Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Amino acids and glycine ethyl ester as new crystallization reagents for lysozyme

Len Ito,[a,b]* Kentaro Shiraki[c] and Hiroshi Yamaguchi[b]

[a]Japan Synchrotron Radiation Research Institute (SPring-8), Japan, [b]School of Science and Technology, Kwansei Gakuin University, Japan, and [c]Institute of Applied Physics, University of Tsukuba, Japan

Correspondence e-mail: l-ito@spring8.or.jp

Received 7 October 2009
Accepted 14 April 2010

© 2010 International Union of Crystallography
All rights reserved

Several amino acids and their derivatives are prominent additives in the field of protein chemistry. This study reports the use of charged amino acids and glycine ethyl ester as precipitants in protein crystallization, using hen egg-white lysozyme (HEWL) as a model. A discussion of the crystallization of HEWL using these reagents as precipitating agents is given.

1. Introduction

X-ray crystal structures of proteins provide a clear understanding of the relationship between tertiary structure and function. To date, the crystallization step has remained a major obstacle in X-ray crystallography of proteins. Various crystallization techniques have been developed in an attempt to facilitate and improve crystal nucleation, such as temperature control (Adachi, Takano, Yoshimura *et al.*, 2003) and the use of gravity (McPherson *et al.*, 1999), magnetic fields (Sazaki *et al.*, 1997), pressure (Suzuki *et al.*, 1994), ultrasonic irradiation (Luft & DeTitta, 1999), laser irradiation (Adachi, Takano, Hosokawa *et al.*, 2003) and solution flow (Adachi *et al.*, 2002), together with other efforts to develop narrow screening cocktails (Kempkes *et al.*, 2008) and to promote initial crystallizations hits, *e.g.* by using additives such as metals (Trakhanov & Quiocho, 1995), nucleants (Sugahara *et al.*, 2008) *etc*. Although various studies have been conducted, obtaining high-quality single crystals is still difficult because of the large number of factors that influence protein crystallization, such as protein concentration, temperature, buffer type, pH, ionic strength and the type and concentration of precipitants. The large number of parameters leads to large amounts of precious protein being required to determine the crystallization conditions.

Amino acids and their derivatives, such as amino-acid ethyl esters and amino-acid amides, offer new perspectives in the field of protein chemistry because of their unique combination of solubilization and stabilization properties. Researchers have recently utilized amino acids and their derivatives by using additives such as Arg for the refolding of recombinant proteins (Buchner & Rudolph, 1991) and the separation and recovery of proteins in chromatography (Ejima *et al.*, 2005) and by using Arg and Arg derivatives for the prevention of heat-induced aggregation and inactivation (Shiraki *et al.*, 2002, 2004; Matsuoka *et al.*, 2007; Hamada & Shiraki, 2007). These results led us to investigate the role of amino acids and their derivatives in other methods within the field of protein crystallography. Recently, we have reported that protein crystallization is promoted by the use of amino acids and their derivatives as additives (Ito, Hidaka *et al.*, 2008; Ito, Kobayashi *et al.*, 2008). They function as a fourth component by decreasing protein aggregation and increasing the probability of crystallizing proteins in solutions containing precipitant, buffer and salt (Ito *et al.*, 2010). These unique properties make amino acids and their derivatives intriguing reagents and potential crystallization reagents. This study focuses on using new aggregation suppressors such as amino acids and GlyEE as precipitants by using high concentrations to crystallize proteins over large concentration ranges.

2. Materials and methods

2.1. Reagents and preparation

L-Lysine–HCl, glycine ethyl ester–HCl and aspartic acid–NaOH were purchased from Sigma–Aldrich Co. (St Louis, Missouri, USA). Other amino acids, such as ornithine–HCl, glycine, serine, glutamic acid–NaOH and arginine–HCl, and other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). Sixfold-crystallized HEWL was purchased from Seikagaku Co. (Tokyo, Japan). All chemicals used were of high-quality analytical grade.

