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Regular arrangement of periodates bound to lysozyme

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The structure of tetragonal hen egg-white lysozyme soaked in a periodate solution has been determined to a resolution of 1.8 Å. Four high-occupancy periodate positions have been identified on the basis of the anomalous signal of the I atoms. The four periodates exhibit a regular rectangular arrangement on the surface of the lysozyme molecule. No similar regular arrangement was found either in lysozyme crystals soaked in other heavy-atom anions or in other structures from the Protein Data Bank. Depending on their position on the surface of the protein, the periodate ions deviate to a varying extent from ideal octahedral geometry.

1. Introduction

Many complexes of proteins with inorganic oxoanions can be found in the Protein Data Bank (PDB). As of 10 January 2005 there are complexes of proteins with sulfates (2960 entries; PDB chemical component ID SO4), nitrates (70 entries; ID NO3), phosphates (804 entries; ID PO4), carbonates (81 entries; ID CO3) *etc*. Only 63 PDB entries containing heavy-atom oxoanions have been found (Table 1): 31 for vanadate (IDs VO3, V4O, V7O, VO4), 21 for tungstate (ID WO4), eight for molybdate (ID MOO), two for perrhenate (ID REO) and one for selenate (ID SE4); no entries were found for bromate, tellurate or periodate. In the Heavy Atom Databank, only sodium perrhenate and tungstates are mentioned.

The chemistry of protein–heavy atom complexes in which the heavy atoms comprise inorganic oxoanions has as yet been little studied. Some of the above-mentioned heavy-atom anions have a simple and regular shape: perrhenate and selenate are tetrahedra, bromate is a deformed tetrahedron and tellurate and periodate are octahedra. In addition, vanadate, tungstate and molybdate can polymerize. The structure of the resulting polymeric anion depends strongly on both the local pH and the surface contour of the protein. In the protein crystal, polymeric ions can be formed that may differ from those present in solution. Thus, new inorganic compounds may be obtained inside protein crystals (Ondráček & Mesters, 2002). Similarly, the protein surface may stabilize intermediates and catalyse chemical reactions.

Anomalously scattering heavy atoms can also be exploited for experimental phase determination. Recently, the quick soaking of protein crystals with a solution containing 0.25–1.0 *M* Br^- or I^- was proposed as a new and universal method for protein phase determination (Dauter & Dauter, 1999, 2001; Dauter *et al.*, 2000, 2001). Bromine has an absorption edge at 0.92 Å and is therefore suitable for MAD analysis (Hendrickson & Ogata, 1997). With $\Delta f'' = 1.28$ e^- at $\lambda = 1.54$ Å, it can be used successfully for structure solution by

Table 1
Protein structures containing selected heavy-atom oxoanions as found in the PDB as of 10 January 2005.

Oxoanion	PDB code	No. of sites	Resolution (Å)
Selenate SeO_4^{2-}	1fga	2	2.2
Perrhenate ReO_4^{2-}	1hnu	2	2.1
	1k4j	7	2.5
Molybdate MoO_4^{2-}	1h9m	8	1.6
	1h9s	2	1.8
	1amf	1	1.7
	1eoi	3	2.4
	1gun	8	1.8
	1guo	8	2.5
	1h9j	2	1.8
	1o7l	5	2.8
Tungstate WO_4^{2-}	3kbp	4	3.0
	1e3p	1	2.5
	1dko	1	2.4
	1gug	16	1.6
	1h9r	2	1.9
	1lgp	1	2.0
	1fez	2	3.0
	1fr3	16	1.5
	1h9k	4	1.8
	2ush	4	2.2
	1atg	1	1.2
	1bys	1	2.0
	1ckj	5	2.5
	1cws	1	2.0
	1j9k	2	2.1
	1wod	1	1.8
	1ytw	1	2.4
	2hnq	1	2.8
	1mu7	2	2.0
	1ohd	2	1.9
	1u7p	2	1.9
Vanadate $V_4O_{12}^{4-}$ (V4O)	1l7v	2	3.2
	1uiz	1	1.9
Vanadate $V_4O_{12}^{4-}$ (VO3)	1e59	1	1.3
Vanadate $V_7O_{19}^{3-}$ (V7O)	1dkt	1	2.9
	1rxs	20	2.8
Vanadate VO_4^{3-} (VO4)	1bo6	2	2.1
	1vnc	1	2.1
	3rnt	1	1.8
	1dfl	2	4.2
	1idq	1	2.0
	1idu	1	2.2
	1rpt	1	3.0
	1qi9	2	2.1
	1j9l	2	1.9
	1vnf	1	2.4
	1vne	1	2.2
	1vng	1	2.2
	1vom	1	1.9
	1vni	1	2.2
	1vnh	1	2.1
	1lkx	4	3.0
	1mu9	2	2.1
	1nop	2	2.3
	1ovi	1	2.5
	1rff	2	1.7
	1rfi	2	2.2
	1rg1	2	2.1
	1uzi	3	1.89
	1rg2	2	2.1
	1rgt	2	2.0
	1rgu	2	2.2
	1rho	2	2.3

SIRAS (Dauter & Dauter, 1999). Iodine, with $\Delta f'' = 6.84$ e$^-$ at $\lambda = 1.54$ Å, has been used successfully for structure solution by SAD (Chen *et* al., 1991). The use of I^- was extended recently by the use of I_3^- (Evans & Bricogne, 2002). The study has been expanded to include lanthanides (Nagem *et al.*, 2003) and uranyl compounds (Djinovic Carugo *et al.*, 2005) and may also be applicable to heavy-atom oxoanions.

This study is a contribution to establishing a relation between the size, shape and chemical properties of the various heavy-atom oxoanions and their binding modes to the proteins. In addition, it may be possible to exploit the anomalous signal for the determination of new protein structures. The structure and binding mode of periodate anion is the first result presented.

Table 2
Data-collection and processing parameters.

Values in parentheses correspond to the last resolution shell.

