is felt that the error of the data on unit-cell dimensions is less than 1%, the error of the density measurements is less than 3%, and the error of the amount of water removed at 100 °C. is about 10%.

Experimental

β-Lactoglobulin: Aschaffenberg and his collaborators (Aschaffenberg & Drewry, 1955; Green, North & Aschaffenberg, 1956) have shown that β-lactoglobulin is ordinarily a mixture (β_m) of two components (β_1 and β_2), which they have isolated and studied crystallographically. The β_1- and β_2-lactoglobulins were found to form orthorhombic crystals that were nearly isomorphous with β_m-lactoglobulin. In addition, β_2-lactoglobulin was obtained by them in a monoclinic form.

Dr Henry Klostergaard of the Division of Biology of this Institute has now prepared a new crystalline form of β_m-lactoglobulin. This form was obtained from whey by letting the filtrate from a precipitation at pH 3·5 and NaCl concentration of 14 g. per 100 ml., stand for several days. The crystals were repeatedly recrystallized under the same conditions. Reflections were observed corresponding to interplanar spacings as small as 4 Å. Data for the new crystal are given in Table 1 along with similar data from Green, North & Aschaffenberg (1956) for the monoclinic and orthorhombic forms.

Lysozyme chloride: The preparation of tetragonal and orthorhombic forms of lysozyme chloride has been described by Alderton, Ward & Fevold (1945), and crystallographic data for both forms have been published by Palmer (1947). In the present work, the tetragonal form was prepared from a 3% solution of lysozyme (Armour & Company) at pH 4·5 by the addition of 5% sodium chloride; the orthorhombic form was similarly obtained at pH 10. Reflections have been observed from the tetragonal crystals corresponding to minimum spacings of about 1·7 Å.

Lysozyme nitrate: A monoclinic form of lysozyme nitrate has been described by Crick (1953). This form was crystallized by the procedure of Alderton & Fevold (1946); a 1% solution of lysozyme was adjusted to pH 4·5 by the addition of nitric acid, and 3% sodium nitrate was added. In this Laboratory this procedure yielded large flat rectangular plates that gave reflections corresponding to spacings as small as 1·9 Å. The unit-cell parameters determined here were the same as those given by Crick. However, if the angle β is rechosen to bring it closer to 90°, photographs of the $[hk0]$, $[h0l]$, and $[0kl]$ zones show that the crystal has a strong tendency toward a B-centered structure.

A new, triclinic form of lysozyme nitrate was crystallized from a 1% solution of lysozyme containing 2% sodium nitrate and acetate buffer to provide 0·025 M sodium acetate and 0·025 M acetic acid, the pH being finally adjusted to 4·5 by addition of dilute nitric acid. Upon seeding the solution, large prisms were obtained which gave good reflections to spacings as small as 1·6 Å.

Lysozyme iodide: Large flat plates of a monoclinic form of lysozyme iodide were obtained by the procedure of Alderton, Ward & Fevold. The crystals were obtained by the addition of 3% sodium iodide to a 1% solution of lysozyme at pH 4·5, and were very similar in appearance to monoclinic lysozyme nitrate. The reflections were also similar in general features to the nitrate, and were observed out to 2·2 Å.

Lysozyme sulphate: Long thin plates of lysozyme sulphate were obtained after about two weeks at room temperature from a solution containing 3% lysozyme, 10% sodium sulphate, and acetate buffer to provide 0·5 M sodium acetate and 0·5 M acetic acid, the pH being adjusted to 4·5 by addition of dilute sulphuric acid. Reflections were observed out to 2·6 Å, and the pattern showed some similarity to that of monoclinic lysozyme nitrate. Because of the high concentration of sodium sulphate and the extreme fragility of the crystals, it was not possible to obtain a satisfactory value for the water content.

Discussion

One of the more obvious shapes for the lysozyme molecule that is suggested by the data for the triclinic, monoclinic, and orthorhombic forms is one having the dimensions $28 \times 32 \times 30$ Å. These molecular dimensions are not suggested by the dimensions of the tetragonal crystals. It is especially interesting that the water content of the tetragonal crystals appears to be significantly greater than that of the other forms. Assuming 128 amino acid residues per molecule of lysozyme, one can calculate that the average number of water molecules per amino acid residue is approximately 3 for the tetragonal forms and 2 for the other forms.

