Protein crystallization in microgravity

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Abstract A space experiment involving protein crystallization was conducted in a microgravity environment using the space shuttle "Endeavour" of STS-47, on a 9-day mission from September 12th to 20th in 1992. The crystallization was carried out according to a batch method, and 5 proteins were selected as flight samples for crystallization. Two of these proteins : hen egg-white lysozyme and ω -amino acid: pyruvate aminotransferase from *Pseudomonas* sp. F-126, were obtained as single crystals of good diffraction quality. Since 1992 we have carried out several space experiments for protein crystallization aboard space shuttles and the space station MIR. Our experimental results obtained mainly from hen egg-white lysozyme are described below, focusing on the effects of microgravity on protein crystal growth.

Introduction

When using protein engineering techniques for studies on enzyme reaction mechanisms and the modification of enzyme functions, we need to use X-ray crystallography to obtain essential information about the three-dimensional structure of proteins. However, X-ray crystallographic studies cannot start without the preparation of single crystals of good diffraction quality. Since the inception of synchrotron radiation, X-ray crystallographic studies have made rapid progress, and recently the dynamic structure of the whole protein molecule has been intensively studied using the technique of time-resolved X-ray crystallography (Cruickshank, D.W.J. *et al.* 1992). The first requisite of this method is preparing single crystals of lower mosaicity. The accuracy of the three dimensional structure determined by X-ray crystallographic analysis is dependent upon the accuracy of the reflection data. Therefore, the quality of the protein single crystals is very important. On the ground, as crystal nuclei grow larger, the increase in weight causes sedimentation of the crystals, and crystals sometimes pile up on top of each other at the bottom of the crystallization apparatus and finally become deformed (Aibara, S., and Morita, Y. 1992). Moreover, there is no general theory for preparation of protein single crystals of good crystallinity, in which protein molecules are well ordered. Consequently, there is a bottleneck in this research field; we are at present unable to prepare protein single crystals of good diffraction quality, and it is extremely important to establish both a practical method of preparing protein single crystals and a theory of protein crystal growth.

In 1983, Professor Littke of Freiburg University first carried out a space experiment involving the crystallization of hen egg-white lysozyme and β -galactosidase from *E.*

coli aboard a space shuttle (STS-9), which was reported to have succeeded in preparing larger crystals than ground-grown crystals (Littke, W. and John, C. 1983). Since 1985, Professor Bugg and Professor DeLucas of the University of Alabama at Birmingham have headed a team which also conducted programmatic space experiments on protein crystallization using space shuttles (DeLucas, L.J. *et al.* 1989), and have now obtained fruitful results. In Japan, a space experiment involving protein crystallization was carried out in 1992 using the space shuttle "Endeavour" (Aibara, S., and Morita, Y. 1993). In this mission the payload specialist Dr. Mohri of NASDA conducted a space experiment on the Japanese theme FMPT. Protein crystallization in a microgravity environment is distinctly different from that on the ground. As the effects of microgravity on protein crystal growth have been brought to light, it has been revealed that sedimentation of protein crystallites does not occur, and, as results show, crystal nuclei grow larger being suspended. Furthermore, in a microgravity environment, the transport of protein molecules through the solution is limited by diffusion control so that crystal growth, including both the nucleation and growth stages, proceeds at a slower rate. These conditions of a microgravity environment in space are suitable for protein crystallization, and this is one of the reasons why protein single crystals of good crystallinity are obtained in space. Moreover, if we are going to use a microgravity field in a space environment for protein crystallization, we should not simply aim to produce single crystals, but should make efforts to prepare single crystals of better diffraction quality. It is therefore necessary to improve crystallization apparatus for space experiments and to produce the appropriate crystallization devices.

In this paper, we will mainly present the results of FMPT (STS-47), and will also refer to our more recent results in protein crystallization in space experiments.

Materials and Methods

Chemicals

Hen egg-white lysozyme (3 x crystallized, dialyzed and lyophilized; L-6876) was purchased from Sigma Chemical

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Co. The other chemical reagents used were also purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Crystallization

Crystallization of hen egg-white lysozyme was achieved under two different sets of conditions in space: one condition used 40 mM acetate buffer, pH4.7, with sodium chloride as the precipitating agent, and the other used 25 mM acetate buffer, pH4.5, with sodium nitrate as the precipitating agent. In all space shuttle experiments except STS-47 and the space station MIR, vapor diffusion apparatuses were used; the enzyme crystallization vessel was employed in STS-47, and the Biokrist model 4 in the space station MIR.