Unit-cell parameters	
a (Å)	77.03
c (Å)	37.12
Wavelength	1.54178
Crystal-to-detector distance (mm)	100
Resolution (Å)	99.0–1.83 (1.88–1.83)
No. of images	2 × 118
Exposure time per image (s)	900
Φ rotation per image (°)	1
Completeness (%)	99.3 (99.3)
No. of unique reflections	10310
No. of unique reflections, $F > 4\sigma(F)$	9837
$R_{\rm merge}$ (%)	7.3 (15.6)
$R_{\rm anom}$ (%)	6.0 (8.3)
$I/\sigma(I)$	6.6 (4.4)
Redundancy	15.9 (15.7)

2. Materials and methods

2.1. Crystal soaking

Tetragonal crystals of lysozyme (lysozyme chloride from egg white, dried; Roanal, Budapest, Hungary) of size 0.1–0.2 mm grown by the hanging-drop method from 50 m*M* sodium acetate pH 4.5, 1–1.2 *M* NaCl were used for this study. Lysozyme crystals were soaked at room temperature for 5 min in solutions made up from one part of aqueous saturated $NaIO_4$ and 11, five and two parts of 50 m*M* acetate pH 4.5, and then for 15 min in a solution made up from two parts aqueous saturated $NaIO_4$ and one part 50% PEG 2000. Before flash-cooling, crystals were briefly immersed in paraffin oil.

2.2. Data collection and refinement

Diffraction data were collected on a Cu rotating-anode generator (FR591, Nonius, The Netherlands) operated at 50 kV and 80 mA equipped with a cold nitrogen-stream generator (Oxford Cryosystems, UK) at 150 K and a MAR imaging-plate detector (MAR345, X-ray Research, Germany). The inverse-beam technique was used for data collection. The raw data were integrated using *DENZO* (Otwinowski & Minor, 1997) and scaled using *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) using the SCALA-Det scaling protocol as described in Mueller-Dieckmann *et al.* (2004). Further data reduction and refine-

Table 3
HipHop refinement parameters.

The values given are for reflections with $F > 4\sigma(F)$.

Refinement	Input	1	2	3	4	5	6	7	8	9	10	Mean (e.s.d.)	Average structure
R, full data (%)	17.35	15.63	15.79	15.79	15.57	15.78	15.79	15.73	15.68	15.77	15.67	15.72 (7)	15.31
R (90% set) (%)	16.89	15.38	15.41	15.48	15.28	15.52	15.43	15.45	15.42	15.54	15.36	15.43 (7)	15.03
$R_{\rm free}$ (10% set) (%)	24.4	21.8	23.2	22.4	21.9	22.1	22.6	22.2	22.1	22.1	21.9	22.2 (4)	21.0
wR^2, full data	43.53	39.08	39.49	39.53	39.00	39.47	39.56	39.57	39.30	39.40	39.25	39.37 (19)	—
S, full data	2.98	2.653	2.676	2.679	2.646	2.671	2.677	2.677	2.661	2.671	2.659	2.668 (11)	—
Waters	155	185	176	176	185	173	172	170	175	178	178	177 (5)	—
$R_{\rm free} - R$ (%)	7.55	6.4	7.8	6.9	6.6	6.6	7.2	6.8	6.7	6.6	6.5	6.8 (4)	6.0

Table 4
Bond lengths (Å) in periodate I_A and possible hydrogen bonds (Å).

The bond lengths in the periodate I_A correspond to the structure $[I(HO)_4O_2]^-$.

Refinement	1	2	3	4	5	6	7	8	9	10	Mean
I—O1	1.40	1.58	1.58	1.57	1.65	1.52	1.62	1.52	1.48	1.52	1.54 (7)
I—O2	1.96	2.01	2.04	1.94	1.85	1.96	2.07	1.97	2.01	1.89	1.97 (6)
I—O3	1.91	1.89	1.87	1.74	1.76	1.88	1.89	1.77	1.84	1.80	1.84 (6)
I—O4	1.78	1.87	1.84	1.77	1.83	1.79	1.75	1.80	1.83	1.86	1.81 (4)
I—O5	1.66	1.67	1.65	1.60	1.51	1.74	1.70	1.68	1.74	1.65	1.66 (6)
I—O6	1.80	1.88	1.88	1.83	1.86	1.80	1.88	1.85	1.86	1.82	1.85 (3)
O1···Asn93 OD1	3.39	3.26	3.28	3.48	3.41	3.31	3.09	3.17	3.31	3.19	3.29 (11)
O1···Asn93 ND2	3.60	3.37	3.32	3.48	3.19	3.48	3.35	3.28	3.45	3.34	3.39 (11)
O2···His15 ND1	2.87	2.68	2.61	2.72	2.59	2.76	2.56	2.74	2.78	2.91	2.72 (11)
O2···Arg128 NH2$^{\rm i}$	2.70	2.87	3.01	2.90	3.29	2.79	2.69	2.50	2.52	2.52	2.78 (24)
O3···His15 ND1	3.27	3.27	2.98	3.11	3.31	2.63	2.63	2.83	2.65	2.68	2.94 (27)
O4···Lys96 NZ	3.45	3.45	3.39	3.47	3.48	3.47	3.36	3.42	3.47	3.50	3.45 (4)
O4···Arg128 NH2$^{\rm i}$	2.52	2.46	2.58	2.56	2.58	2.63	2.57	2.55	2.59	2.52	2.56 (4)
O4···His15 O	3.66	3.57	3.56	3.64	3.55	3.79	3.73	3.59	3.68	3.61	3.64 (7)
O6···His15 O	3.40	3.36	3.32	3.22	3.27	3.30	3.38	3.35	3.34	3.34	3.33 (5)
O6···His15 ND1	3.27	3.27	3.33	3.41	3.29	3.39	3.39	3.35	3.33	3.32	3.34 (5)
O6···Asn93 ND2	2.83	2.75	2.81	2.86	2.90	2.88	2.80	2.81	2.81	2.78	2.82 (4)
O6···Lys96 NZ	3.60	3.61	3.46	3.34	3.54	3.38	3.24	3.44	3.43	3.57	3.46 (11)
O1···water 1173	—	—	3.30	—	2.71	—	—	3.30	—	—	3.10 (28)
O1···water 1179	—	—	—	—	—	—	—	—	—	3.37	—
O3···water 1179	—	—	—	—	—	—	—	—	—	3.21	—

Symmetry code: (i) $-y, -x, \frac{1}{2} - z$.

ment of the structure were carried out with other programs from the *CCP*4 suite. Unit-cell parameters, data-collection details and statistics are given in Table 2.

The PDB entry 1hel (Wilson *et al.*, 1992) without solvent molecules was used as the starting model for structure determination. For preliminary refinement, the conjugate-gradient algorithm (CGLS) was used as implemented in the program *SHELX*97 (Sheldrick & Schneider, 1997). After determination of the whole structure of lysozyme and the positions of periodates and nearly all waters, the full-matrix (least-squares) refinement was also used. The parameters of this refinement are given in Table 3 in the 'input' column.