Solutions of glycine (Gly), serine (Ser), aspartic acid (Asp), glutamic acid (Glu), arginine (Arg), ornithine (Orn), lysine (Lys) and glycine ethyl ester (GlyEE) were prepared to evaluate their effect on HEWL crystallization. The solutions containing these reagents were adjusted to pH 4.5, 6.5 or 8.5 by a conventional pH electrode using 100 m*M* sodium acetate, sodium phosphate or Tris–HCl, respectively. All the solutions were centrifuged at 15 000*g* for 20 min at 293 K before crystallization.

2.2. Crystallization

Crystallization of HEWL was performed as follows. Protein solution containing 50 or 150 mg ml^{-1} HEWL in 0.1 *M* buffer at pH 4.5 (sodium acetate), pH 6.5 (sodium phosphate) or pH 8.5 (Tris–HCl) was prepared in a 1.5 ml microtube. The protein concentration was determined from the absorbance at 280 nm with an appropriate blank using an extinction coefficient of 2.63 ml mg^{-1} cm^{-1} (Saxena & Wetlaufer, 1970). The hanging-drop vapour-diffusion method was used for crystallization at 293 K. Hanging drops were prepared by mixing 1.5 µl protein solution with 1.5 µl reservoir solution, with three different buffer conditions, eight different amino-acid concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 *M*) and ten different GlyEE concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25 and 2.5 *M*). The volume of reservoir solution was 500 µl for each setting. Reproducibility was evaluated by repeating each experiment at least three times. Drops were carefully examined under a stereoscopic microscope every day.

2.3. X-ray characterization

All data sets were collected under cryogenic conditions from crystals soaked in Paratone-N (Hampton Research) and cooled to 100 K in a nitrogen-gas stream. Single-pass φ-axis scan oscillation images were recorded on a Rigaku R-AXIS IV imaging-plate detector mounted on a Rigaku rotating-anode generator operating at 50 kV and 100 mA with Cu $K\alpha$ radiation. The crystals obtained using Asp, Lys and GlyEE as precipitants at pH 6.5 were examined on the BL44XU beamline of SPring-8 at 100 K using a DIP6040 image-plate detector. Diffraction intensities were integrated with the program *MOSFLM* (Leslie, 1992) and scaled with the program *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results

Fig. 1 shows the results of HEWL crystallization using Gly, Ser, Asp, Glu, Arg, Orn, Lys and GlyEE as precipitants at pH 4.5, 6.5 and 8.5. At pH 4.5 Orn, Lys and GlyEE led to the crystallization of HEWL at a wide range of concentrations, while Gly and Ser did not. Arg only led to the crystallization of HEWL at a high protein concentration (Fig. 1*a*). The data for Orn, Lys, GlyEE and Arg at pH 6.5 showed a similar pattern to those at pH 4.5 (Figs. 1*a* and 1*b*). At pH 8.5, Lys, Orn, Arg, Glu, Asp and Gly led to HEWL crystallization (Fig. 1*c*). High concentrations of Glu and Asp did not produce any crystals at pH 8.5 because of the appearance of precipitate (Fig. 1*c*). These data indicate that amino acids and GlyEE have the potential to crystallize HEWL. Owing to the low solubility of Asp, Glu and GlyEE, the crystallization experiments could not be performed at pH 4.5 and 8.5.

Fig. 2 shows representative pictures of HEWL crystals obtained using amino acids and GlyEE as precipitants. Many crystals appeared and grew in the presence of amino acids such as Gly, Asp, Glu, Orn and Lys. In contrast, the use of Arg and GlyEE resulted in a decrease in the number of crystals.