Results and Discussion

FMPT mission

At the beginning of 1980's we planned a space experiment and started preliminary experiments for selection of the protein samples and optimization of the crystallization conditions on the ground. In an FMPT mission of STS-47 in 1992, five proteins and enzymes were finally selected for the crystallization samples: hen egg-white lysozyme (pH4.5 & pH4.7), horse skeletal muscle myoglobin, *Pseudomonas* sp. F-126 ω -amino acid: pyruvate aminotransferase, and *Rhizopus niveus* lipase (Aibara, S. and Morita, Y. 1993), and we made use of an

enzyme crystallization vessel made of polycarbonate which we had produced according to the principle of the batch method. Four of the five protein samples were obtained as crystals in the FMPT mission, of which hen egg-white lysozyme at pH4.5 and ω -amino acid: pyruvate aminotransferase grew large single crystals of good diffraction quality. Since 1992 we have carried out space experiments using space shuttles and the space station MIR at 20°C in a microgravity as shown in Table 1. Crystallization of hen egg-white lysozyme has also been carried out on each mission under the two different sets of conditions: 40 mM sodium acetate at pH 4.7 and 25 mM sodium acetate

Fig.1. Hen egg-white lysozyme monoclinic crystals grown on the ground

Table 1 Protein samples for space crystallization experiments

				protein	method	crystal
SPACE SHUTTLE						
STS-42	IML-1	(1992)	Discovery	Hen egg-white lysozyme (pH4.5 & pH4.7)	VDA	Yes
STS-47	Spacelab-J	(1992)	Endeavour			
	FMPT			Hen egg-white lysozyme (pH4.5 & pH4.7)	Batch	Yes
				Horse skeletal muscle myoglobin	Batch	No
				<i>Pseudomonas</i> sp. F-126 w-APT	Batch	Yes
				<i>Rhizopus niveus</i> lipase	Batch	Yes
	PCG			Hen egg-white lysozyme (pH4.5 & pH4.7)	VDA	Yes
				<i>Arthromyces ramosus</i> peroxidase	VDA	No
STS-62	USMP-2	(1994)	Columbia	Hen egg-white lysozyme (pH4.5 & pH4.7)	VDA	Yes
				<i>Rhodococcus rhodocrous</i> nitrile hydratase	VDA	No
STS-63		(1995)	Discovery	Hen egg-white lysozyme (pH4.5 & pH4.7)	VDA	Yes
STS-73	USML-2	(1995)	Columbia	Hen egg-white lysozyme (pH4.7)	VDU	Yes
STS-72		(1995)	Endeavour	Hen egg-white lysozyme (pH4.5 & pH4.7)	VDA2	Yes
STS-77		(1996)	Endeavour	Hen egg-white lysozyme (pH4.5 & pH4.7)	CVDA	Yes
STS-79		(1996)	Atlantis	Hen egg-white lysozyme (pH4.5 & pH4.7)	VDA	Yes
				Human urine trypsin inhibitor	VDA	No(gel)
STS-84		(1997)	Atlantis	Hen egg-white lysozyme (pH4.5 ,pH4.7& pH8.6)	CVDA	Yes
				Human urine trypsin inhibitor	CVDA	No(gel)
SPACE STATION						
MIR 13		(1993)		Hen egg-white lysozyme (pH4.5 & pH4.7)	Biokrist	Yes
				<i>Rhodococcus rhodocrous</i> nitrile hydratase	Biokrist	Yes
				Wheat gliadin	Biokrist	Yes
				Rice dwarf virus	Biokrist	Yes
MIR 16		(1994)		Hen egg-white lysozyme (pH4.5 & pH4.7)	Biokrist	Yes
				HTLV protease	Biokrist	No

at pH 4.5, using a precipitant NaCl and NaNO₃, respectively. In two experiments aboard the space station MIR, wheat γ -gliadin and other proteins were crystallized in addition to hen egg-white lysozyme (Aibara, S. 1995).