Although the reliability parameters for the preliminary refinement appear to be satisfactory for the resolution, it was impossible to determine the individual iodine–oxygen distances in the periodate structures. The structure was therefore re-refined using the *HipHop* refinement method (Ondráček, 2005; Ondráček & Mesters, 2004; http://www.img.cas.cz/hiphop). A paper with further details of *HipHop* is currently in preparation (Ondráček *et al.*, 2005).

Briefly, in the 'Hip' step 150 maxima in the difference Fourier synthesis were filled with water molecules provided they fulfilled the geometrical criteria for water molecules. The 'Hop' steps were run five times and waters not fulfilling the ball shape or criteria of density greater than 0.09, 0.13, 0.18, 0.23 and 0.25 e^- Å^{-3} were gradually removed. Every Hip and every Hop step was followed by 1000 cycles of CGLS in *SHELXH*.

During the preliminary *HipHop* cycles the structure was inspected and some side chains were reoriented manually. In this way the static disorder in Ile55, Asn77 and Lys97 and the different side-chain orientations of Leu83 and Val109 were found. After stabilization of the model, the final ten cycles were run and statistics of *HipHop* refinement were calculated.

Table 3 shows the final refinement results of the final ten *HipHop* cycles and their mean values. In the column 'Average structure' crystallographic (vector structure factors) averages of all ten structures are given. In the multi-conformer PDB entry 1hc0 the single structures are labelled by different MODEL numbers.

2.3. Structure comparisons

Three-dimensional structure superpositions were carried out using the program *LSQKAB* (Collaborative Computational Project, Number 4, 1994). Since the *HipHop* refinement protocol yields an ensemble of ten models describing the refined structure, the values for the superpositions given are average values over all ten structures.

3. Results

3.1. The overall structure of lysozyme

The global refinement parameters (Table 3) are comparable in quality with the parameters for other published structures of native hen egg-white tetragonal lysozyme (Table 9). The

Table 5
Bond lengths (Å) in periodate I_B and possible hydrogen bonds (Å).

The bond lengths in the periodate I_B correspond to the structure $[I(HO)_5O]$.

Refinement	1	2	3	4	5	6	7	8	9	10	Mean
I—O1	1.59	1.61	1.56	1.60	1.64	1.63	1.66	1.72	1.77	1.82	1.66 (8)
I—O2	1.81	1.89	1.88	1.85	1.88	1.84	1.83	1.84	1.87	1.84	1.85 (2)
I—O3	1.92	1.98	2.01	1.96	1.89	1.97	1.90	1.95	1.92	1.96	1.95 (4)
I—O4	1.99	1.95	2.00	1.98	1.90	2.07	2.02	1.98	1.99	2.02	1.99 (4)
I—O5	1.97	2.02	1.99	2.01	2.02	1.97	2.06	2.07	2.04	2.06	2.02 (3)
I—O6	1.88	2.05	2.10	1.96	1.91	1.84	1.90	1.99	1.93	1.98	1.95 (8)
O1···Glu7 OE2^{i}	2.80	2.75	2.72	2.75	2.76	2.75	2.65	2.58	2.59	2.48	2.68 (10)
O1···Arg14 NH2	2.49	2.49	2.49	2.49	2.49	2.50	2.49	2.49	2.50	2.50	2.49 (1)
O2···Ile88 N	2.83	2.84	2.86	2.91	2.90	2.91	2.92	2.98	2.94	3.00	2.91 (5)
O2···Ser86 O	3.39	3.32	3.35	3.36	3.32	3.33	3.33	3.30	3.36	3.33	3.34 (3)
O3···His5 NE2	3.70	3.65	3.59	3.72	3.75	3.74	3.74	3.71	3.73	3.62	3.70 (5)
O4···Glu7 OE2^{i}	3.54	3.44	3.38	3.46	3.54	3.58	3.51	3.48	3.49	3.42	3.48 (6)
O4···Asp87 OD1	3.69	3.69	3.64	3.65	3.69	3.64	3.59	3.64	3.61	3.61	3.65 (3)
O4···I_F O2	2.41	2.38	2.53	2.49	2.56	2.37	2.46	2.51	2.44	2.49	2.46 (6)
O5···Arg14 NH2	3.20	3.29	3.32	3.22	3.27	3.24	3.35	3.37	3.31	3.26	3.28 (5)
O5···His15 NE2	2.79	2.84	2.90	2.83	2.78	2.82	2.87	2.79	2.77	2.70	2.81 (5)
O5···Asp87 OD1	2.96	2.78	2.69	2.80	2.83	2.93	2.87	2.89	2.90	2.90	2.86 (8)
O5···Ile88 N	3.48	3.47	3.49	3.54	3.50	3.57	3.43	3.51	3.61	3.60	3.52 (6)
O1···water 1080	2.75	2.84	2.85	2.84	2.92	2.93	2.99	3.02	3.02	3.16	2.93 (11)
O1···water 1064^{i}	3.45	3.54	3.49	3.51	3.49	3.47	3.51	3.52	3.51	3.51	3.50 (2)
O2···water 1181	2.90	2.78	2.79	2.73	2.66	2.73	2.78	2.75	2.73	2.72	2.76 (6)
O3···water 1080	2.35	2.33	2.32	2.30	2.53	2.49	2.60	2.53	2.58	2.61	2.46 (12)
O6···water 1064	2.83	2.47	2.53	2.63	2.82	2.86	2.70	2.63	2.71	2.65	2.68 (12)
O6···water 1064^{i}	3.09	3.29	3.29	3.19	3.16	3.09	3.17	3.11	3.26	3.17	3.18 (7)
O6···water 1181	2.67	2.59	2.51	2.54	2.56	2.60	2.78	2.86	2.74	2.56	2.64 (11)

Symmetry code: (i) $-y, -x, \frac{1}{2} - z$.

Table 6
Bond lengths (Å) in periodate_C and possible hydrogen bonds (Å).

The bond lengths in the periodate correspond to the structure $[I(HO)_3O_3]^{2-}$.

