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Interaction between Protein and Sugar during Frozen Storage

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Abstract

Interaction between protein and sugar during frozen protein was described from the view points of gel-filtration of protein solution containing sugar, effect of glucose on salting-out of actomyosin and effect of cryoprotectant on changes of secondary structure of protein. From these investigation it was assumed that the initial denaturation of protein by freezing was occurred by exposing hydrophobic side chains of protein and then thiol bond made protein insoluble.

It was found that the protective effect of sugar on protein during frozen storage was affected by the molecular weight, structure and number of hydroxyl group of sugar.¹⁾ However very little is known about the mechanism of protective effect. NOGUCHI²⁾ pointed out that when cryoprotectant having OH-group attached the side chain of protein molecule, hydration of protein was increased. Namely he concluded that the protective mechanism of sugar on protein during frozen storage was to protect the side chain of protein. On the other hand it was found from the experiments performed by authors that the effect of sorbitol was different from that of inositol. So it was investigated whether protein formed a complex with sugar.

This report describes about a trial of the separation of the complex by gel-filtration, the effect of sugar on salting-out of actomyosin and the effect of cryoprotectant on changes of secondary structure of protein.

Experimental Procedure

Experimental procedure except determination of radioactivity was described in previous report.¹⁾

Determination of radioactivity. Sample (0.5 ml) was added to 5 ml of toluene containing 4 g of 2, 5-diphenyloxazole (PPO), 0.1 g of 1, 4-bis[2-(5-phenyloxazol)] benzen (POPOP) and 500 ml of Triton X-100 per litre and the radioactivity was determined in a liquid scintillation spectrometer (Beckman LS-230).

Results

Gel-filtration of protein solution containing ¹⁴C-glucose. Glucose at a concentration of 3.24 mg/ml and trace amounts of ¹⁴C-glucose were added to protein

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solution (3.5 mg protein/ml) and stirred slowly overnight in a refrigerator. 0.5 ml of the mixture was applied to a column of Sephadex G-50 and eluted with 0.6 M KCl (pH 6.8) at a flow rate of 10 ml of per hr. Fractions of 10 ml were collected. Result was shown in Fig. 1.

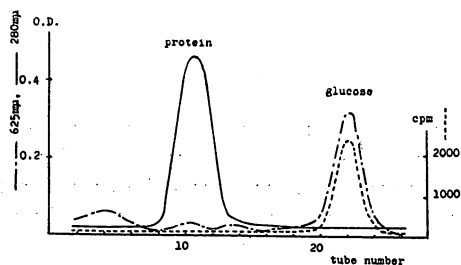


Fig. 1. Gel-filtration of protein containing ^{14}C -glucose ($38.2 \times 10^4 \text{cpm/mg}$) by Sephadex G-50. Column size: $1.5 \times 75 \text{ cm}$.

Gel-filtration of protein solution containing N-acetyl glucosamine. It was assumed that hydrophobic area of sugar binded to hydrophobic area of protein. So N-acetyl glucosamine containing hydrophobic group (acetyl group) was added to protein solution as cryoprotectant. N-acetyl glucosamine at a concentration of 2.21 mg/ml was added to protein solution (3.45 mg protein/ml) and stirred slowly overnight in a refrigerator. 0.5 ml of the mixture was applied to a column of Sephadex G-50. Result was shown in Fig. 2.

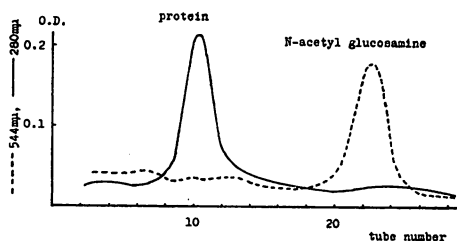


Fig. 2. Gel-filtration of protein containing N-acetyl glucosamine by Sephadex G-50. Column size: $1.5 \times 75 \text{ cm}$.

Experimental of salting-out. It is presumed that sugar makes to dissolve protein in salt solution easy. So experiments of salting-out of protein which was dissolved in 0.6 M KCl (pH 6.8) were performed.

Saturated ammonium sulfate solution was added to the solution (2 ml) containing 3.5 mg of protein and 36.0 mg of glucose per ml. The sample was stirred to avoid bubbling slowly and the turbidity of the sample was determined by the absorbance at $640 \text{ m}\mu$. The degree of salting-out was observed by the turbidity. Result was shown in Table 1.

Table 1. Effect of glucose on salting-out.

Glucose mM	Saturated Ammonium Sulfate (ml)			
	0.0	0.5	1.0	2.0
0	0.2132	0.1898	0.3615	0.2958
20	0.2147	0.1851	0.2888	0.1979

O. D. at 640 m μ **Effect of cryoprotectant for changes of secondary structure of protein.**

It was presumed that protective effect of sugar did not depend on the direct interaction between protein and sugar. So thiol group numbers of soluble and precipitated protein, fluorescent analysis as hydrophobic index of protein and intrinsic viscosity of protein were determined. Results were shown in Fig. 3.

Discussion

As shown in Fig. 1, protein was separated from glucose clearly and trace amounts of glucose did not bind to protein. The same result was obtained in the experiment using frozen protein for 1 week. It can be presumed that glucose does not affect the denaturation of protein directly, and that the interaction between glucose and water affects the denaturation of protein during frozen storage.