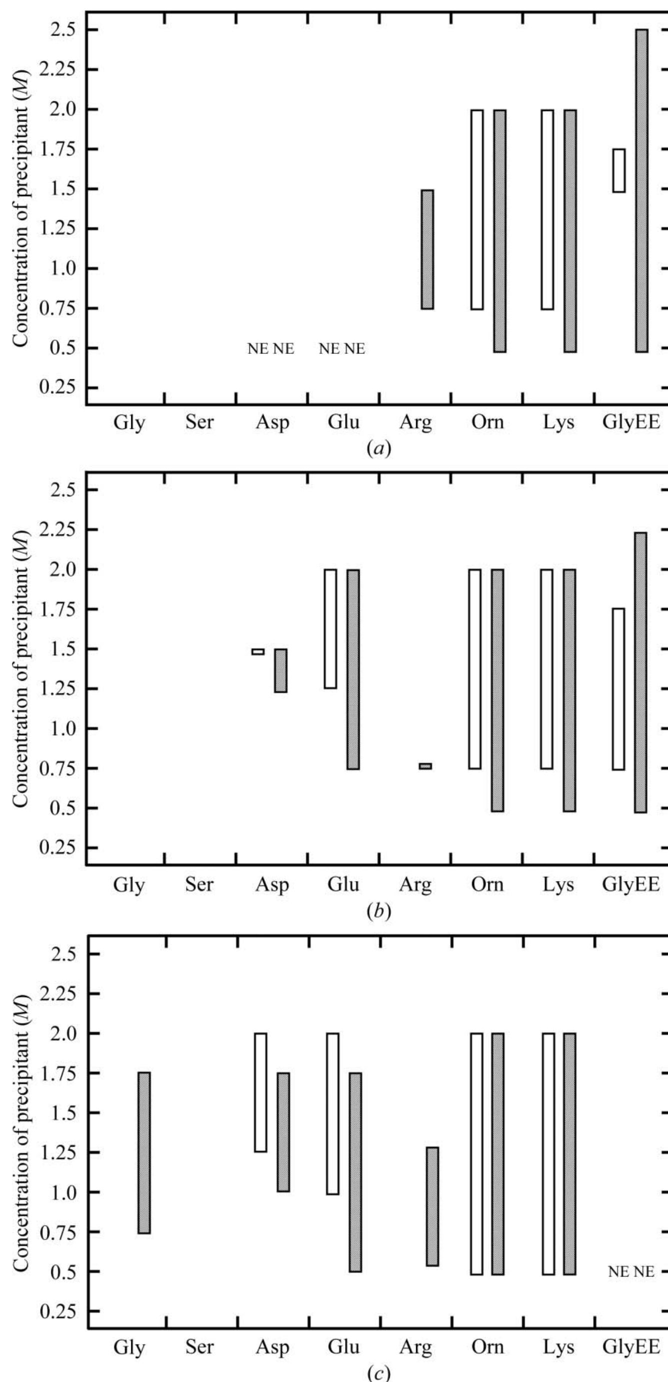

Figure 1
Crystallization experiments with HEWL using amino acids as precipitants at three pH values: (*a*) pH 4.5, (*b*) pH 6.5, (*c*) pH 8.5. The bars show the precipitant concentration ranges in which crystals were formed. Open bars, 50 mg ml^{-1} HEWL; solid bars, 150 mg ml^{-1} HEWL. NE indicates that the reagents could not be used because of low solubility.

Table 1
Data-collection statistics at pH 4.5.

Values in parentheses are for reflections in the highest resolution shell.

Precipitant	Arg	Orn	Lys	GlyEE
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Growth pH	4.5	4.5	4.5	4.5
Unit-cell parameters				
a (Å)	76.91	78.56	78.64	78.86
c (Å)	37.23	37.26	37.09	36.88
Unit-cell volume (Å^3)	2.20×10^5	2.30×10^5	2.29×10^5	2.29×10^5
Resolution (Å)	30.86–1.70	30.93–1.80	30.86–1.70	33.41–1.80
Wavelength (Å)	1.54	1.54	1.54	1.54
Crystal-to-detector distance (mm)	100	100	100	100
Oscillation angle (°)	2.5	3.0	3.0	3.0
Exposure time (s per frame)	210	210	210	210
Total oscillation range (°)	125	150	150	120
No. of reflections				
Observed	8160	123160	114847	92740
Unique	2150	11218	13341	11154
Completeness (%)	88.1 (88.8)	99.3 (99.7)	100.0 (99.9)	99.1 (99.8)
$R_{\rm merge}$†	0.094 (0.232)	0.047 (0.183)	0.045 (0.202)	0.056 (0.231)
$I/\sigma(I)$	15.7 (4.8)	34.6 (31.2)	29.4 (7.6)	26.6 (9.3)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation and $\langle I(hkl)\rangle$ is the mean intensity of the reflection.