Refinement	1	2	3	4	5	6	7	8	9	10	Mean
I—O1	1.68	1.60	1.64	1.64	1.64	1.61	1.59	1.56	1.65	1.56	1.62 (4)
I—O2	1.56	1.40	1.45	1.46	1.49	1.51	1.48	1.38	1.45	1.49	1.47 (5)
I—O3	2.03	2.03	2.04	2.06	2.04	2.07	2.02	2.06	2.07	2.07	2.05 (2)
I—O4	1.92	1.87	1.97	1.85	1.97	1.93	1.83	1.98	1.92	1.95	1.92 (5)
I—O5	1.66	1.71	1.64	1.58	1.55	1.41	1.71	1.67	1.46	1.67	1.61 (10)
I—O6	2.07	2.13	2.02	2.08	2.00	2.11	2.19	2.08	2.05	2.08	2.08 (5)
O1···Arg68 NH1^{ii}	3.17	3.17	3.14	3.23	3.17	3.12	3.16	3.16	3.10	3.14	3.16 (3)
O3···Glu35 O	2.65	2.66	2.72	2.66	2.68	2.62	2.58	2.57	2.56	2.56	2.63 (5)
O3···Asn44 ND2	2.76	2.74	2.76	2.70	2.71	2.72	2.79	2.75	2.75	2.71	2.74 (3)
O5···Asn44 OD1	3.30	3.24	3.36	3.45	3.50	3.54	3.30	3.31	3.51	3.39	3.39 (10)
O5···Asn44 ND2	2.88	2.87	2.89	2.89	3.04	2.94	2.87	2.85	2.91	2.98	2.91 (6)
O5···Arg45 NH2^{ii}	3.00	2.96	2.99	3.00	3.06	3.19	2.89	2.99	3.11	2.99	3.02 (8)
O5···Arg68 NH1^{ii}	3.61	3.51	3.43	3.63	3.56	3.69	3.58	3.50	3.54	3.57	3.56 (7)
O6···Ser36 O	3.53	3.37	3.37	3.32	3.39	3.26	3.13	3.21	3.27	3.22	3.31 (11)
O6···Asn37 N	3.45	3.33	3.40	3.37	3.38	3.31	3.24	3.34	3.34	3.34	3.35 (5)
O1···water 1024	2.66	2.81	2.65	2.63	2.68	2.77	2.78	2.82	2.71	2.94	2.75 (9)
O2···water 1039	3.18	3.30	3.17	3.23	3.37	3.29	3.55	3.40	3.40	3.34	3.32 (11)
O2···water 1094	3.57	3.56	3.49	3.57	3.30	3.29	3.45	3.69	3.35	3.40	3.47 (13)
O3···water 1094	3.94	3.93	3.81	3.90	3.64	3.54	3.55	3.94	3.60	3.55	3.74 (17)
O5···water 1067	2.60	2.67	2.71	2.68	2.63	2.73	2.50	2.59	2.80	2.54	2.65 (9)
O6···water 1039	2.75	2.83	2.85	2.98	3.00	3.12	3.33	3.19	3.10	3.11	3.03 (17)
O6···water 1024	3.69	3.59	3.48	3.49	3.54	3.50	3.41	3.49	3.41	3.48	3.51 (8)

Symmetry code: (ii) $y, x, 1 - z$.

three-dimensional structure of lysozyme is very similar to other published hen egg-white lysozyme structures. A comparison of the ten models generated by *HipHop* refinement yielded r.m.s. deviations in the range of the expected coordinate error of the structure (Table 9).

The static disorder of Arg14 and the dynamic disorder of Trp62 will be discussed in detail in the paper describing the basis of *HipHop* refinement (Ondráček *et al.*, 2005). Other cases of disorder were observed for Lys97, Asn103, Arg21 and Arg125. These and the side-chain disorder observed for Ile55, Asn77 and Lys97 were not present in PDB entry 1hel, which was used as the starting model for refinement. Furthermore, PDB entry 1hel significantly differs from our refined model in the side-chain orientations of Lys13, Arg21, Asn59, Arg61, Trp62, Ser86, Lys97, Asn103 and Arg125.

In PDB entry 1dpx, the disorder observed for Arg14 (atoms NE, CZ, NH1 and NH2) differs from that found in our final ten models (only the CG and CD atoms are disordered; the remaining atom positions are again similar). The disorder for Lys1 NZ observed in 1dpx was not found in our models. The disorder of Asn77 determined in 1dpx is practically identical to that found in the first steps of *HipHop* refinement.

No disordered residues were observed in 1jis and 1lz8. In 1jis, the orientation of Arg14 is identical to that found in one set of our models, the orientations of Ile55, Asn77 and Lys97 are similar and the orientations of Arg21, Asn103 and Arg125 are different. In 1lz8, the orientations of Ile55 and Asn77 are identical with that found in one set of our models, the orientations of Arg21, Lys97 and Arg125 are similar and the orientations of Arg14 and Asn103 are again different.

More interesting is the comparison with the high-resolution structure 1iee (resolution 0.94 Å). The disorder observed for Ile55, Asn77 and Lys97 found and refined during *HipHop* refinement in our model is almost identical to that found in 1iee. The side-chain orientations for Asn59, Ser86 and Lys97 in our models are identical to one of the multiple side-chain conformations in 1iee, while the side-chain orientations for Lys13, Arg14, Arg21, Arg61, Trp62, Asn103, Arg125 and Arg128 differ between the two structures.

With respect to the water molecules, a total of 291 water molecules were found in different positions in the ten models. The average number of waters per model is 177 (5) (Table 3). The distribution of water molecules is as follows: 112 water molecules occurred in all ten models, 18 waters in nine, ten in

Table 7
Bond lengths (Å) in periodate_D and possible hydrogen bonds (Å).

The bond lengths in the periodate correspond to the structure $[I(HO)_6]^+$.

