Studies on Rainbow Trout Aldolase I. Purification of the Aldolase from Rainbow Trout Muscle

Tohoku Journal of Agricultural Research
Vol. 21, No. 1, March, 1970
Printed in Japan

Studies on Rainbow Trout Aldolase
I. Purification of the Aldolase from Rainbow Trout Muscle

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(Received, March 3, 1970)

Summary

1. An enzyme, aldolase, was isolated from rainbow trout muscle in the form of needles by crystallization with ammonium sulfate at pH 7.5.
2. About 50 per cent of the aldolase activity and 9 per cent of the protein in an aqueous extract from rainbow trout muscle could be recovered as aldolase crystals by fractionation between 0.5 and 0.6 saturation with ammonium sulfate at pH 7.5.
3. Rainbow trout aldolase was about 60 per cent on the specific activity of rabbit aldolase.
4. Rainbow trout aldolase was electrophoretically and ultracentrifugally homogeneous.

That aldolase, an enzyme of the glycolytic chain, splits fructose-1,6-diphosphate into D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, was first described and investigated by Meyerhof and Lohmann (1) in 1934. Herbert, Gordon, Subrahmanyan and Green (2) purified aldolase from rabbit muscle as an amorphous product which was electrophoretically homogeneous. Warburg and Christian (3) crystallized aldolase from rat muscle and reported a value for the turnover number which was about twice the value found by Herbert et al. for their best preparation. Successively Taylor, Green and Cori (4) obtained aldolase in crystalline form from rat and rabbit muscles. In 1942 Engelhardt (5) reproted that myogen A, isolated from rabbit muscle by the method of Baranowski (6), contained the aldolase activity. Baranowski and Niederland (7) investigated this problem and concluded that myogen A of 15 or 35 per cent of aldolase activity was electrophoretically and ultracentrifugally homogeneous but neither myogen A was identical with aldolase.

On the other hand, there have been very little investigations on fish muscle aldolase. Shibata (8), and Kwon and Olcott (9) reported their studies on the purification and properties of aldolase from carp and tuna muscles respectively. They found that the aldolase activity of fish muscle was lower than that of rabbit.

But fish muscle aldolase has not yet been formed into crystals. Henrott (10) crystallized "myogen I" in the fraction between 0.48 and 0.60 saturation with ammonium sulfate fractionation from carp muscle, but he could not find any aldolase activity in it.

The present paper describes some studies on the crystallization and properties of aldolase from rainbow trout muscle. The best preparations were electrophoretically and ultracentrifugally homogeneous, but the specific activity was lower than that of rabbit aldolase.