Tables 1, 2 and 3 give the crystallographic data for HEWL crystals obtained using amino acids and GlyEE as precipitants. The HEWL crystals obtained using amino-acid and GlyEE solutions belonged to the same space group, with the exception of those obtained using Arg at pH 8.5. The space group of the HEWL crystals was $P4_32_12$, with unit-cell parameters $a = b = 78.54$, $c = 37.77$ Å and one molecule in the crystal asymmetric unit (Vaney *et al.*, 1996; PDB code 193l). The unit-cell parameters showed systematic changes on using amino acids at pH 8.5. The other crystals belonged to the same space group, $P4_32_12$, with identical unit-cell parameters (Table 3), with the exception of those obtained in the presence of Arg. The dimensions of the a axis of the crystals obtained from Arg solutions at pH 4.5 and 6.5 were the smallest. The volume of the unit cell calculated from the unit-cell dimensions had a minimum value for the crystals obtained from Arg solutions at pH 4.5 and 6.5. At pH 8.5, the space group of the crystals from the Arg solution differed from that of the others, indicating that HEWL crystals obtained using Arg as a precipitant have the most compact packing of those grown with any other amino acids or GlyEE. The unit-cell dimensions of the Arg-derived crystal were slightly smaller than those of the published orthorhombic form (PDB codes 1aki, 1f0w and 1jj1; Artymiuk *et al.*, 1982; Biswal *et al.*, 2000; Datta *et al.*, 2001). Moreover, these results suggest that Arg induces different protein–protein interactions at pH 8.5 and inhibits the growth of certain orientations compared with the other reagents.

4. Discussion

This paper describes the effect of amino acids and GlyEE as new protein-crystallization precipitants. HEWL crystals were obtained under wide concentrations of these reagents. We selected these reagents as precipitants because they are reasonable for the design of protein-crystallization solutions. The following properties are demonstrated by our results: (i) amino acids and GlyEE can act as new crystallization reagents, (ii) these reagents not only suppress protein aggregation but also increase the probability of obtaining protein crystals under a wider range of concentrations, (iii) Arg and