It was found from Fig. 2 that N-acetyl glucosamine did not bind to protein, and that the frozen protein for 1 week did not also bind to N-acetyl glucosamine. As shown in Fig. 1 and 2, both sugars, glucose and N-acetyl glucosamine, did not bind to protein.

Salting-out of protein was prevented by sugar (Table 1). It is said that the mechanism of salting-out is the phenomenon following the increased solvation. Taking into consideration that sugar did not bind to protein, the mechanism to prevent the denaturation of fish muscle protein containing substantial quantity of salt during frozen storage by sugar was the prevention of increasing of solvation by salt.

The quantity of soluble protein decreased rapidly during initial frozen storage and then scarcely changed (Fig. 3-A). Sorbitol prevented apparently protein from aggregation.

Numbers of thiol group of the whole sample containing sorbitol were little difference from that of the sorbitol-free sample (Fig. 3-B) and decreased gradually in both samples. However numbers of thiol group of the supernatant protein increased gradually. Furthermore it was assumed that SH-group in supernatant protein obtained from the sample containing sorbitol was protected (Fig. 3-C). On the other hand numbers of thiol group of the precipitated protein decreased rapidly initial 2 weeks and decreased gradually. As shown in Fig. 3-E, changes of quantity of hydrophobic area was significantly large and occurred quickly. It was presumed that freezing of protein brought about exposure of hydrophobic area of protein and disintegration of secondary

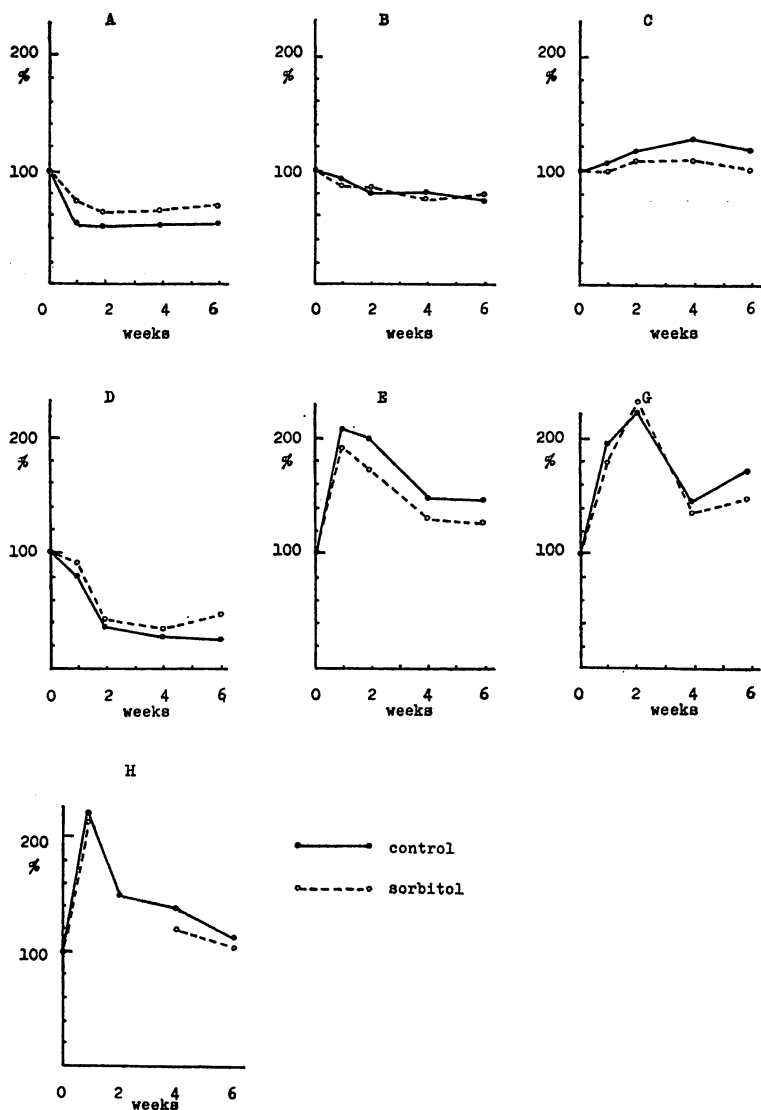


Fig. 3. Effect of cryoprotectant for changes of secondary structure of protein.

Protein containing 3.6 mg of sorbitol per ml and the sorbitol-free protein as control were frozen for appropriate periods of weeks. Whole sample was the suspension containing soluble protein and insoluble protein. After centrifugation of whole sample at $5,000 \times g$ for 20 min, the supernatant was used as fraction of soluble protein. Precipitated protein was obtained by calculation.

A: Soluble protein, B: SH-group in whole protein C: SH-group in supernatant protein D: SH-group in precipitated protein E: Hydrophobic area in whole protein F: Hydrophobic area in supernatant protein G: Hydrophobic area in precipitated protein

structure of protein occurred. Then thiol group of the supernatant protein was exposed.

The authors consider about the denaturation of protein during frozen storage as follows. Hydrophobic side chains of protein are exposed by freezing. Protein solubility decreases quickly owing to the aggregation of protein. Then protein precipitates step by step. The precipitated protein is combined successively each other by thiol bond.

References

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