Refinement	1	2	3	4	5	6	7	8	9	10	Mean
I—O1	1.84	1.80	1.79	1.77	1.77	1.78	1.82	1.77	1.78	1.70	1.78 (4)
I—O2	1.99	2.01	1.94	1.83	1.96	1.88	1.96	1.84	1.93	1.82	1.92 (7)
I—O3	1.93	1.89	1.86	1.91	1.82	1.83	1.83	1.75	1.82	1.84	1.85 (5)
I—O4	2.01	2.03	2.04	2.06	2.09	2.08	2.07	2.00	2.04	2.02	2.04 (3)
I—O5	1.80	1.88	1.85	1.86	1.92	1.77	1.95	1.90	1.91	1.91	1.88 (5)
I—O6	1.74	1.91	1.89	1.84	1.91	1.85	1.89	1.95	1.96	1.86	1.88 (6)
O1···Glu35 OE1	2.59	2.62	2.68	2.68	2.65	2.65	2.62	2.67	2.69	2.76	2.66 (4)
O1···Asp52 OD2	3.14	3.15	3.12	3.17	3.19	3.19	3.19	3.19	3.14	3.13	3.16 (3)
O2···Ala107 O	3.41	3.34	3.39	3.44	3.34	3.39	3.35	3.42	3.31	3.50	3.39 (5)
O2···Val109 N	2.64	2.64	2.69	2.79	2.74	2.75	2.71	2.75	2.69	2.74	2.71 (5)
O3···Glu35 OE1	3.11	3.14	3.09	3.14	3.05	3.10	3.10	3.05	3.07	3.12	3.10 (3)
O3···Glu35 OE2	2.30	2.30	2.30	2.30	2.29	2.30	2.29	2.30	2.30	2.32	2.30 (1)
O3···Ala109 N	2.90	2.84	2.89	2.79	3.00	2.91	2.96	3.06	2.99	2.95	2.93 (8)
O3···Ala110 N	2.89	2.92	2.98	2.91	2.98	2.98	2.97	3.07	3.01	2.98	2.97 (5)
O4···Asp52 OD2	2.32	2.30	2.30	2.29	2.29	2.30	2.30	2.30	2.29	2.30	2.30 (1)
O5···Glu35 OE1	3.30	3.36	3.41	3.34	3.42	3.32	3.41	3.37	3.40	3.45	3.38 (5)
O5···Glu35 OE2	3.11	3.10	3.19	3.13	3.20	3.12	3.18	3.17	3.07	3.14	3.14 (4)
O5···Gln57 O	3.20	3.10	3.10	3.07	3.05	3.19	3.03	3.03	3.05	3.05	3.09 (6)
O5···Asp52 OD2	3.63	3.61	3.53	3.54	3.55	3.54	3.52	3.51	3.50	3.50	3.54 (4)
O1···water 1007	2.99	3.08	3.02	3.01	3.07	3.01	3.00	3.10	3.10	3.14	3.05 (5)
O1···water 1146	3.16	—	—	—	—	—	—	—	—	—	3.16
O1···water 1168	—	—	—	—	—	3.01	3.08	3.13	3.20	3.21	3.13 (7)
O2···water 1169	—	2.51	2.63	2.66	2.49	—	—	—	—	—	2.57 (7)
O2···water 1184	—	—	—	—	—	2.63	2.61	2.64	2.61	2.63	2.62 (1)
O3···water 1146	3.41	—	—	—	—	—	—	—	—	—	3.41
O4···water 1059	2.29	2.30	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29 (1)
O4···water 1181	—	—	—	—	—	2.25	—	—	—	—	2.25
O4···water 1184	—	—	—	—	—	3.06	3.12	3.11	3.06	3.18	3.11 (4)
O4···water 1169	—	2.99	3.10	3.12	3.00	—	—	—	—	—	3.05 (6)
O4···water 1169	—	—	—	—	—	3.27	3.22	3.21	3.33	3.29	3.26 (4)
O6···water 1146	3.33	—	—	—	—	—	—	—	—	—	3.33
O6···water 1168	—	2.89	2.67	2.78	—	—	—	—	—	—	2.78 (9)
O6···water 1169	—	—	—	—	—	2.89	2.94	2.89	3.09	2.98	2.96 (7)
O6···water 1168	—	—	—	—	—	3.37	3.33	3.33	3.40	3.21	3.33 (6)

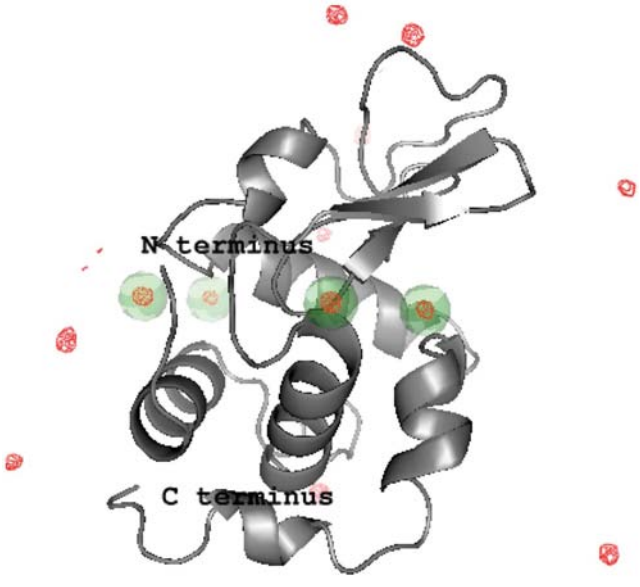

Figure 1
Anomalous difference electron-density map (contoured at 3 standard deviations above the mean value) and regular geometric arrangement of periodates (green spheres). The additional maxima are from symmetry-related molecules. The figure was produced using the program *PyMol* (DeLano, 2002).

eight, 11 in seven, nine in six, 12 in five, 12 in four, 17 in three, 26 in two and 64 in only one. The stable water-molecule content and the limited water capacity is in agreement with our other results.

3.2. The structure of the periodate ions

Nine possible periodate positions, four maxima at the sulfur positions of Cys6, Cys76, Cys80 and Cys115 and one for a chloride corresponding to the chloride 205 in 1lz8 were found on the basis of the anomalous diffraction (Fig. 1). Four periodates exhibited higher occupancy than 0.5. These were termed periodates I_A, I_B, I_C and I_D. After *HipHop* refinement and on the basis of the static evaluation it was possible to study their structures in detail.

The bond lengths in periodate I_A (occupancy 0.6) and the possible hydrogen bonds to protein atoms and water molecules are shown in Fig. 2(*a*) and the distances are given in Table 4. The values show that two of the iodine–oxygen bonds seem to be significantly shorter then the other four. Thus, this structure corresponds to the formula $[I(OH)_4O_2]^-$, with two short I—O bonds and four longer I—OH bonds. One of the negative charges of the periodate is in the neighbourhood of the positively charged Arg128 of a symmetry-related molecule described by the symmetry operator $-y, -x, \frac{1}{2} - z$.