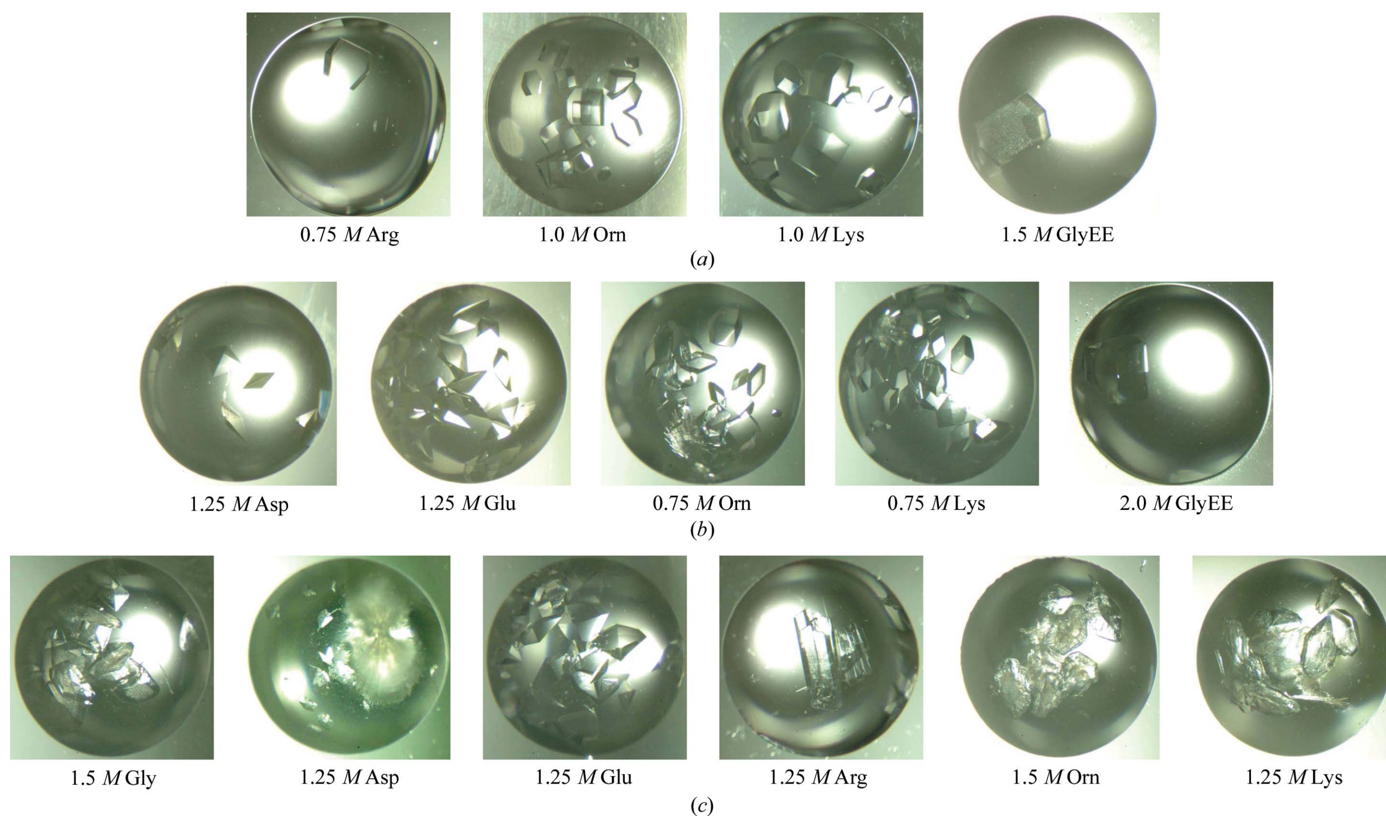

Figure 2
Representative pictures of HEWL crystals obtained using amino-acid solutions. The results of the crystallization of 150 mg ml^{-1} HEWL (a) at pH 4.5 in 0.1 M sodium acetate, (b) at pH 6.5 in 0.1 M sodium phosphate and (c) at pH 8.5 in 0.1 M Tris–HCl are shown.

Table 2
Data-collection statistics at pH 6.5.

Precipitant	Asp	Glu	Arg	Orn	Lys	GlyEE
Growth pH	6.5	6.5	6.5	6.5	6.5	6.5
Unit-cell parameters						
a (Å)	78.41	78.76	77.28	78.74	78.60	78.94
c (Å)	37.36	36.82	38.04	37.08	37.01	37.11
Unit-cell volume (Å^3)	2.30×10^5	2.28×10^5	2.27×10^5	2.30×10^5	2.29×10^5	2.31×10^5
Resolution (Å)	19.60–1.70	30.71–2.20	31.22–1.90	30.86–1.80	31.22–1.90	19.15–1.40
Wavelength (Å)	0.90	1.54	1.54	1.54	0.90	0.90
Crystal-to-detector distance (mm)	200	100	100	100	200	200
Oscillation angle (°)	2.0	3.0	2.0	2.0	2.0	2.0
Exposure time (s per frame)	1.0	210	210	210	1.0	1.0
Total oscillation range (°)	80	120	100	100	80	80
No. of reflections						
Observed	82829	45201	68417	79663	98154	146461
Unique	13325	6200	9256	11203	14715	23381
Completeness (%)	99.9 (100.0)	98.8 (92.9)	97.6 (98.6)	99.1 (94.5)	93.5 (95.9)	99.0 (99.9)
R_{merge}†	0.109 (0.276)	0.081 (0.169)	0.068 (0.380)	0.049 (0.201)	0.121 (0.260)	0.115 (0.294)
$I/\sigma(I)$	16.1 (6.5)	19.2 (7.5)	19.2 (4.4)	26.9 (7.9)	15.7 (7.3)	14.4 (5.5)

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th observation and $\langle I(hkl) \rangle$ is the mean intensity of the reflection.

