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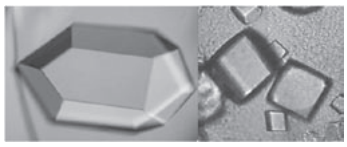
Analysis of crystal growth of trigonal ribonuclease A from bovine pancreas

Hideaki Takusagawa, Shigefumi Yamamura, Shigeru Endo, Koh Ida, Yoko Sugawara
Kitasato University, School of Science, 1-15-1 Kitasato, Sagamihara, Kanagawa, 228-8555, Japan, E-mail：ms06818u@st.kitasato-u.ac.jp

Mechanism of crystal growth is an object to understand and control crystal size and quality. Ribonuclease A (RNaseA) is known to crystallize in the trigonal form using 3 M NaCl and 30% $(NH_4)_2SO_4$ as precipitants. Crystal growth of trigonal RNaseA was investigated. Solubility curves were determined at 10, 20, and 35℃. It was found that solubility decreases at higher temperature. Negative correlation between solubility and temperature is known for hydrophobic proteins. However, RNaseA is not hydrophobic protein, and high concentration of the precipitants attributes temperature dependence of solubility curve. Crystal morphology changed from a hexagonal bipyramidal surrounded by {1 0 0} and {1 0 1} to a truncated cube surrounded by {1 0 1} with increment of concentrations of the precipitants (Fig. 1). The salt effect on crystal morphology and surface microtopograph will be discussed based on the intermolecular interactions. Besides impurity effect will be discussed, because RNaseA of commercial source includes slight amount of deamidated protein and dimeric protein.



Keywords: ribonuclease A, crystal growth, solubility curve

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Protein crystallization in the presence of amino acids and their derivatives: (2) The mechanism

Len Ito[1], Gen Sazaki[2], Kentaro Shiraki[3], Tomohisa Shibano[1], Atsushi Matsuoka[1], Hiroshi Yamaguchi[1]
[1]Kwansei Gakuin University, School of Science and Technology, 2-1 Gakuen, Sanda, Japan/Hyogo, 669-1337, Japan, [2]The Institute of Low Temperature Science, Hokkaido University, N19-W8, Kita-ku, Sapporo 060-0819, Japan, [3]Institute of Applied Physics, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan, E-mail：len@ksc.kwansei.ac.jp

Preventing protein aggregation is necessary for the formation of single crystals under aggregation-prone solution conditions. Recently, we showed that the addition of a certain amino acid and amino acid derivative expanded the range of precipitant concentration in which crystals formed without aggregation. In order to reveal the mechanism of promotion of protein crystallization in the presence of them, we evaluate the aggregate formation and the solubility of HEWL under the crystallization conditions. The formation of aggregation was measured as turbidity by a spectrometer and the solubility of proteins was measured by a Michelson interferometer. The results showed that these additives decreased the formation of protein aggregation during the crystallization and crystals appeared even from high concentration condition that crystal was not appeared in the absence of them (Fig). In addition, these additives also decrease the solubility of HEWL. Therefore, these additives expand the crystallization conditions for both lower and higher concentration of precipitant.

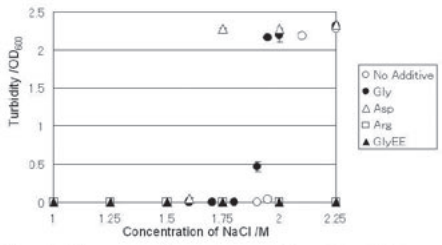

Figure Turbidity measurements via absorbance monitoring at 600 nm during crystallization using amino acids and amino acid derivatives at pH 6.5. (A) (○) No additive, (●): glycine (Gly), (△): aspartic acid (Asp), (□): arginine (Arg), (▲): glycine ethyl ester (GlyEE).

1.L. Ito et al., J. Synchrotron Rad. (2008), 15, 316-318
2.T. Kobayashi et al., Peptide Science (2007), 481-482

Keywords: amino acid and amino acid derivative, solubility, aggregation

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Protein crystallization in the presence of amino acids and their derivatives: (1) The effect

Tomohisa Shibano[1], Len Ito[1], Kentaro Shiraki[2], Yuhei Yamazaki[1], Hiroshi Yamaguchi[1]
[1]Kwansei Gakuin University, School of Science and Technology, 2-1 Gakuen, Sanda, Hyogo, 669-1337, Japan, [2]Institute of Applied Physics, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan, E-mail：tomohisa@kwansei.ac.jp

High quality single crystals are difficult to obtain. Preventing protein aggregation is necessary for the formation of single crystals under aggregation-prone solution conditions. Recently, amino acids and amino acid derivatives, which are non-denaturating reagents, such as arginine and glycine ethyl ester are the most widely used additives for increasing refolding yields by decreasing aggregation. In this study, we investigated the effect of amino acids and amino acids derivatives on protein crystallization. As a result, protein crystals were obtained in expanded concentration range of the known precipitant in the presence of some kind of these reagents. Especially, crystals were obtained from several conditions containing precipitants that crystal did not appear in the absence of these reagents. For example, in the presence of arginine and glycine ethyl ester, HEWL crystals were obtained from ammonium sulfate solution. These results show that these reagents are very effective for crystallization and suggest a new strategy to improve the performance of the protein crystallization.

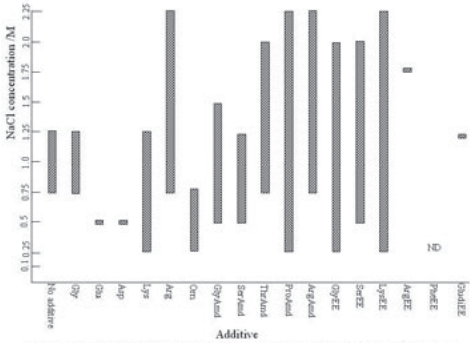

Figure The concentration range of NaCl in the presence of 0.25 M additives, in which HEWL crystals were observed at pH 6.5. The solution of 50 mg/mL HEWL was used for crystallization.

Keywords: amino acid and amino acid derivative, protein crystallization, promotion of crystallization

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Protein crystallization strategy in microgravity

Sachiko Takahashi[1], Hiroaki Tanaka[1], Masaru Sato[2], Koji Inaka[3],