

コイ筋肉冷凍貯蔵中の乳酸脱水素酵素の失活防止

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Short Paper

Inactivation of Lactate Dehydrogenase Derived from Carp Muscle During Frozen Storage and Its Prevention*¹

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In the study of carp muscle lactate dehydrogenase (LDH, [EC 1.1.1.27]), we found that sodium glutamate prevented the inactivation of LDH during the process of extraction and purification from carp muscle.¹⁾ Sodium glutamate also prevented the freeze denaturation of fish muscle proteins²⁾ and purified LDH.³⁾ Based on these observations, the effect of sodium glutamate on the inactivation of LDH in carp muscle during frozen storage was investigated in this paper.

Live carp was decapitated and its dorsal lateral muscle (10 g, 2 cm × 2 cm × 2 cm) was stored for one week at -20°C and thawed at 4°C. Various amounts of sodium glutamate (0-0.5 g/g wet muscle) were added to the surfaces of carp muscle before freezing and the extracting solution. The methods of extraction and activity measurement of LDH were described previously.¹⁾

The protein concentrations of LDH samples were almost the same (13.8 mg/ml ± 2.36, n=17), although carp muscles were treated with sodium glutamate before freezing and/or extraction. Little difference of protein concentrations was also found between LDH sample extracted from fresh carp muscle and that extracted from freeze-thawed carp muscle. The specific activity of LDH extracted from fresh carp muscle was 30.1 units/mg. The activity of LDH extracted from freeze-thawed muscle was 28.5% of the activity extracted from fresh muscle. When 10 g of muscle was treated with 2.25 g of sodium glutamate (0.225 g/g wet muscle) before freezing, the preventive effect of sodium glutamate on freezing reached its maximum. Under this condition, extracted LDH retained 62.5% of the activity extracted from fresh muscle. Sodium glutamate was also effective against the inactivation of LDH during extraction from freeze-thawed carp muscle. The specific activity of LDH extracted with water was 7.1 units/mg, whereas the activity of LDH extracted with sodium glutamate solution (more than 0.05 M) was about 10.5 units/mg. When sodium glutamate was added to the muscle (0.225 g/g wet muscle) before

freezing, the concentration of sodium glutamate at successive extraction with water was 0.134 M. This concentration was high enough to prevent the inactivation of LDH during the extraction. As sodium glutamate seemed to prevent the inactivation of LDH during frozen storage and/or extraction, 5 different experiments illustrated in Table 1 (A-E) were performed. In this combination of experiments, the difference between the results of experiments A and B demonstrated the preventive effect of sodium glutamate on inactivation of LDH in the muscle during extraction. The difference between the results of experiments B and D demonstrated the effect during frozen storage. Final concentrations of sodium glutamate in the extraction media of experiments B and D were the same; those of C and E were also the same. Although the activities obtained from experiments B and C were almost the same, the activity obtained from experiment E was higher than that of D. These results indicate that sodium glutamate has a multiple preventive effect on inactivation of LDH in carp muscle during frozen storage and extraction.

The effect of sodium glutamate on freeze denaturation of proteins was attributed to the increment of the stability of the protein molecules by the association of sodium glutamate to the protein molecules or to the decrement of the ice crystal formation in the water phase caused by the presence of sodium glutamate molecules.²⁾ In the non-freeze condition, such as extraction and purification, association of sodium glutamate molecules onto the protein molecules was likely to occur.¹⁾ The results of the present study suggest that sodium glutamate is effective against the freeze denaturation of water soluble proteins in muscle. This effect is considered to be the same effect as occurs in fish myofibrillar proteins.

References

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Table 1. Test for the effective point of sodium glutamate on inactivation of LDH in carp muscle during frozen storage

Sample	Amount of sodium glutamate			Protein concn. (mg/ml)	Specific activity (units/mg)
	Added to muscle before freezing (g/g muscle)	Added to extracting solution (g/100 ml)	Final concn. (mol/l)		
A (Control)	0	0	0	12.1	4.40
B	0	2.25	0.134	14.9	5.85
C	0	4.50	0.267	14.3	6.09
D	0.225	0	0.134	13.7	9.18
E	0.225	2.25	0.267	12.0	12.2

Carp muscle was stored at -20°C for one week. After thawing at 4°C, LDH was extracted from the frozen stored muscle and the specific activity of the extracted LDH was measured.

*¹ Studies on denaturation of enzymes—II.

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