Table 3
Data-collection statistics at pH 8.5.

Precipitant	Gly	Asp	Glu	Arg	Orn	Lys
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P2_12_12_1$	$P4_32_12$	$P4_32_12$
Growth pH	8.5	8.5	8.5	8.5	8.5	8.5
Unit-cell parameters						
a (Å)	78.48	77.12	78.56	30.33	77.44	76.93
b (Å)				57.45		
c (Å)	37.09	38.47	37.10	67.03	37.41	38.23
Unit-cell volume (Å^3)	2.28×10^5	2.29×10^5	2.29×10^5	1.17×10^5	2.24×10^5	2.27×10^5
Resolution (Å)	33.54–1.80	31.43–2.10	30.86–2.00	33.52–2.00	30.89–1.80	31.28–1.80
Wavelength (Å)	1.54	1.54	1.54	1.54	1.54	1.54
Crystal-to-detector distance (mm)	100	100	100	100	100	100
Oscillation angle (°)	2.5	3.0	2.5	3.0	2.0	3.0
Exposure time (s per frame)	210	210	210	210	210	210
Total oscillation range (°)	125	150	125	150	100	150
Number of reflections						
Observed	94518	81767	74616	43721	48861	119720
Unique	10851	7182	8310	8295	7749	11104
Completeness (%)	96.4 (75.6)	100.0 (100.0)	100.0 (100.0)	99.0 (97.4)	96.6 (95.4)	99.7 (98.1)
R_{merge}†	0.065 (0.293)	0.079 (0.300)	0.083 (0.288)	0.068 (0.210)	0.075 (0.284)	0.051 (0.233)
$I/\sigma(I)$	23.7 (4.8)	23.2 (8.2)	20.5 (7.5)	18.1 (7.4)	18.7 (5.8)	30.8 (7.9)

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th observation and $\langle I(hkl) \rangle$ is the mean intensity of the reflection.

GlyEE decrease crystal clusters or nuclei and (iv) Arg induces different protein–protein interactions and inhibits the growth of certain orientations compared with the other reagents.

The experiment without additives showed that HEWL crystals were obtained in the range 0.75–1.25 *M* sodium chloride at pH 6.5 (Ito, 2008). However, the range of concentration conditions under which crystals could be obtained using positively charged amino acids (Lys and Orn) and GlyEE as precipitants is wider than the range using sodium chloride as a precipitant at pH 6.5 (Fig. 1*b*). This is very important in determining the initial crystallization condition in the initial screening because if the crystallization conditions of a certain protein were in an extremely narrow range then researchers would find it difficult to determine the crystallization condition; on the other hand, amino acids and GlyEE as precipitants have a much wider concentration range in which protein crystals can be obtained, indicating that these reagents might increase the success rates of initial screening in protein crystallization. Moreover, using amino acids and their derivatives as precipitants might decrease the crystallization parameters because the crystallization conditions, including protein concentration, pH, ionic strength and precipitant concentration, influence these parameters.

High ionic strength can weaken hydrogen bonding and ionic interactions, but may enhance hydrophobic interactions. Conversely, organic solvents can weaken hydrophobic interactions, but may strengthen hydrogen-bonding and ionic interactions. The solubility measurements of amino acids in Arg showed that Arg favourably interacts with almost all amino-acid side chains and peptide bonds, indicating that it can reduce both electrostatic and hydrophobic interactions (Arakawa *et al.*, 2007). We suggest that these properties are useful for crystallizing various proteins and that traditional reagents do not possess these properties. In previous reports, Arg was more effective at higher concentrations. For example, increasing the arginine concentration to 2 *M* resulted in a higher recovery of antibodies from Protein-A columns above pH 4.0 (Ejima *et al.*, 2005). A requirement for additives at high concentrations means that the interactions between the additive and protein are weak. Our data, in which the unit-cell parameters changed when using Arg as a precipitant, might elucidate the mechanism of the effect of Arg on protein aggregation. Currently, structural determination and refinement are under study.