The bond lengths of periodate I_B (occupancy 0.7) and possible hydrogen bonds are summarized in Table 5 and shown in Fig. 2(*b*). Only one bond length in this periodate differs statistically from the others. Thus, this periodate corresponds to the formula $[I(OH)_5O]$. This formula is also supported by its neighbourhood: the two negatively charged side chains Glu7 (position $-y, -x, \frac{1}{2} - z$) and Asp87, and one positively charged side chain Arg14 are close to I_B. The interaction of Arg14 with I_B may explain the static disorder observed for Arg14. The side chain of Arg14 exhibits two different orientations with relative frequencies of 6:4 in the ten final models.

The geometry and interactions of periodate I_C (occupancy 0.5) are given in Table 6 and Fig. 2(*c*). The bond lengths of this periodate correspond to the formula $[I(OH)_3O_3]^{2-}$. The two negative charges of this periodate are stabilized by Arg45 and Arg68 (both in position $y, z, 1 - z$).

The structure of periodate I_D (occupancy 0.6) is described in Table 7 and Fig. 2(*d*). The bond lengths correspond to the formula $[I(OH)_6]^+$. The positive charge of the periodate is

stabilized by the presence of Glu35 and Asp52 in its neighbourhood.

The I—O bond lengths given in Tables 4–7 are in good agreement with the published mean value of 1.803 Å for the distance $I^{7+}—O^{2-}$ (Wilson & Prince, 1999).

3.3. Exploitation of anomalous signal for structure solution

Our data measured were more than good enough for the determination of periodate positions, the verification of these positions on the basis of the anomalous difference map and the determination of the periodate oxygen positions. However, attempts to use the anomalous signal for phase determination were unsuccessful. No easily interpretable map was obtained by the SAD method using the programs *MLPHARE* and *DM* (Collaborative Computational Project, Number 4, 1994). However, it is planned to reperform the experiment more carefully and to enhance the anomalous signal by carrying out data collection at longer wavelengths (Mueller-Dieckmann *et al.*, 2004, 2005). This will be the subject of a separate study.

3.4. Geometric arrangement of the four periodates with high occupancy

Selected distances, angles and the plane through the I atoms of the periodates I_A, I_B, I_C and I_D are described in Table 8. The I atoms of I_A, I_B, I_C and I_D form a deformed rectangle *ABCD* (Fig. 1). The deformation occurs because of the deviation of I_A of about 3 Å in the direction *DA*. The angles *BCD* and *CDA* may be considered to be regular, the sides *AB* and *CD* as equivalent and the side *BC* as double this value.

The regular arrangement of the periodate ions is in good agreement with the unit-cell parameters of several minerals and may help to explain the phenomenon of epitaxy of lysozyme on the minerals lepidolite and fluorite (McPherson & Shlichta, 1988). The only commonly accepted necessary condition for epitaxy of one compound on another is the presence of the same *d* value in both compounds. In the case of proteins, the *d* value of one compound can be changed by the distance of possible binding points, represented by periodate positions. The average value of 10.23 Å obtained from *AB*, *BD* and *CD* used for this evaluation is in excellent agreement with the unit-cell parameters c = 10.13 Å in lepidolite $C2/m$ (Bernard & Rost, 1992), c = 20.20 Å in lepidolite $C2/c$ or a = 5.20 Å in lepidolite $P3_112$. The agreement is evident for fluorite $P3_121$ with a = 4.913 Å or for fluorite $P4_12_12$ with a = 4.97 Å.

The epitaxy of proteins on minerals has been described (McPherson & Shlichta, 1988) for proteins such as concanavalin B, catalase and canavalin, but a similar analysis for these proteins is so far lacking.

3.5. Comparison of periodate positions with positions of other ions

Various ions bound to lysozyme are often found in structures deposited in the PDB. The positions of chlorides in three selected structures compared with the positions of the periodate ions in our model yields the following results: chloride ions 201, 202 and