Space-grown monoclinic crystals of hen egg-white lysozyme

The space experiments on hen egg-white lysozyme crystallization were mainly conducted under two different sets of conditions at 20-22°C. In the crystallization conditions of 25 mM acetate, pH4.5, using NaNO₃ as a precipitant, monoclinic crystals of rectangular shape or thin plate grew on the ground as shown in Fig. 1. On the other hand, space-grown crystals were obtained as indicated in Table 1. However, some of them were extremely thin plate. Especially those of FMPT mission were unusually thin plate in shape (Fig. 2a), which clearly distinguishes them from the thin plates or rectangular shape of the ground-grown monoclinic crystals. It has been noted that a lot of microcrystals often adhere to the space-grown crystals during observation, as shown in Fig. 2a. Thin plates of

monoclinic crystal were also obtained from the other space shuttle flights (Fig. 2b). In the space experiments aboard the space station MIR 13 in 1993, both crystal shapes appeared (Fig. 3), whereas only crystals of rectangular shape grew in the reference experiments on the ground (Aibara, S. 1994; Aibara, S. and Morita, Y. 1994). Later, these two types of monoclinic space-grown crystals were revealed to have the same molecular packing in spite of the different morphology. Recently the reflection data at 1.7Å resolution was collected using a R-axis IIC from a space-grown monoclinic crystal obtained from the space flight of STS-84 mission.

In a microgravity environment, orthorhombic crystals happened to grow under the same conditions described above. On the ground, two forms of orthorhombic crystals with different crystal packing have been reported. One such crystal form was reported by Artymiuk *et al.* (1982), but this does not have such a good diffraction quality, although it has the same crystal packing as the space-grown crystals. The other appears under different conditions at temperatures above 37°C using sodium chloride instead

(a) STS-47

(b) STS-42

Fig.2. Space-grown hen egg-white lysozyme monoclinic crystals

Thin Plate

Rectangular

Fig.3. Hen egg-white lysozyme monoclinic crystals grown aboard space station MIR

of sodium nitrate as a precipitant (Jollès, P. and Berthou, J. 1972). In the case of our space experiment, the orthorhombic crystal form was crystallized without the need for such a high temperature, using sodium nitrate as a precipitant. Moreover, these ground-grown orthorhombic crystals vary slightly in their lattice constants as shown in Table 2. They also vary in their inner molecular packing.

These facts suggest that the difference in morphology of space-grown hen egg-white lysozyme was caused by the environmental conditions derived from the microgravity field. In other words, the growth rate of particular planes of the crystal may be limited in the microgravity field depending on the particular arrangement of protein molecules in the crystal surface. Furthermore, this arrangement influences the molecular packing in the nucleation process of the protein crystal growth and results in the generation of two types of single crystals with different space groups.

Space-grown tetragonal crystals of hen egg-white lysozyme

In experiments on the ground, under conditions of 40 mM acetate buffer, pH4.7, using NaCl as the precipitant, hen egg-white lysozyme usually produced tetragonal crystals. In such conditions, however, space-grown crystals could not be obtained because of a slower rate of crystal growth in the short space-shuttle flights STS-42 and STS-47 within 10 days. However, in space experiments aboard the space station MIR and in the succeeding 16-day space-shuttle flight STS-73, tetragonal crystals of good diffraction quality were obtained using a glove box crystallization facility. The lattice constants and the molecular packing of the space grown crystal were very similar to those of the ground-grown crystal, as listed in

Table 2. These results suggest that the reason why we could not obtain tetragonal crystals in the early space flights, STS-42 and -47, was that the drop solution was not adequately mixed due to high viscosity of the protein solution.

Tetragonal crystals with a similar shape and similar crystal packing to ground-grown crystals grew in a microgravity field. However, the fact that the crystals grown in a microgravity field gave better reflection data suggests that the crystallinity of the space-grown crystals is higher than that of the ground-grown crystals. The behavior of protein molecules in a microgravity environment in space is different from that on the ground, and this causes significant differences in the generation and growth of crystal nuclei in space and on the ground. Although the details of the molecular mechanism of protein crystal growth in a microgravity field are still obscure, it is

Fig.4. Hen egg-white lysozyme tetragonal crystal grown aboard space shuttle STS-84