When attempting to grow crystals from spontaneous nuclei in a supersaturated liquid sample, it is necessary to enter the labile region. In the far reaches of the labile region of supersaturation, not only crystal nuclei might form but also amorphous precipitate (aggregated forms). In any case, these aggregates, whether ordered or otherwise, may be incorporated into the crystals (Land *et al.*, 1995). If incor-

porated, clusters will introduce misoriented molecules into the lattice, leading to defects and dislocations. Thus, the prevention of protein aggregation plays a key role in the formation of single crystals in solution conditions that are prone to aggregation (McPherson *et al.*, 1986). Traditional precipitants such as ammonium sulfate and polyethylene glycol (PEG) have the potential to disrupt electrostatic and hydrophobic interactions and to enhance the hydrophobic and electrostatic interactions that lead to protein aggregation. Recently, some papers have proposed that small molecules be used in the crystallization of proteins and viruses (Sauter *et al.*, 1999; Jeruzalmi & Steitz, 1997; McPherson & Cudney, 2006; Larson *et al.*, 2007). The underlying hypothesis of this approach was that small molecules could form reversible cross-links in the crystal lattice through intermolecular electrostatic hydrogen bonding, and perhaps hydrophobic interactions, but these molecules have a low potential to suppress protein aggregation.

We have previously reported that the addition of amino acids and their derivatives improves protein crystallization (Ito, Hidaka *et al.*, 2008; Ito, Kobiyashi *et al.*, 2008) when used as a fourth component. However, this is the first study to report that amino acids and their derivatives can be used as precipitants in protein crystallization. Amino acids and their derivatives have many potential applications in protein crystallography. Hydrophobic ligands that are generally difficult to introduce into a protein crystal may dissolve in solutions of amino acids and their derivatives and be more easily delivered into protein crystals grown using amino acids and their derivatives. For example, coumarin, which is not very soluble in water, has been shown to be soluble in solutions containing a little Arg (Hirano *et al.*, 2008). Some proteins, especially those with exposed hydrophobic regions that do not respond to traditional precipitating agents such as peripheral or integral membrane proteins, may have improved solubility in the presence of amino acids and their derivatives. Many researchers now adopt a sparse-matrix sampling method that utilises commercially available crystallization-screening kits (Jancarik & Kim, 1991). However, these kits are unsuitable in many cases owing to the limited conditions that are examined. In contrast, amino acids and their derivatives have the potential to crystallize proteins under a wider range of concentrations and may increase the probability of obtaining protein crystals and reduce time, cost and effort.

We thank Dr Takashi Kumasaka, Dr Hiroshi Komatsu and Dr Gen Sazaki for useful discussions, and Ms Satomi Uramoto, Mr Toyoaki Kobayashi, Mr Tomohisa Shibano, Mr Makoto Kanou, Ms Tomoko Miyashita and Ms Akiko Oosuka for performing many crystallization experiments. We are grateful to Dr A. Nakagawa and Dr E. Yamashita, Institute for Protein Research, Osaka University, at BL44XU (SPring-8) for their kind help during data collection.

