

Denaturation of *Labeo rohita* (Rohu) Actomyosin on Frozen Storage — Preventive Effect of Carbohydrates

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The preventive effect of sucrose and glucose on the denaturation of frozen rohu actomyosin at -20°C for 7 weeks was examined using an *invitro* test model. The rate of denaturation was followed by estimating percentage salt extractability, Ca^{2+} ATPase activity and the clearing response test. Sucrose and glucose showed cryoprotective action for all concentration of actomyosin. Higher actomyosin concentration was preserved better than lower concentration. Post-rigor actomyosin was preserved to a greater extent than pre-rigor actomyosin. Correlation between percentage salt extractability and enzyme activity could not be observed in all samples of frozen actomyosin studied.

Meat can be preserved frozen for a long time without any appreciable spoilage. However, muscle myofibrillar proteins are denatured on frozen storage and this phenomenon is easily recognised in frozen fish. Dyer (1951), Connell (1960, 1962) have reported the deterioration of fish meat quality during frozen storage. Matsumoto & Noguchi (1971), Noguchi & Matsumoto (1975), Tsuchia *et al.* (1975) made a successful attempt to study the cryoprotective effect of various chemical additives to prevent denaturation of frozen carp actomyosin using an *invitro* model. The use of sugar compounds to prevent denaturation of frozen carp actomyosin was also reported by Arai & Takashi (1973).

However, some factors known to influence the extent of denaturation of fish actomyosin have yet to be studied using *invitro* model of actomyosin mixed with chemical additives. The present paper reports the effectiveness of carbohydrates in preventing denaturation of rohu (*Labeo rohita*) myofibrillar proteins (actomyosin) extracted from pre and post-rigor fish and with varying concentration of actomyosin on frozen storage.

Materials and Methods

Rohus were caught from a fresh-water lake with a hand net and instantaneously killed by a blow on the head. They were brought to the laboratory packed in polythene bag

filled with ice and used for extraction of pre-rigor actomyosin within one hour of their death. Their weight ranged between 400–500 g and none were spawning. Actomyosin was extracted by the method of Deng (1977) modified by Rao (1982). Post-rigor actomyosin was extracted from fish stored at $1-3^{\circ}\text{C}$ for 6 days after catch.

The protein concentration of the extract was estimated by the biuret method as described by Wootton (1974) using bovine serum albumin as standard. The actomyosin was mixed with carbohydrates in a final concentration of 0.1 M, pH 7. Some amount of actomyosin was withdrawn for assay of Ca^{2+} ATPase activity and clearing response test and the rest kept frozen at -20°C .

The Ca^{2+} ATPase activity was measured as described by Noguchi & Matsumoto (1970). The inorganic phosphate liberated due to ATPase activity was measured by the Fiske & Subbarow (1925) method.

The clearing response test was done as suggested by Noguchi & Matsumoto (1971) with some modification. In tubes 1 cm diameter and 10 cm length were added 1 ml actomyosin, 4 ml distilled water, 1 ml Tris-acetate buffer, pH 7 (0.24 M), 0.3 ml ATP (Na salt) each in a final concentration of 1 mM. The tubes were incubated at 20°C for 30 min. The clearing response was classified as follows.

- (a) Time for complete settling of actomyosin noted upto 10 min
- (b) Settling beyond 10 min, recorded as 10
- (c) Partial and incomplete settling was reported as +ve
- (d) No clearing response was shown as +ve

Protein extractability in salt solution of frozen actomyosin was studied after thawed samples (12 h, 5–7°C) were homogenised in a glass-homogeniser for 2 min, centrifuged at 3500 r.p.m. for 15 min and aliquots of supernatant withdrawn for protein estimation. Each experiment of actomyosin stored frozen with chemical additives was done in triplicate and mean of the results was noted.