Table 2 The lattice constants of the space- and ground-grown hen egg-white lysozyme crystals

space group	crystal lattice (Å)			crystal angle(°)			source	
	a	b	c	α	β	γ		
P 2 ₁	28.01	62.94	66.97	90	115.3	90	MIR13	[1]
P 2 ₁	28.0311	62.886	66.3684	90	114.1	90	Ground	[2]
P 2 ₁	28.048	62.883	60.648	90	90.9	90	STS-84*	[3]
P 2 ₁	28.1268	63.0252	60.7566	90	91.2	90	Shuttle	[2]
P 2 ₁	27.9	63.1	60.6	90	90.5	90	Ground	[4]
P 2 ₁	27.99	62.92	60.48	90	90.67	90	PDB*	[5]
P 4 ₃ 2 ₁ 2	79.10	79.10	38.19	90	90	90	MIR13	[1]
P 4 ₃ 2 ₁ 2	79.192	79.192	37.933	90	90	90	STS-84*	[3]
P 4 ₃ 2 ₁ 2	79.13	79.13	37.95	90	90	90	Ground	[6]
P 4 ₃ 2 ₁ 2	79.19	79.19	38.02	90	90	90	PDB*	[7]
P 2 ₁ 2 ₁ 2 ₁	59.21	68.20	30.36	90	90	90	MIR16*	[1]
P 2 ₁ 2 ₁ 2 ₁	59.4	68.7	30.8	90	90	90	Ground	[8]
P 2 ₁ 2 ₁ 2 ₁	56.3	73.2	30.6	90	90	90	PDB*	[9]

[1] Aibara, S. (1994), [2] Aibara, S. & Morita, Y. (1993), [3] This paper (1997), [4] Steinrauf, L.K. (1959), [5] Young, A.C.M. *et al.* (1993), [6] Ashida, T. (1996), [7] Young, A.C.M. *et al.* (1994), [8] Artymiuk, P.J. *et al.* (1982), [9] Rypniewski, W.R. *et al.* (1993),

possible to obtain protein single crystals of good diffraction quality, which possess relatively good molecular arrangement and a lower mosaicity than ground-grown crystals (Snell, E. H. *et al.* 1995). In this respect, the microgravity environment in space is a suitable field for protein crystal growth. Recently, reflection data above 1.5Å resolution were collected from the tetragonal space-grown crystals grown in space flight STS-84 (Fig. 4), using the R-axis IV system.

Other proteins employed in the FMPT experiment in STS-47 mission

Horse skeletal muscle myoglobin, *Pseudomonas* sp. F-126 w-amino acid:pyruvate aminotransferase, and *Rhizopus niveus* lipase were used as protein samples in addition to hen egg-white lysozyme. The latter two enzymes were crystallized. w-Amino acid:pyruvate aminotransferase grew single crystals of good diffraction quality up to 2.0Å (Fig. 5). However, the size and shape of the space-grown crystals were not always suitable for X-ray diffraction experiment. Although crystal quality was high, the frequency with which we obtained adequately sized crystals with a good shape was still lower in a microgravity environment than on the ground. Thus, it is thought that characteristics of the protein molecule such as electric charges and hydrophobic regions on the molecular surface influence the inter-molecular contacts during the formation of crystal nuclei and their subsequent growth more profoundly than they would do on the ground.

Thermodynamic aspects of protein crystal growth in space

Formation of the crystal nuclei occurs through transfer from liquid, l, to solid, s, thus the change in the free energy of the crystal nuclei formation, ΔG , at constant temperature and pressure is expressed in the form of equation (1) using chemical potential, μ , and using the initial and the super-saturated concentrations of protein, c and s , respectively.

$$-\Delta G = \Delta n(\mu_s - \mu_l) = \Delta n kT \ln(c/s) \quad (1)$$

where Δn is the increment of protein molecules composed of crystal nuclei, k is Boltzmann's constant, and T is absolute temperature. The energy of transferring from the liquid phase to the solid phase is compensated by the gap between the supersaturated and initial concentrations of protein.