References

Adachi, H., Takano, K., Hosokawa, Y., Inoue, T., Mori, Y., Matsumura, H., Yoshimura, M., Tsunaka, Y., Morikawa, M., Kanaya, S., Masuhara, H., Kai, Y. & Sasaki, T. (2003). *Jpn J. Appl. Phys.* **42**, L798–L800.
Adachi, H., Takano, K., Yoshimura, M., Mori, Y. & Sasaki, T. (2002). *Jpn J. Appl. Phys.* **41**, L1025–L1027.
Adachi, H., Takano, K., Yoshimura, M., Mori, Y. & Sasaki, T. (2003). *Jpn J. Appl. Phys.* **42**, L384–L385.
Arakawa, T., Ejima, D., Tsumoto, K., Obeyama, N., Tanaka, Y., Kita, Y. & Timasheff, S. N. (2007). *Biophys. Chem.* **127**, 1–8.
Artymiuk, P. J., Blake, C. C. F., Rice, D. W. & Wilson, K. S. (1982). *Acta Cryst.* B**38**, 778–783.
Biswal, B. K., Sukumar, N. & Vijayan, M. (2000). *Acta Cryst.* D**56**, 1110–1119.
Buchner, J. & Rudolph, R. (1991). *Biotechnology*, **9**, 157–162.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Datta, S., Biswal, B. K. & Vijayan, M. (2001). *Acta Cryst.* D**57**, 1614–1620.
Ejima, D., Yumioka, R., Tsumoto, K. & Arakawa, T. (2005). *Anal. Biochem.* **345**, 250–257.
Hamada, H. & Shiraki, K. (2007). *J. Biotechnol.* **130**, 153–160.
Hirano, A., Arakawa, T. & Shiraki, K. (2008). *J. Biochem.* **144**, 363–369.
Ito, L. (2008). PhD thesis. Kwansei Gakuin University, Japan.
Ito, L., Hidaka, Y., Okumura, M., Konishi, H. & Yamaguchi, H. (2008). *Acta Cryst.* F**64**, 531–532.
Ito, L., Kobayashi, T., Shiraki, K. & Yamaguchi, H. (2008*b*). *J. Synchrotron Rad.* **15**, 316–318.
Ito, L., Shiraki, K. & Yamaguchi, H. (2010). *Acta Cryst.* F**66**, 744–749.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jeruzalmi, D. & Steitz, T. A. (1997). *J. Mol. Biol.* **274**, 748–756.
Kempkes, R., Stofko, E., Lam, K. & Snell, E. H. (2008). *Acta Cryst.* D**64**, 287–301.
Land, T. A., Malkin, A. J., Kuznetsov, Y. G., McPherson, A. & De Yoreo, J. J. (1995). *Phys. Rev. Lett.* **75**, 2774–2777.
Larson, S. B., Day, J. S., Cudney, R. & McPherson, A. (2007). *Acta Cryst.* D**63**, 310–318.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **26**.
Luft, J. R. & DeTitta, G. T. (1999). *Acta Cryst.* D**55**, 988–993.
Matsuoka, T., Tomita, S., Hamada, H. & Shiraki, K. (2007). *J. Biosci. Bioeng.* **103**, 440–443.
McPherson, A. & Cudney, B. (2006). *J. Struct. Biol.* **156**, 387–406.
McPherson, A., Koszelak, S., Axelrod, H., Day, J., Williams, R., Robinson, L., McGrath, M. & Cascio, D. (1986). *J. Biol. Chem.* **261**, 1969–1975.
McPherson, A., Malkin, A. J., Kuznetsov, Y. G., Koszelak, S., Wells, M., Jenkins, G., Howard, J. & Lawson, G. (1999). *J. Cryst. Growth*, **196**, 572–586.
Sauter, C., Joseph, D., Ng, J., Lorber, B., Keith, G., Brion, P., Hosseini, M. W., Lehn, J. M. & Giegé, R. (1999). *J. Cryst. Growth*, **196**, 365–376.
Saxena, V. P. & Wetlaufer, D. B. (1970). *Biochemistry*, **9**, 5015–5023.
Sazaki, G., Yoshida, E., Komatsu, H., Nakada, T., Miyashita, S. & Watanabe, K. (1997). *J. Cryst. Growth*, **173**, 231–234.
Shiraki, K., Kudou, M., Fujiwara, S., Imanaka, T. & Takagi, M. (2002). *J. Biochem.* **132**, 591–595.
Shiraki, K., Kudou, M., Nishikori, S., Kitagawa, H., Imanaka, T. & Takagi, M. (2004). *Eur. J. Biochem.* **271**, 3242–3247.
Sugahara, M., Asada, Y., Morikawa, Y., Kageyama, Y. & Kunishima, N. (2008). *Acta Cryst.* D**64**, 686–695.
Suzuki, Y., Miyashita, S., Komatsu, H., Sato, K. & Yagi, T. (1994). *Jpn J. Appl. Phys.* **33**, L1568–L1570.
Trakhanov, S. & Quiocho, F. A. (1995). *Protein Sci.* **4**, 1914–1919.
Vaney, M. C., Maignan, S., Riès-Kautt, M. & Ducruix, A. (1996). *Acta Cryst.* D**52**, 505–517.