An Evaluation of Effect of Temperature on Denaturation of Protein in Frozen KCl Solution*1

Previously, we reported that the apparent first order rate constant (Kf) for denaturation of carp actomyosin in frozen KCl solution decreased with increasing concentration of protein in the liquid portion (CIP) within the frozen solution, and that the reaction order for the decrease of CIP due to freeze-denaturation varied according to temperature.1) We also suggested that the slope of the plot of log Kf vs. log CIP may be useful for evaluating the effect of temperature on the denaturation of protein in the frozen KCl solution.

In this study, data reported by other workers 2-6) were analyzed to confirm the foregoing observations and suggestions. The Kf was calculated from the change of ATPase activity or solubility of protein. The relation of log Kf vs. log CIP was examined upon replacing CIP with the reciprocal of KCl concentration or the concentration of protein in the test solution, since CIP was directly proportional to the concentration of protein and inversely to the concentration of KCl in the solution. An analysis was made of the published data showing the rates of denaturation of myosin or actomyosin at three or more different concentrations of KCl or protein in the test solution.

As seen in Fig. 1, in all cases, when log Kf was plotted against log [protein] or [KCl]-1, a straight line was obtained, though the slopes of the plots differed according to the experimental conditions under which the data were obtained. These results were similar to those previously shown by us.1) The finding strongly suggests that the effect of temperature on protein denaturation in frozen KCl solution may be more reasonably evaluated from the slope of the plot (denoted as Df) of log Kf vs. log CIP than from Kf alone. The slope of the plot, i.e. the Df value, is considered as revealing the overall extent of denaturation occurring through the reaction of CIP at different concentrations with KCl of a fixed concentration in the liquid portion of the solution frozen at a given temperature, while Kf represents only the rate of reaction between CIP of a variable concentration and KCl of a definite concentration. The difference of Kf between two different temperatures therefore varies according to CIP value.

Fig. 2 illustrates approximate Df values obtained from published data7) representing the rates of denaturation of carp actomyosin when protein solutions in KCl at different temperatures were frozen at various temperatures. These results indicate that the freeze-denaturation at -5, -11 and -20°C lessened in that order, but the denaturation at -3 and -4°C was much less than that at -5°C and was about the same as that at -20°C.

Thus, we inferred that the effect of temperature on denaturation of protein in the frozen solution system should be evaluated, with such a measure as Df value, from the viewpoint of the denaturation rate in the liquid phase of the frozen solution.

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References

*1 破綻 KCl 溶液中のたん白質の変性に対する温度効果の評価について。
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