The crystal nuclei formed in a microgravity environment grew in suspension without convection, and sometimes crystal nuclei with a different molecular packing were formed simultaneously. If there is not much difference between the thermodynamical free energy of the crystal nuclei, it is possible for the less stable type of crystal nuclei to be kinetically generated in a meta-stable form and to grow single crystals by exceeding the critical size. Because the surface charges of the protein molecules and of the crystal are involved in the formation of the protein crystal nucleus and in the crystal growth thereafter, the surface energy of the crystals has to be considered. Thus,

total free energy of a crystal, ΔG_t , can be expressed in the form of the following equation using free energy, ΔG , as described above.

$$\Delta G_t = 4/3 \pi r^3 \Delta G + 4 \pi r^2 \sigma \quad (2)$$

where σ is the surface energy of the crystal. When ΔG_t has a maximum value at $r = r_c$, these values are represented by the following equations:

$$r = r_c = 2\sigma / RT \ln(c/s) \quad (3)$$

$$\Delta G_{\max} = 16\sigma^3 / (RT)^2 [\ln(c/s)]^2 \quad (4)$$

where r_c is the critical radius of the crystal nucleus. Equation (2) shows that the uptaking of protein molecules into the crystal nucleus is influenced by the surface energies of the molecules in the crystal nucleus, and so the rate of generation of the crystal nucleus, J , is represented by the following equations:

$$J = A \exp(-\Delta G_{\max} / kT) \quad (5)$$

$$= A \exp\{-16M^2\sigma^3N / 3\rho^2(RT)^3[\ln(c/s)]^2\} \quad (6)$$

Equation (6) also shows that the rate of generation of the crystal nucleus is profoundly dependent on the surface energy of the crystal.

We could therefore conclude that in a microgravity environment in space, not only is the orientation of protein molecules controlled by the surface charges, but the surface energy also makes a more important contribution to the formation of different crystal nuclei than it would on the ground. This strong influence exerted by the surface energy may in turn affect the space group of the crystal nuclei as well as the crystallinity. In conclusion, it can be thought that crystal nuclei with different molecular packing, even though they are thermodynamically unstable, are kinetically generated irrespective of the difference in the energy level, and could exist in stable crystals as the crystals exceed the critical size.

Crystallographic Analysis

Fig.5. ω -Aminitransferase crystals grown aboard space shuttle STS-47

Crystallographic refinement of the three space-grown crystals from hen egg-white lysozyme was carried out : the statistics of crystallographic refinements are shown in Table 3 together with the lattice constants of related ground- and space-grown crystals. The refinement of the orthorhombic crystal was accomplished at 1.8Å resolution, providing the first refinement data for this crystal which has a different molecular packing from the high temperature form of hen egg-white lysozyme orthorhombic crystal which has already been reported (Rypniewski, W. R. *et al.* 1993). The refinement of the monoclinic and tetragonal crystals at 1.8 and 1.6Å resolution, respectively, is under way as shown in Table 3. Regarding tetragonal crystals, the results of refinement at 1.33Å resolution from the ground-grown crystal and at 1.4Å resolution from the space-grown crystal were compared in a paper by Vaney *et al.* (1996). The relevant resolution values of these structures are, however, assumed to be lower, taking into account the completeness of the reflection data ($I > 3\sigma$).

Conclusions

From the space experiments in protein crystallization that have been conducted since 1992, a number of interesting behavioral features have been observed in the microgravity environment:

1. Crystal growth proceeded at a slower rate in the microgravity environment in space than on the ground.
2. Single crystals of good crystallinity were obtained because the precise orientation of protein molecules in the crystal were preserved in a microgravity field, but these crystals were not always of an optimal shape and size.
3. The nucleation process was more profoundly affected

by the characteristics of the crystal nuclei (*cf.* the surface energies of protein molecules and the crystals, and the characteristics of the molecular arrangement in the crystal) than on the ground.

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References

- Aibara, S.1994. Crystal growth of enzymes in microgravity. *Proc. Intl. Symp. Space Technol. Sci.*, **19**: 887-891, Yokohama.
- Aibara, S.1995. Crystallization of wheat γ -gliadin under a microgravity environment using space station MIR, *J. Crystal Growth*, **155**: 247-253.
- Aibara, S., and Morita, Y.1992. Crystal growth of hen egg-white lysozyme using a crystallization vessel produced for a spacelab experiment, *J. Crystal Growth*, **116**: 289-293.

