Denaturation of *Labeo rohita* (Rohu) Actomyosin on Frozen Storage-Preventive Effect of Dicarboxylic Acids

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The preventive effect of fumarate, maleate, tartrate and oxalate on the denaturation of frozen rohu actomyosin at-20°C in 0.7 M KC1 for 7 weeks was examined using an *in vitro* test model. The rate of denaturation was followed by estimating percentage salt extractability, $Ca^{2}+$ ATP-ase activity and the clearing response test. Fumarate, maleate and tartrate showed cryoprotective effect for higher concentration of pre-rigor rohu actomyosin of 10 mg/ml and 20 mg/ml. At actomyosin concentration of 6 mg/ml, maleate and tartrate showed some preventive effect whereas fumarate enhanced denaturation. Oxalate showed poor cryoprotective action. Post-rigor rohu actomyosin was preserved frozen without denaturation to a greater extent than pre-rigor actomyosin.

The deterioration of fish meat quality during frozen storage has been studied by many workers (Dyer & Dingle, 1961; Connell, 1960; 1962). Matsumoto & Noguchi (1971), Noguchi & Matsumoto (1975a), Tsuchiya *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 *in vitro* model. However, some of the factors known to influence the denaturation of fish actomyosin on frozen storage has not been studied by them.

Rao (1983a) reported that sucrose and glucose showed different cryoprotective effect on varying concentration of actomyosin and pre and post-rigor actomyosin. This paper reports the cryoprotective action of some dicarboxylic acids and rohu actomyosin.

Material and Methods

Unless otherwise stated methods and material used were similar to that reported by Rao (1983a). Actomyosin was extracted from rohu by a modified method described by Rao (1983 b). The clearing response test was done as described previously and was classified as follows.

a) Time for complete settling of actomyosin noted upto 10 min at invervals of 2 min.

b) Settling beyond 10 min and upto 60 min recorded as 10 +

c) Partial and incomplete settling was reported as + ve.

d) No clearing response was shown as -ve.

e) Super precipitation seen before clearing response was indicated by an asterisk along with the clearing response values.

Results and Discussion

The dicarboxylic acids maleate, fumarate and tartrate showed preventive effect on the denaturation of pre-rigor rohu actomyosin stored frozen at 10 mg/ml and 20 mg/ml protein concentration. At 6 mg/ml actomyosin concentration the cryoprotective effect was negligible for fumarate and tartrate whereas maleate showed some effect. These conclusions were drawn from the extent of solubility of actomyosin after frozen storage.

However, residual $Ca^{2}+$ ATP-ase activity showed enhanced denaturation of frozen stored actomyosin of 6 mg/ml concentration in the presence of fumarate as compared to controls without them. For higher rohu actomyosin concentration preventive effect on denaturation was observed with all the dicarboxylic acids as seen from residual $Ca^{2}+$ ATP-ase activity.

Table 1. *Percentage salt extractability of rohu actomyosin stored at-20°C in 0.7 M KCl in the presence of dicarboxylic acids*

Additives (0.1 M)	Storage period (weeks)				
	1	3	5	7	
A	66.7	55.8	48.4	40.3	Pre-rigor actomyosin 6mg/ml
B	66.7	55.0	50.2	48.4	
C	70.0	67.3	65.4	66.0	
D	66.7	61.3	56.3	50.4	
A	54.0	50.0	41.0	32.0	Pre-rigor actomyosin 10 mg/ml
B	72.0	72.0	71.1	50.0	
C	84.0	80.0	68.0	66.0	
D	63.3	62.9	56.0	50.0	
E	59.0	42.0	36.0	30.0	
A	56.9	46.5	36.8	32.9	Pre-rigor actomyosin 20 mg/ml
B	97.8	83.6	68.3	68.0	
C	90.7	80.0	75.6	71.1	
D	80.0	61.5	68.0	65.2	
E	60.4	35.6	27.9	28.4	
A	64.7	52.0	44.0	35.0	Post-rigor actomyosin 20 mg/ml
B	98.3	98.3	95.4	80.5	
C	98.3	95.4	90.6	92.4	
D	95.4	96.8	87.0	83.4	