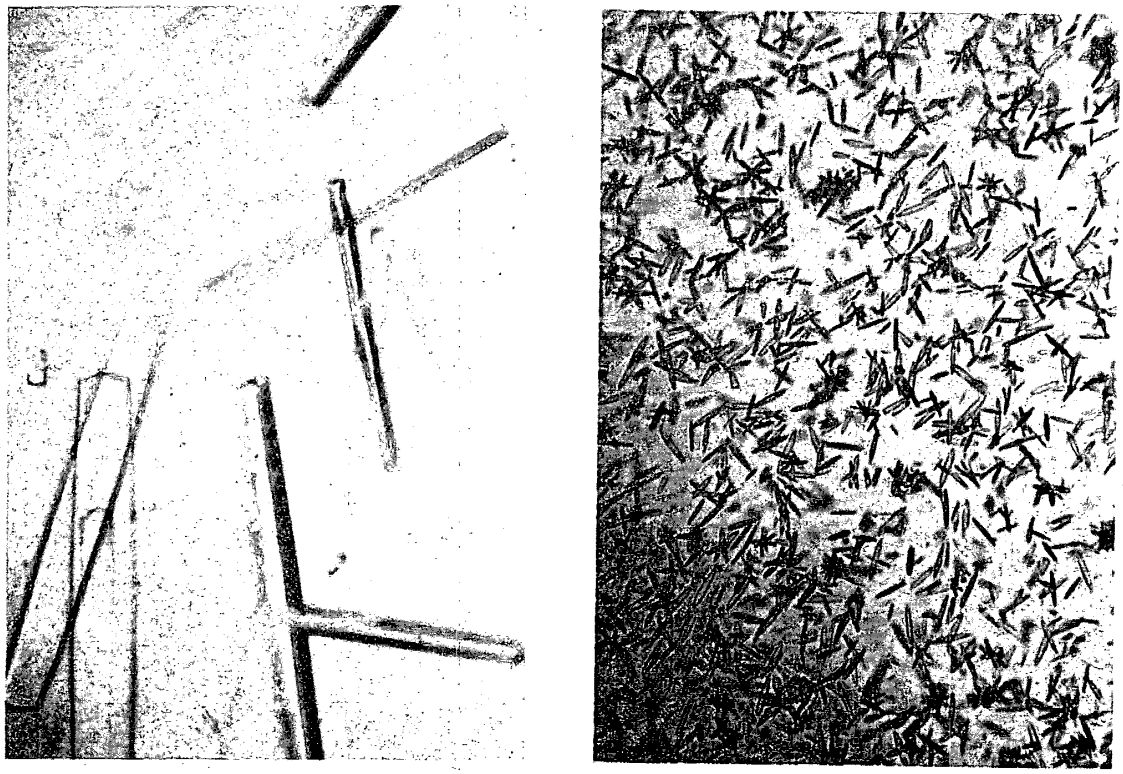
Experimental and Results

Materials

Live rainbow trouts (Salmo gairdnerii irideus) were obtained from the Zao Rainbow Trout Breeding Station, Miyagi Prefecture. Rabbits were provided for our study from the laboratory of Fisheries Biology in Faculty of Agriculture, Tohoku University.

Preparation of Aldolase

The method outlined by Taylor et al. (4) was used for the preparation of aldolase. A rainbow trout, which was from 800 g to 1000 g in body weight, was stunned by a blow on the head, decapitated, skined and filleted. The dark muscle



a b

FIG. 1. Crystals of rainbow trout aldolase in ammonium sulfate solution at neutral pH. a) crude crystals (magnification 150), b) recrystallized (magnification 150)

located under the lateral line was removed. The muscle thus obtained was cut into very small pieces with a knife at room temperature without delay. The remaining steps were carried out at 0–5°C. The minced flesh was continually stirred with 1.5 volume of cold water or cold 0.03 N KOH solution for one hour and centrifuged for 10 minutes at 8,000×g. The extraneous matter which accumulated at the top of the extract was removed through gauze. The filtrate was first brought to pH 7.5 with dilute NH_4OH and then to 0.5 saturation by the addition of an equal volume of ammonium sulfate solution saturated at room temperature and adjusted to pH 7.5 by the addition of concentrated NH_4OH. The solution stood at 5°C with stirring for an hour, and the precipitate was removed by centrifugation. The supernatant was brought to 0.6 saturation with solid ammonium sulfate (the ammonium sulfate saturation was calculated with a monogram of Brenner-Holzach and Staehelin (11)) and the precipitate was separated by centrifugation at 8,000×g for 20 minutes.

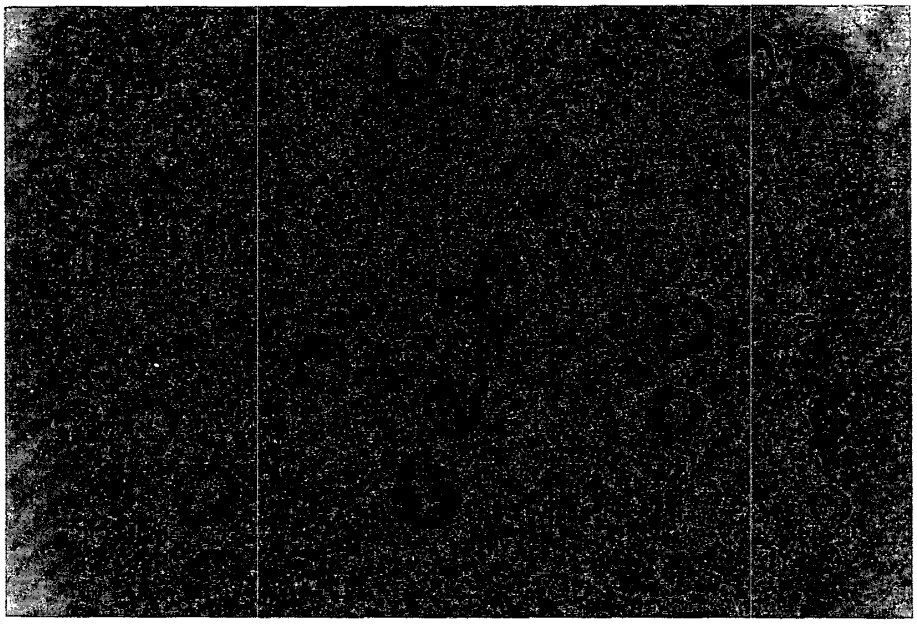


FIG. 2. Crystals of rabbit aldolase in ammonium sulfate solution at neutral pH. (magnification 150)

