Short Paper

Influence of KCl on the Decrease of Ca^{2+} -ATPase Activity and Solubility of Carp Myofibrils during Frozen Storage

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A model system containing KCl was used in a previous investigation of freeze denaturation of fish myofibrillar proteins.^{1,2)} The maximum rate constant for the freeze inactivation of myofibrils (Mf) ATPase in a buffer containing 0.1 M KCl occurred at about -11°C in a temperature range from -3 to -30°C .³⁾ From these results, it has been considered that the freeze denaturation is related to the volume and concentration of unfrozen KCl solution. It is important to know what effect the presence of KCl has on the denaturation in the model system. However, there are few reports where the freeze denaturation in the presence of 0.1 M KCl is compared to samples in the absence of KCl. Ohta and Yamada⁴⁾ showed that phosphate buffer made of K_2HPO_4 – NaH_2PO_4 did not accelerate freeze denaturation of actomyosin. In this paper, we therefore compare freeze denaturation of Mf between in both the presence and absence of 0.1 M KCl in the model system containing a phosphate buffer (K_2HPO_4 – NaH_2PO_4).

Carp Mf were prepared using a modified method of Kato *et al.*,⁵⁾ and suspended in 0.1 M KCl–20 mM phosphate buffer (with KCl, pH 6.8) or 20 mM phosphate buffer (without KCl, pH 6.8). The subsequent procedure was the same as the method of Takahashi *et al.*³⁾ Storage temperatures were -4 , -11 and -26°C . Thawed Mf suspension was dialyzed against 0.6 M KCl–20 mM Tris–HCl buffer (pH 7.0) for 24 h at 4°C . Ca^{2+} -ATPase activity assay was the same as that used by Takahashi *et al.*,³⁾ except a 20 mM Tris–HCl buffer (pH 7.0) was used in this case. The dialyzed suspension (the inner suspension after dialysis) was centrifuged at $20,000 \times g$ for 15 min. Solubility was the ratio of proteins in supernatant to the proteins in the dialyzed suspension.

In the case of Mf with KCl, the decrease of the Ca^{2+} -ATPase activity (Fig. 1) and the solubility (Fig. 2) at -11°C were greatest in the three storage temperatures. This temperature-dependence of the ATPase inactivation and decrease of the solubility with KCl could be ascribed to affect by the highly concentrated solution of K^+ and Na^+ condensed under the frozen conditions³⁾ in the presence of KCl and phosphates above the eutectic point [The eutectic point may be below -13.7°C under this experimental conditions, because K_2HPO_4 has the lowest one (-13.7°C) among three salts used, KCl, K_2HPO_4 , and NaH_2PO_4]. The freeze denaturation, however, seems to be mainly due to the effect of KCl, because the concentration of K^+ dissociated from KCl could be larger than that of K^+ and Na^+ dissociated from phosphates.

Fig. 1. Ca^{2+} -ATPase inactivation of myofibrils during frozen storage.

Fig. 2. Changes in solubility of myofibrils during frozen storage.

While in the case of Mf without KCl, a smaller decrease of the solubility was found when the storage temperature was lower. However, no difference in the ATPase activity was observed among storage temperatures and the extent of the inactivation was less than that observed with KCl. These phenomena may be based on the absence of KCl, and may possibly be explained by the mobility of water⁶⁾ and the volume of unfrozen solution under lower temperature.

Thus from the above facts, it can be ascribed that the Mf in the model system with KCl are affected by concentration effect of KCl, while the Mf in the model system without KCl are affected by the temperature effect.

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