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

Key: A = control (without additives)
B = Sodium fumarate
C = Sodium maleate
D = Sodium potassium tartrate
E = potassium oxalate

Post-rigor actomyosin was preserved better than pre-rigor actomyosin by fumarate, maleate and tartrate. Further, higher the concentration of actomyosin better the preventive effect of these cryoprotective compounds against denaturation due to frozen storage. These conclusions were drawn from the data of solubility in salt solution, residual Ca^{2+} ATP-ase activity and the clearing response test.

It has been reported by Noguchi & Matsumoto (1975b) that among the steroisomers fumaric and maleic, the former showed no preventive effect whereas the later showed a marked protective action against denaturation of frozen stored actomyosin. These workers had mixed carp actomyosin (2.5-4 mg/ml) with these acids to a final concentration of 0.1 M. For 6 mg/ml protein concentration the results reported in this study for rohu actomyosin with fumarate and maleate agreed with those of Japanese workers for carp actomyosin.

Fumaric acid is less soluble in water than maleic acid. Noguchi & Matsumoto (1975b) suggested this property of fumaric acid to be responsible for its poor cryoprotective action. However, this does not explain as to why fumaric acid has similar cryoprotective action as maleic acid when mixed with higher concentration of pre-rigor or post-rigor actomyosin. These observations have not been reported earlier.

Table 2. *Percentage residual Ca^2 + ATP-ase activity of rohu actomyosin stored at*-20°C *in* 0.7 *M KCI in presence of dicarboxylic acid*

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	27.7	18.6	15.2	9.3	
C	56.5	44.9	38.6	35.8	
D	52.7	43.2	35.7	30.6	
A	25.4	18.3	11.7	5.6	Pre-rigor actomyosin 10 mg/ml
B	45.2	30.7	33.0	28.8	
C	50.7	32.9	29.7	28.6	
D	56.2	34.9	29.8	28.7	
E	21.9	13.7	3.2	4.1	
A	35.7	25.9	12.8	7.4	Pre-rigor actomyosin 20 mg/ml
B	76.9	61.2	53.9	51.4	
C	66.7	56.7	51.2	48.2	
D	76.9	46.2	25.7	23.1	
E	28.1	9.4	4.2	6.3	
A	49.4	38.0	26.8	12.5	Post-rigor actomyosin 20mg/ml
B	79.3	71.3	62.1	58.3	
C	78.1	66.3	58.4	48.9	
D	80.3	65.3	46.2	38.7	

Ca^2 + ATP-ase activity of fresh actomyosin was taken as 100.

Key: A = control (without additives), B = Sodium fumarate
C = sodium maleate, D = sodium potassium tartrate,
E = potassium oxalate

Oxalate showed poor cryoprotective action on actomyosin as seen from results of percentage solubility, residual Ca^2+ ATP-ase activity and clearing response test. Maleic and fumaric acid differ in structure from oxalic acid in having an additional CH-CH chain separating the two carboxylic groups. This may suggest that the presence of double bond and increased chain length could be important in a compound for its cryoprotective ability.

Tartrate showed some protection against deterioration of actomyosin due to frozen storage. However this effect was less compared to those of fumarate and maleate at higher rohu actomyosin concentration, whereas at lower concentration the preventive effect on denaturation was similar to that seen for maleate. Tartaric acid has two additional hydroxyl groups to satisfy the carbon valency in the double bond in maleic acid. The additional hydroxyl groups does not seem to confer any additional cryoprotective ability on the compound. This was also reported earlier in the case of sucrose and glucose (Rao, 1983 a). But the presence of double bond in a compound could be advantageous for cryoprotective action.