This precipitate was dissolved in a minimum amount of water. The solution was saturated with purified solid ammonium sulfate until a small amount of precipitate formed, and stood at 0–5°C. The presence of crystals can be detected by the "sheen" when the solution is stirred. The crystals were separated and precipitated in a lower layer of the solution within a few hours or a day in the form of needles (Fig. 1a). The same method was used for the preparation of rabbit aldolase and the crystals obtained were in the form of hexagonal bipyramid (Fig. 2).

Recrystallization: The precipitate of crude crystals was dissolved in a little

water and brought to pH 7.5. A small amount of amorphous materials was removed by filtration. Crystals began to separate upon the cautious addition of saturated $(NH_4)_2SO_4$ solution or purified solid $(NH_4)_2SO_4$ at less than 0.5 saturation in the cold (Fig. 1b). The yield of aldolase from rainbow trout muscle was about 2,000 mg per kg. Preparations of the crystallized enzyme were stored at 0–5°C in 0.6 saturated ammonium sulfate solution. Aldolase activity was determined by the method of Taylor et al. (4). The protein concentration (mg per ml) of the solution of crystalline aldolase was determined by the absorption at 280 mμ using the relation of (-log T)/Kl, where K=0.806 and 1 is length in cm. 1 unit represents 1 mg of inorganic phosphate transformed in 1 minute under the experimental conditions.

TABLE 1. *Partial Purification of Rainbow Trout Aldolase*

		Protein	Enzyme activity	Specific activity
Extract from 374g of muscle		mg 8,216	units 550	units/mg 0.067
Fractionation with $(NH_4)_2SO_4$	Saturation			
	50% supernatant	7,030	534	0.076
	50–60%	1,980	312	0.158
	60% supernatant	4,992	195	0.039
50–60% Fraction, Recrystallized				0.396

The results obtained from an ammonium sulfate fractionation of aldolase from rainbow trout muscle are recorded in Table 1. The fraction obtained between 0.5 and 0.6 saturation with $(NH_4)_2SO_4$ contained 57 per cent of the original aldolase activity and 24 per cent of the total protein in an aqueous extract. Kwon and Olcott (9) obtained 16 per cent and 3 per cent respectively in the fraction between 0.50 and 0.55 saturation with $(NH_4)_2SO_4$ from tuna muscle. While Taylor et al. (4) obtained 75 per cent and 10 per cent respectively in the fraction between 0.50 and 0.52 saturation in rabbit muscle. Therefore our data indicate that rainbow trout aldolase precipitates within a wider range of ammonium sulfate concentrations than that of Kwon and Olcott. The specific activity of crystalline aldolase from rainbow trout muscle was 60 per cent of that of rabbit aldolase. This agrees with the results of Shibata (8), and Kwon and Olcott (9) (Table 2).

Homogeneity of Crystalline Aldolase.

Electrophoresis: The electrophoretic homogeneity of crystalline preparations of rainbow trout and rabbit aldolase was investigated by Tiselius electrophoresis using an apparatus of the Hitachi HTB-II type and starch-gel electrophoresis. Experi-

TABLE 2. *Aldolase Activity of Various Preparations*

Investigators	Source	Specific activity
Shibata	carp	0.386
Kwon and Olcott	tuna	0.45
Taylor et al.	rabbit	0.69
Authors	rat	0.69
	rabbit	0.69
	rainbow trout	0.39