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Table 1. *X-ray data*

Crystal form	*a* (Å)	*b* (Å)	*c* (Å)	Space group	Molecules per unit cell	Volume of asymmetric unit (Å^3)	Density g.cm.$^{-3}$	Molecular weight (one asymmetric unit)	Water removed at 100 °C.	Dry molecular weight	Source
β-Lactoglobulin											
Hexagonal	67	67	141	$P6_3$	6	91,000	—	—	—	—	This work
Orthorhombic	69·3	70·6	156·6	$P2_12_12_1$	8	2×86,000	—	—	—	—	Green, North & Aschaffenberg (1956)
Monoclinic	36·1	127·5	36·0	$P2_1$	2	82,000	—	—	—	—	Green, North & Aschaffenberg (1956)
Lysozyme chloride											
Tetragonal	79·1	79·1	37·9	$P4_12_1$	8	29,600	1·233	22,000	—	—	Palmer (1947)
	79·1	79·1	37·9	$P4_12_1$	8	29,600	1·242	22,100	33·5 %	14,700	This work
Orthorhombic	56·3	65·2	30·6	$P2_12_12_1$	4	28,000	1·304	22,000	—	—	Palmer (1947)
Lysozyme nitrate											
Monoclinic	27·9	63 $\beta = 114\frac{1}{4}°$	66·3	$P2_1$	4	2×26,600	—	—	—	—	Crick (1953)
	27·9	63·1 $\beta = 90° 30'$	60·6	$P2_1$	4	2×26,600	1·239	2×20,000	24 %	2×15,200	This work
Triclinic	27·5 $\alpha = 88° 20'$	32·1 $\beta = 109° 0'$	34·4 $\gamma = 111° 0'$	$P1$	1	26,500	1·269	20,200	26 %	15,000	This work
Lysozyme iodide											
Monoclinic	28·1	63·1 $\beta = 91° 0'$	60·4	$P2_1$	4	2×26,900	1·300	2×21,100	24 %	2×16,000	This work
Lysozyme sulphate											
Monoclinic	28·6	63·0 $\beta = 93° 30'$	61·6	$P2_1$	4	2×26,800	1·299	2×21,000	24 %*	2×15,500*	This work

* Estimated values.

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New high pressure modifications of BPO_4 and $BAsO_4$.

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In a recent study of phase transformations in various silica isomorphs, Dachille & Roy (1957) found that at pressures and temperatures up to 35,000 atmospheres and 550 °C., only BeF_2 yielded a dense structure corresponding to coesite–SiO_2. The compounds BPO_4 and $BAsO_4$, analogous to silica as $SiSiO_4$, are known only in the high-cristobalite modification (Schulze, 1934), a reported quartz form of BPO_4 being still unconfirmed (Shafer *et al.*, 1956). As comparatively higher pressures and temperatures are now available for phase transformation studies, the preparation of the denser polymorphs of these oxides has been reattempted. This note describes the formation of new dense modifications of BPO_4 and $BAsO_4$.

BPO_4 was obtained from Victor Chemical Works and $BAsO_4$ prepared from aqueous solutions of H_3BO_3 and As_2O_5. Chemical analysis showed the purity of the two oxides to be > 99·5% and spectroscopic analysis revealed the absence of heavy metals. The polycrystalline samples were contained in graphite capsules and subjected to pressures of 85,000 atmospheres at 1000–1200 °C. for about 10 min. The compressed materials were crystalline and optically uniaxial. Repeated attempts have failed to produce single crystals of dimensions > 30 μ. Marked increases over the starting materials in density, by sink-float method, and refractive indices, by optical immersion, were found (Table 1). No transformations were observed by heating the dense oxides in air at 100 °C. for 24 hours. The X-ray powder diffraction data for both compounds, obtained from Debye–Scherrer and spectrometer methods, are similar and can be indexed on the basis of a hexagonal unit cell, the dimensions of which are presented in Table 1. The spectrometer data are given in Table 2. The c_0/a_0 ratios are appreciably different from those of α-quartz (1·10) and coesite (1·73). However, allowing for the differences between the Si–O and B–O, P–O, and As–O bond lengths, the volumes of individual BPO_4 and $BAsO_4$ groups are approximately equal to that of 2 SiO_2 in α-quartz.

Table 1. *Unit-cell dimensions, density and refractive indices of dense* BPO_4 *and* $BAsO_4$

	BPO_4	$BAsO_4$
a_0 (Å)	7·75 ±0·02	7·91 ±0·02
c_0 (Å)	9·95 ±0·02	10·32 ±0·02
c_0/a_0	1·29	1·31
N (molecules/unit cell)	9	9
$\varrho_{\text{X-ray}}$ (g.cm.$^{-3}$)	3·05	3·98
$\varrho_{\text{obs.}}$ (g.cm.$^{-3}$)	3·05 ±0·05	3·9 ±0·2
ω	1·636±0·002	1·740±0·002
ε	1·648±0·002	1·748±0·002

Table 2. *X-ray powder diffraction data for dense* BPO_4 *and* $BAsO_4$

(Co $K\alpha$, λ = 1·5405 Å)

BPO_4		$BAsO_4$		
d_0 (Å)	*I*	d_0 (Å)	*I*	*hkl*
3·895	*s*	3·964	*m*	110
3·626	*m*	3·699	*s*	111
3·363	*vvw*			200
3·318	*w*	3·452	*w*	003
3·204	*vvw*			201
3·060	*vs*	3·145	*vs*	112
2·519	*vvw*	2·598	*vw*	113
2·239	*m*	2·281	*w*	300
2·183	*vw*	2·228	*w*	301
2·092	*m*	2·162	*m*	114
2·040	*w*	2·087	*vw*	302
1·936	*vvw*	1·976	*w*	220
		1·941	*vvw*	221
1·852	*vvw*	1·901	*vw*	303
1·805	*vw*			222
1·768	*vvw*	1·831	*w*	115
1·671	*w*			223
1·662	*m*	1·710	*m*	304
1·527	*w*	1·568	*vw*	224
1·485	*vvw*	1·530	*vw*	305
1·448	*vw*	1·476	*vw*	411
1·403	*w*			412
		1·433	*w*	216
		1·381	*vvw*	117
1·339	*vvw*	1·357	*vvw*	413
1·333	*vvw*			306
1·280	*vvw*	1·307	*vvw*	331
1·260	*vw*	1·296	*vw*	226

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