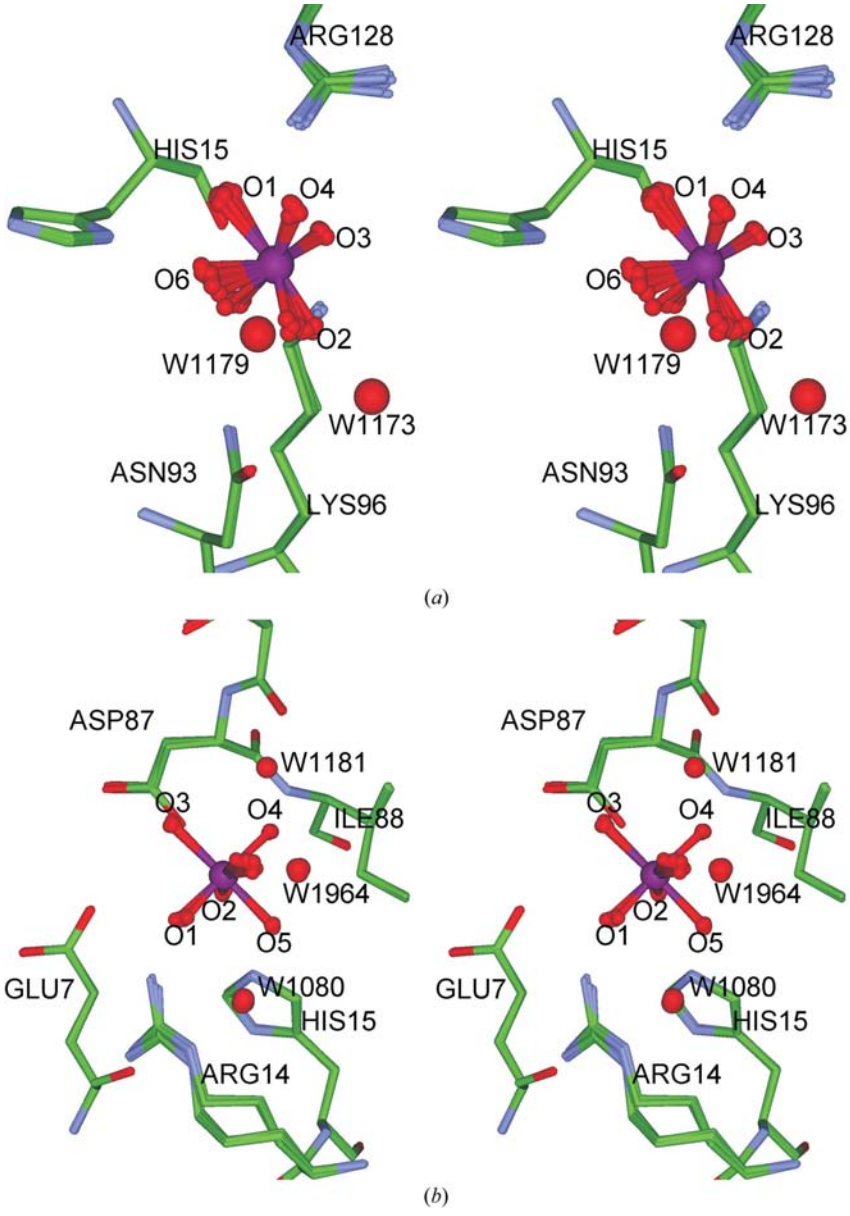

Figure 2
Stereoscopic views of the four periodate ions bound to lysozyme exhibiting ocupancy >0.5. The figure was prepared using the programme *Viewer Lite* 4.2. (*a*) Binding position of periodate I_A. (*b*) Binding position of periodate I_B. (*c*) Binding position of periodate I_C. (*d*) Binding position of periodate I_D.

Table 8
Geometric parameters of the relative arrangement of the periodate ions I_A–I_D on the surface of lysozyme.

Refinement	1	2	3	4	5	6	7	8	9	10	Mean
Distances (Å)											
I_A···I_B	10.00	10.01	10.03	10.01	10.01	9.99	9.99	9.96	9.95	9.95	9.99 (3)
I_B···I_C	20.50	20.50	20.50	20.48	20.50	20.48	20.49	20.50	20.49	20.50	20.49 (1)
I_C···I_D	10.41	10.41	10.40	10.39	10.39	10.39	10.41	10.42	10.42	10.40	10.40 (1)
I_D···I_A	22.94	22.91	22.91	22.91	22.81	22.92	22.92	22.92	22.90	22.91	22.91 (3)
Angles (°)											
I_A···I_B···I_C	107.94	107.78	107.73	107.78	107.63	107.85	107.90	107.94	107.90	107.81	107.83 (9)
I_B···I_C···I_D	86.37	86.34	86.40	86.47	86.36	86.43	86.32	86.32	86.33	86.37	86.37 (5)
I_C···I_D···I_A	91.43	91.50	91.50	91.43	91.54	91.40	91.47	91.37	91.31	91.35	91.43 (7)
I_D···I_A···I_B	74.24	74.36	74.35	74.30	74.44	74.29	74.27	74.35	74.44	74.45	74.35 (7)
Distances from plane (Å)											
I_A	0.061	0.071	0.057	0.077	0.071	0.072	0.074	0.080	0.074	0.070	0.071 (7)
I_B	0.069	0.079	0.064	0.086	0.079	0.081	0.083	0.090	0.082	0.078	0.079 (7)
I_C	0.064	0.073	0.059	0.080	0.073	0.075	0.077	0.083	0.076	0.072	0.073 (7)
I_D	0.056	0.065	0.052	0.071	0.065	0.070	0.068	0.073	0.067	0.064	0.065 (6)
R.m.s. deviation of fitted atoms	0.063	0.072	0.058	0.079	0.072	0.073	0.076	0.082	0.075	0.071	0.072 (7)

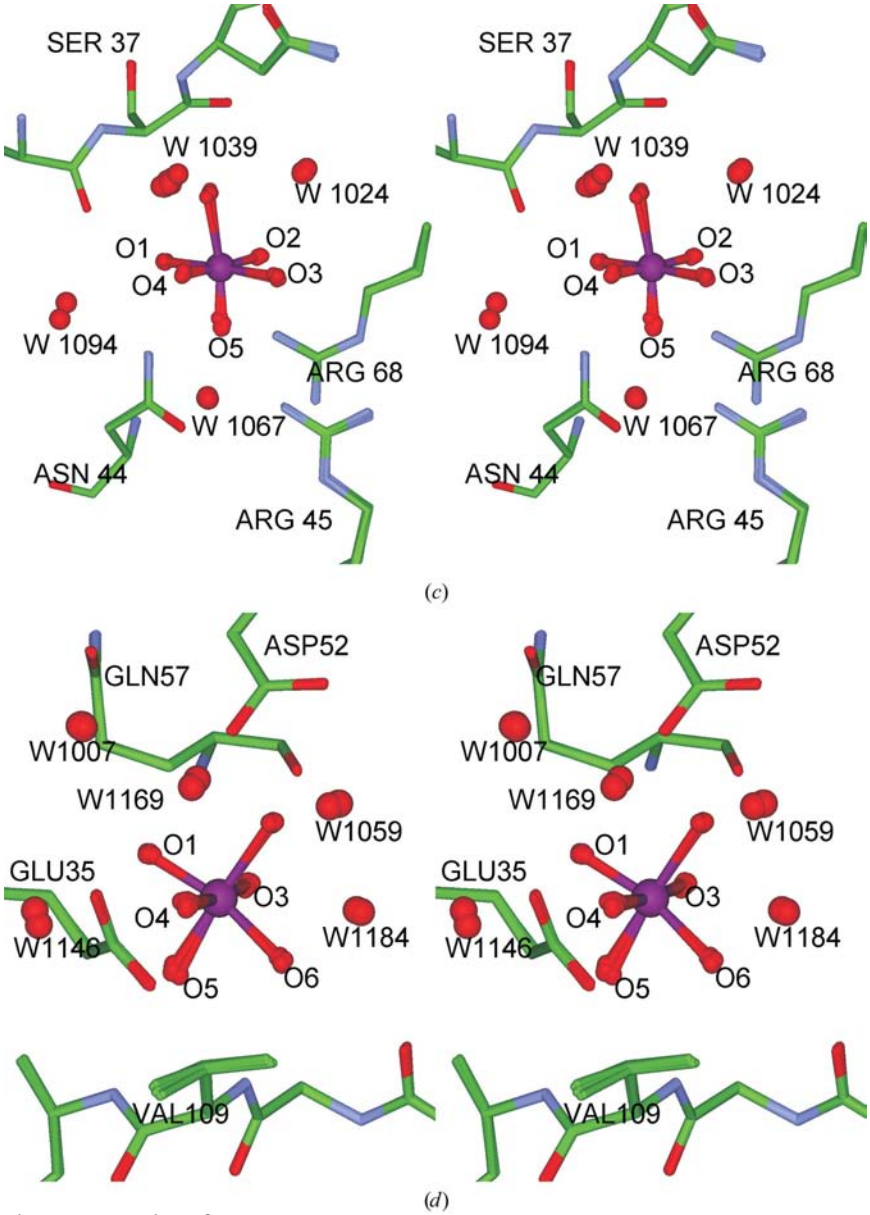

Figure 2 (continued)