Results and Discussion

Percentage salt extractability of rohu pre-rigor actomyosin stored frozen in the presence of sugars was greater at 10 mg/ml protein concentration compared to 6 mg/ml and 20 mg/ml (Table 1). The residual

Ca²⁺ ATPase activity for actomyosin concentration of 6 mg/ml was higher than for 10 mg/ml and 20 mg/ml for a week of frozen storage but at the end of seven weeks the values did not differ significantly (Table 2). Sucrose and glucose showed very similar preventive effect on denaturation of frozen actomyosin. Post-rigor actomyosin was protected from denaturation on frozen storage to a greater extent by carbohydrates as seen from solubility studies and Ca²⁺ ATPase activity than pre-rigor actomyosin (Table 1, 2). This observation was also supported by the results of the clearing response test (Table 3). The clearing response test for pre-rigor actomyosin stored with carbohydrates gave good results as the protein concentration increased.

Noguchi, *et al.* (1976) reported that some carbohydrates could prevent the denaturation of frozen carp actomyosin. Sucrose and glucose were considered good cryoprotective agents. Both were shown to possess similar protective action against denaturation of carp actomyosin during frozen storage. The present study also shows that

Table 1. Percentage salt extractability of rohu actomyosin stored at –20 °C in 0.7 M KC1 in the presence of carbohydrates

Additives (0.1 M)	Storage period (weeks)				
	1	3	5	7	
A	66.7	55.8	48.4	40.3	Pre-rigor actomyosin 6 mg/ml
B	72.2	65.3	60.3	56.3	
C	68.7	60.8	56.7	55.9	
A	54.0	50.0	41.0	32.0	Pre-rigor actomyosin 10 mg/ml
B	81.0	77.8	75.0	74.0	
C	77.8	76.0	74.0	71.0	
A	56.9	46.5	36.8	32.9	Pre-rigor actomyosin 20 mg/ml
B	69.5	66.5	64.7	61.1	
C	61.2	59.9	57.9	53.9	
A	64.7	52.0	44.0	35.0	Post-rigor actomyosin 20 mg/ml
B	98.6	71.5	64.0	62.0	
C	86.0	63.6	61.2	60.0	

Salt extractability of fresh actomyosin with respective additives was taken as 100

Key: A = Control (without additives), B = Sucrose
C = Glucose

Table 2. *Percentage residual Ca^{2+} ATPase activity of rohu actomyosin stored at -20°C in 0.7 M KCl in presence of carbohydrates*

Additives (0.1 M)	Storage period (weeks)				
	1	3	5	7	
A	36.0	21.8	18.6	12.7	Pre-rigor actomyosin 6 mg/ml
B	71.6	49.3	42.6	35.3	
C	69.7	52.4	40.4	31.8	
A	25.4	18.3	11.7	5.6	Pre-rigor actomyosin 10 mg/ml
B	48.2	40.8	35.2	21.6	
C	55.8	39.7	28.7	18.7	
A	35.7	25.9	12.9	7.4	Pre-rigor actomyosin 20 mg/ml
B	53.5	60.1	48.2	23.6	
C	52.3	53.3	45.1	28.8	
A	49.4	38.0	26.8	12.5	Post-rigor actomyosin 20 mg/ml
B	91.2	84.7	74.3	53.8	
C	73.3	66.7	57.1	56.7	

Ca^{++} ATPase activity of fresh actomyosin was taken as 100

Key: A = Control (without additives), B = Sucrose,
C = Glucose

Table 3. *Clearing response of fresh actomyosin and after storage at -20°C in 0.7 M KCl in the presence of carbohydrates*

Storage period (weeks)	Actomyosin	Control	Additives (0.1 M)	
			Sucrose	Glucose
0	A	10	+	+
	B	10	10	10
	C	8	8	8
	D	4	4	4
1	A	—	—	—
	B	—	+	+
	C	+	+	8
	D	10	6	4
3	A	—	—	—
	B	—	+	+
	C	—	10	10
	D	+	4	6
5	A	—	—	—
	B	—	—	—
	C	—	+	+
	D	—	6	6
7	A	—	—	—
	B	—	—	—
	C	—	—	—
	D	—	8	8

Key: A, B, C represents pre-rigor actomyosin 6, 10 & 20 mg/ml respectively, D represents post-rigor actomyosin 20 mg/ml

the preventive effect of sucrose and glucose on frozen storage denaturation does not differ significantly. This suggests that cryoprotective effect of different sugars on different fish actomyosin could be very similar.