The results of the clearing response test supported the results of the residual Ca^2+ ATP-ase activity to indicate the cryoprotective ability of different dicarboxylic acids mixed with rohu actomyosin. It is interesting to observe that the control samples without additives before frozen storage gave clearing response within 60 min and samples with additives without storage gave partial clearing response. As the clearing response test indicates Mg^2+ATP-ase activity it could be suggested that the additives may interfere

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

Storage period (weeks)	Actomyosin	Control	Sodium fumarate	Sodium maleate	Sodium potassium tartrate	Potassium oxalate
0	A	*10+	+	+	+	NS
	B	*10+	*10+	*10+	*10+	10+
	C	8	4	4	4	4
	D	4	4	4	4	NS
1	A	—	—	—	—	NS
	B	—	*10+	*10+	*10+	—
	C	+	4	8	4	—
	D	10	8	8*	8*	NS
3	A	—	—	—	—	NS
	B	—	—	*10+	—	—
	C	—	10	*10+	+	—
	D	+	4	6*	6	NS
5	A	—	—	—	—	NS
	B	—	—	—	—	—
	C	—	8	*10+	—	—
	D	—	6	*10+	2	NS
7	A	—	—	—	—	NS
	B	—	—	—	—	—
	C	—	10	*10+	—	—
	D	—	6	+	6	NS

NS = not studied

Key: A, B, C represents pre-rigor actomyosin, 6, 10 and 20 mg/ml respectively, D represents post-rigor actomyosin 20 mg/ml *super precipitation i.e. increase in turbidity was seen before clearing response

with this property of actomyosin. Probably one way of doing so is by sequestering magnesium ions. The poor clearing response of samples of 6 mg/ml proteins as compared to higher concentration of pre or post-rigor rohu actomyosin has been discussed in the previous paper (Rao, 1983a) and is thought to be due to these samples containing more myosin than actomysoin.

It has been reported by Murozuka *et al.* (1976) that a mixture of myosin and actomyosin at a weight ratio of 1:1 from tilapia muscle showed decreased ATP-ase activity at higher temperature to a greater extent than actomyosin alone but to a lesser extent than only myosin. This may explain the varied clearing response for control samples without frozen storage with different pre and post-rigor rohu actomyosin concentrations. The ratio of myosin to actomyosin in these samples could be the limiting factor.

Buttkus (1974) suggested that myosin molecules tend to aggregate during storage by forming disulphide bonds, leading to denaturation. However, actomyosin molecules are found to be less susceptible to this type of denaturation. In dilute solutions of actomyosin, the myosin to actomyosin ration will be usually high. As such, the tendency for this type of denaturation also will be more. The poor protective effect of dicarboxylic acids on dilute solutions of actomyosin thus probably indicate their inability to prevent this aggregations and consequent denaturation of the myosin present in these samples.

In general it was observed that stored actomyosin with residual Ca^{2+} ATP-ase activity of less than 40 percent showed poor clearing response test. However there was poor correlation between the extent of residual Ca^{2+} ATP-ase activity and percentage solubility of actomyosin after frozen storage. The enzyme activity was a more sensitive parameter to indicate denaturation. The percentage solubility data varies with methodology as reported by Oguni *et al.* (1975).

In some samples of rohu actomyosin preserved frozen with maleate and tartrate the clearing response was seen after super precipitation, that is, increase in turbidity. It is difficult to explain this phenomenon at present. These observations have not been reported earlier and needs further investigation.

In the present investigation pre and post-rigor rohu actomyosin stored with fumarate, maleate and tartrate showed a two stage decrease in solubility upto 10 mg/ml protein concentration. An initial rapid stage was followed by second slow stage of myofibrillar protein denaturation. At 20 mg/ml protein concentration of pre and post-rigor actomyosin there was uniform decrease in solubility.

The values of residual Ca^{2+}ATP-ase activity showed in general that there were two stages of loss in enzyme activity on frozen storage for all concentrations of pre and post-rigor actomyosin stored with dicarboxylic acids. However first stage tends to decrease with increased protein concentration.

I am grateful to Dr. B. P. Chakravarty, Retired Professor & Head, Department of Biochemistry, Grant Medical College, Bombay for his constructive criticism and suggestions during this study.

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