205 found in PDB entry 1lz8 (Dauter *et al.*, 1999) correspond to the positions of water molecules in our structure, with deviations of 1.05, 1.22 and 1.57 Å, respectively. No maxima in the anomalous difference Fourier map were found for chlorines 203, 206, 207 and 208. Chlorine 204 is situated at a distance of 2.45 Å from periodate I_B bound to His15. Both chlorides in PDB entry 1dpx (Weiss *et al.*, 2000) are replaced by water molecules in our crystal. Chlorides 201 and 202 found in PDB entry 1iee (Sauter et *al.*, 2001) are again replaced by waters in our crystal, chloride 203 is located 1.13 Å from periodate I_H (occupancy 0.4) and chloride 204 1.41 Å from periodate I_B. Chloride 205 superposes with a chloride in our crystal structure, with a difference in position of 1.67 Å.

The ion positions in two other lysozyme structures determined under xenon and krypton pressure (PDB entries 1c10 and 1qtk; Prangé *et al.*, 1998) were also compared with our model (Table 9). In 1c10, the chlorides 160, 161 and 164 correspond to water molecules in our model, chloride 162 approximately corresponds to the position of the low-occupancy periodate I_H (distance 2.09 Å), chloride 163 roughly superimposes with periodate I_B (distance 2.69 Å) and chloride 165 is 1.54 Å from the position of the chloride in our model. In 1qtk, chlorides 160 and 161 correspond to water molecules in our model and the position of chloride 162 corresponds to the position of the low-occupancy periodate I_H (distance 2.01 Å). As might be expected, all xenon and krypton positions are far from periodate positions.

Table 9
Published structures of native hen egg-white tetragonal lysozyme and average values of r.m.s. deviations from our models (Å).

PDB code	Structure	Resolution (Å)	R (%)	R_{free} (%)	R.m.s.d. (Å)	Reference
1dpx	177 waters, 2 chlorides	1.65	18.7	24.6	0.223 (5)	Weiss *et al.* (2000)
1iee	233 waters, 1 sodium, 5 chlorides	0.94	12.3	15.1	0.271 (6)	Sauter *et al.* (2001)
1jis	141 waters	1.90	19.0	23.4	0.313 (3)	Datta *et al.* (2001)
1lz8	224 waters, 1 sodium, 8 chlorides	1.53	22	31	0.343 (3)	Dauter *et al.* (1999)
1hel	319 waters	1.70	15.2	Not given	0.279 (4)	Wilson *et al.* (1992)
1c10	68 waters, 1 sodium, 6 chlorides and 3 xenons	2.03	17.2	22	—	Prangé *et al.* (1998)
1qtk	77 waters, 1 sodium, 3 chlorides and 1 krypton	2.03	17.3	21.6	—	Prangé *et al.* (1998)
1gwd	103 waters, 1 sodium, 7 chlorides and 5 iodides	1.77	16.8	21.1	—	Evans & Bricogne (2002)

The periodate positions were also compared with ions found in the tri-iodide derivative of tetragonal lysozyme (Evans & Bricogne, 2002; Table 9). Tri-iodide is formed by iodides A1130-A1147-A1130, with the I—I distance being 2.72 Å (A1147 is placed on a special position). Tri-iodide, iodides A1131 and A1133, and chlorides A1137, A1139, A1142 and A1143 are distant from any periodate positions in our model. The iodide A1132 position approximately corresponds to the position of periodate I_E (occupancy 0.21); here, the side chain of the nearby Asp18 exhibits a different orientation. The chlorides A1138 and A1140 practically superimpose with periodate I_H and periodate I_B, with differences in position of 2.01 and 2.41 Å, respectively. The chloride A1141 corresponds to the chloride ion in our model, with a distance difference of 1.39 Å.

The structure of lysozyme in complex with 2-methyl-2,4-pentanediol and 2-amino-2-hydroxymethyl-propane-1,3-diol (Weiss *et al.*, 2000) and in complex with tri-*N*-acetylchitotriose (Cheetham *et al.*, 1992) as well as the structural effects of other monovalent anions such as *para*-toluenesulfonate, NO_3^-, SCN^- and I^- on polymorphic lysozyme crystals (Vaney *et al.*, 2001) have also been studied. No similarities between the binding of these ions and periodates in tetragonal HEW lysozyme were found.

The characteristics of periodate-binding positions are determined by its octahedral shape, size and charge flexibility. Periodate can change its charge and I—O bond lengths and thus adjust itself to the lysozyme surface. Surprisingly, periodate oxidizes neither cysteines or methionines nor any other parts of the lysozyme molecule. No similar structural flexibility of inorganic oxoanions such as bromate, selenate, tellurate *etc.* was found within lysozyme crystals (Ondráček, unpublished results).

4. Conclusions

The soaking of tetragonal lysozyme crystals in periodate solutions led to the binding of periodate in four highly occupied positions. Standard refinement of the structure did not yield the positions of periodate O atoms. Thus, the structures of the periodate ions could not be determined exactly. A standard single model is not capable of describing every significant maximum in the difference Fourier map. However, the oxygen positions in all four periodate ions could be determined using the novel *HipHop* refinement procedure.

The structures of the four bound periodates differ and depend to some extent on the binding position of the periodate on the surface of the lysozyme molecule. The periodate anion is able to accept or release H^+ and balance a partial charge within lysozyme crystal. Thus, the structure of the periodate depends on the local pH in the binding position. Three of four periodates are bound to two neighbouring symmetry-related lysozyme molecules.

Four periodates form a regular rectangular arrangement on the surface of lysozyme. A similar arrangement was not found after soaking tetragonal lysozyme in other heavy-atom oxoanion solutions such as tellurate, bromate, selenate, vanadate, tungstate or perrhenate or in the structure of other complexes of heavy-atom oxoanions with proteins listed in Table 1. The reason can be found in the octahedral symmetry of periodate and its structural flexibility as presented in this article. There is a relationship between the dimensions of this rectangle and the unit-cell parameters of some inorganic minerals that might enable epitaxy of lysozyme on these minerals.

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