The number of hydroxyl groups in sucrose is more than in glucose. Hence the mere presence of additional hydrophilic groups in a molecule does not seem to confer on it any advantage as a better cryoprotective agent against denaturation of frozen actomyosin. The importance of hydrophilic groups for cryoprotective action has been suggested by Tsuchiya *et al.* (1975) and Noguchi *et al.* (1976). Further, sucrose is a non-reducing sugar while glucose has a reducing group. This property does not seem to influence the cryoprotective action of these two carbohydrates.

It has been reported by Arai & Takashi (1973) that denaturation of carp actomyosin Ca^{2+} ATPase on frozen storage, accelerated as the protein concentration decreased below 4 mg/ml. It proceeded at a uniform rate as protein concentration increased above 5 mg/ml. In the present investigation rohu pre-rigor actomyosin stored frozen with and without carbohydrates for seven weeks showed two-stage decrease in solubility and Ca^{2+} ATPase activity for all concentration of protein (Tables 1, 2). In general it was observed that much denaturation occurred within the first week of frozen storage, (Tables 1, 2, 3). The post-rigor actomyosin showed a more uniform process of denaturation when stored with carbohydrates for seven weeks (Tables 1, 2).

Frozen post-rigor actomyosin stored with carbohydrates were preserved from denaturation to a greater extent compared to the pre-rigor actomyosin (Tables 1, 2, 3). Greater amount of actomyosin is extracted from post-rigor fish muscle as reported by Dingle *et al.* (1963). Connell (1960) proposed that myosin component of myofibrillar proteins was most labile to denaturation on frozen storage. Probably the greater amount of actomyosin extracted from post-rigor rohu muscle could be preserved to a greater extent from denaturation on frozen storage by carbohydrates, compared to pre-rigor actomyosin which may contain a large amount of free myosin. Certain changes

reported by Robson *et al.* (1967) to occur during the ageing of myofibrillar proteins in ice may have also contributed to the resistance to denaturation on frozen storage of post-rigor rohu actomyosin.

The clearing response test for low concentration of actomyosin was very poor whereas for 20 mg/ml pre-rigor actomyosin concentration the test gave good results until the fifth week of frozen storage (Table 3). These results are not in agreement with those of Noguchi & Matsumoto (1975) and Noguchi *et al.* (1976). Probably the carp used to extract actomyosin by these workers were purchased from the market and might have passed through rigor mortis and would have behaved like post-rigor samples the results of which are comparable with those of post-rigor rohu actomyosin. The clearing response test was better for post-rigor rohu actomyosin compared to pre-rigor actomyosin after frozen storage with carbohydrates (Tables 3).

Very low clearing response was observed with lower concentration of fresh pre-rigor rohu actomyosin as compared to a higher concentration (Table 3). This could be due to a higher concentration of myosin to actomyosin in the extract as explained earlier. The clearing response test depends on the critical concentration of actomyosin to myosin in solution as reported by Arai *et al.* (1974). Further the actomyosin extract used was diluted with salt solution to obtain solutions of low protein concentration. It is probable that some actomyosin may have split to myosin during dilution due to the effect of salt solution which can break actomyosin bonds to form free actin and myosin as reported by Ellis & Winchester (1959).

A strict correlation between Ca^{2+} ATPase activity, Mg^{2+} ATPase activity (clearing response) and salt extractability could not be observed in this investigation. It could be concluded that salt extractability is the least reliable of parameters to discuss fish actomyosin denaturation due to frozen storage in the presence of cryoprotective agents but the enzymic activity could be more informative. Some discrepancy in the correlation of results of ATPase activity and clearing response test could raise a

question whether these tests could indicate specific mode of denaturation of frozen actomyosin.

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