Table 3 Comparison of the statistics of crystallographic refinement of hen egg-white lysozyme

	Monoclinic		Orthorhombic		Tetragonal	
	$P2_1$		$P2_12_12_1$		$P4_32_12$	
Source	STS-84	4LYT*	MIR16	132L*	STS-84	6LYT*
a (Å)	28.048	27.99	59.21	56.22	79.192	79.19
b (Å)	62.883	62.92	68.210	73.2	79.192	79.19
c (Å)	60.648	60.48	30.36	30.6	37.933	38.02
α (°)	90	90	90	90	90	90
β (°)	90.9	90.67	90	90	90	90
γ (°)	90	90	90	90	90	90
V (Å ³)	106,954	102,541	122,597	126,167	237,892	238,425
Zasym.	2	2	1	1	1	1
Solvent molecule	159	191	79	78	148	100
No. of Reflection Fo>3σ(Fo)	16,786		8,903	10796	15118	
Resolution range (Å)	10.0~1.8	~1.9	7.0~1.9	30~1.8	10.0~1.6	~1.9
Completeness(%)	86.0		91.55	88	92.4	
R-factor	19.74	17.9	18.1	17.3	19.78	17.3

*: Entry name of PDB data, 4LYT, Young, *et al.*(1993); 132L, Rypniewsky *et al.* (1993); 6LYT, Young, *et al.*(1994)

- Aibara, S., and Morita, Y.1993. Crystal growth of enzymes in space microgravity, *Biol. Sci. Space*, **7**: 341-352.
- Aibara, S. , and Morita, Y.1994. Crystal growth of enzymes in space microgravity (in Japanese), *J. Jpn. Assoc. Crystal Growth*, **21**: 467-472.
- Artymiuk, J., Blake, C.C.F., Rice, D.W., and Wilson, K.S.1982. The Structures of the monoclinic and orthorhombic forms of hen egg-white lysozyme at 6 Å Resolution, *Acta Cryst.*, **B38**: 778-783.
- Ashida, T. 1995. Personal Data.
- Cruickshank, D.W.J., Helliwell, J. R., Johnson, L. N., Moffat, K. and Phillips, D. 1992. Time-resolved macromolecular crystallography, *Phil. Trans. R. Soc Lond.* **A340**: 167-334.
- DeLucas, L.J., Smith, C. D., Smith, H. W., Vijay-Kumar, S., Senadhi, S. E., Ealick, S. E., Carter, D. C., Snyder, R. S., Weber, P.C., Salemme, F. R., Ohlendorf, D. H., Einspahr, H. M., Clancy, L. L., Navia, M. A., McKeever, B. M., Nagabhushan, T. L., Nelson, G., McPherson, A., Koszelak, S., Taylor, G., Stammers, D., Powell, K., Darvy, G. & Bugg, C. E.1989. Protein crystal growth in microgravity, *Science*, **246**: 651- 654.
- Jollès, P. and Berthou, J.1972. High temperature crystallization of lysozyme. An example of phase transition, *FEBS Lett.*, **23**: 21-23.
- Littke, W. and John, C.1983. Protein single crystal growth under microgravity, *Science*, **225**: 203-204.
- Rypniewski, W.R., Holden, H.M., and Rayment, I.1993. Structural consequences of reductive methylation of lysine residues in hen egg-white lysozyme. *Biochemistry*, **32**: 9851-9858.
- Snell, E. H., Weisgerber, S. & Helliwell, J. R. 1995. Improvements in lysozyme protein crystal perfection through microgravity growth. *Acta Cryst.*, **D51**:1099-1102.
- Steinrauf, L. K.1959. Preliminary X-ray data for some new crystalline forms of β -lactoglobulin and hen egg-white lysozyme, *Acta Cryst.*, **12**: 77-79.
- Vaney, M.C., Maignan, S., Ries-Kautt, M., and Ducruix, A.1996. High-resolution (1.33Å) of a HEW lysozyme tetragonal crystal grown in the APCF Apparatus. Data and structural comparison with a crystal grown under microgravity from SpaceHab-01 Mission, *Acta Cryst.*, **D52**: 505-517.
- Young, A.C.M., Dewan, J.C., Nave, C. and Tilton, R.F.1993. Comparison of radiation-induced decay and structure refinement from X-ray data collected from lysozyme crystals at low and ambient temperatures, *J. Appl. Cryst.*, **26**: 309-319.
- Young, A.C.M., Tilton, R.F. and Dewan, J.C.1994. Thermal expansion of hen egg-white lysozyme. Comparison of 1.9Å resolution structures of the tetragonal form of the enzyme at 100K and 298K, *J. Mol. Biol.*, **235**: 302-317.