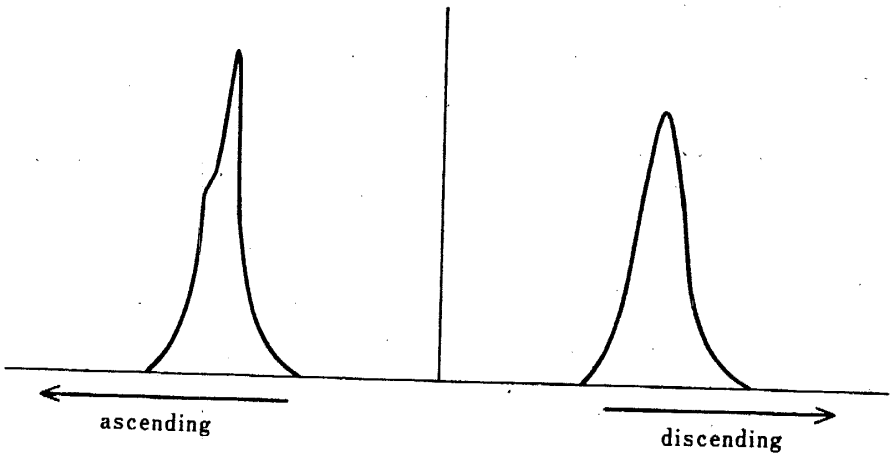


FIG. 3. Tiselius electrophoretic pattern of rainbow trout aldolase in phosphate buffer, pH 7.3, μ 0.15, after 240 min. at 30 volt (protein concentration 1.3%).

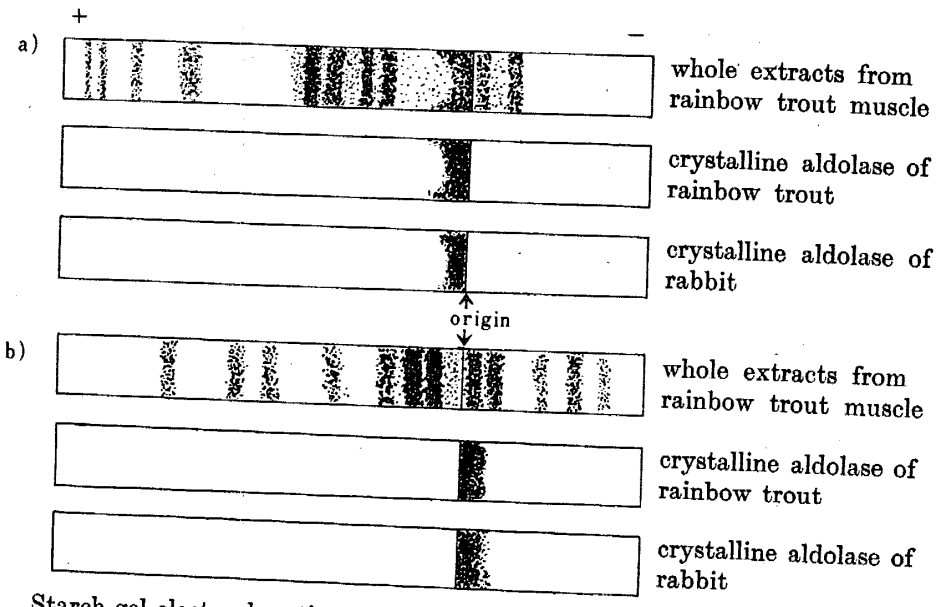


FIG. 4. Starch-gel electrophoretic patterns of crystalline aldolase from rainbow trout and rabbit muscle.
a) tris-citrate buffer, pH 8.6, μ 0.06. b) borate buffer, pH 8.45, μ 0.10.

ments were performed at room temperature or 5°C, in the pH range from 7.3 to 8.6, phosphate, tris-citrate or veronal buffer of ionic strength μ 0.06 or 0.15. The buffer system used was as follows; Tris-citrate buffer (pH 8.6, μ 0.06); 0.076M-tris (hydroxymethyl aminomethane), 0.005M-citrate. Borate buffer (pH 8.45, μ 0.10); 0.3M-H_3BO_4, 0.05M-NaOH. Phosphate buffer (pH 7.3, μ 0.15); 0.032M-Na_2HPO_4, 0.004M-NaH_2PO_4, 0.05M-NaCl.

In the Tiselius electrophoresis, the crystalline preparation of rainbow trout aldolase showed what we considered to be a single peak with a small shoulder (Fig. 3). In the starch-gel electrophoresis, the vertical method of Smithies was used as modified in a variety of ways (12, 13, 14). Both Rainbow trout and rabbit aldolases showed a single zone with similar electrophoretic mobility near the original spot in various buffer systems (Fig. 4). The results agreed with those obtained by Roberts and Tsuyuki (15).

Ultracentrifugation. The homogeneity of the crystalline preparation of rainbow trout aldolase was investigated by using an ultracentrifugal apparatus of the Hitachi UCA-I type. Experiments were performed at 20°C in the phosphate

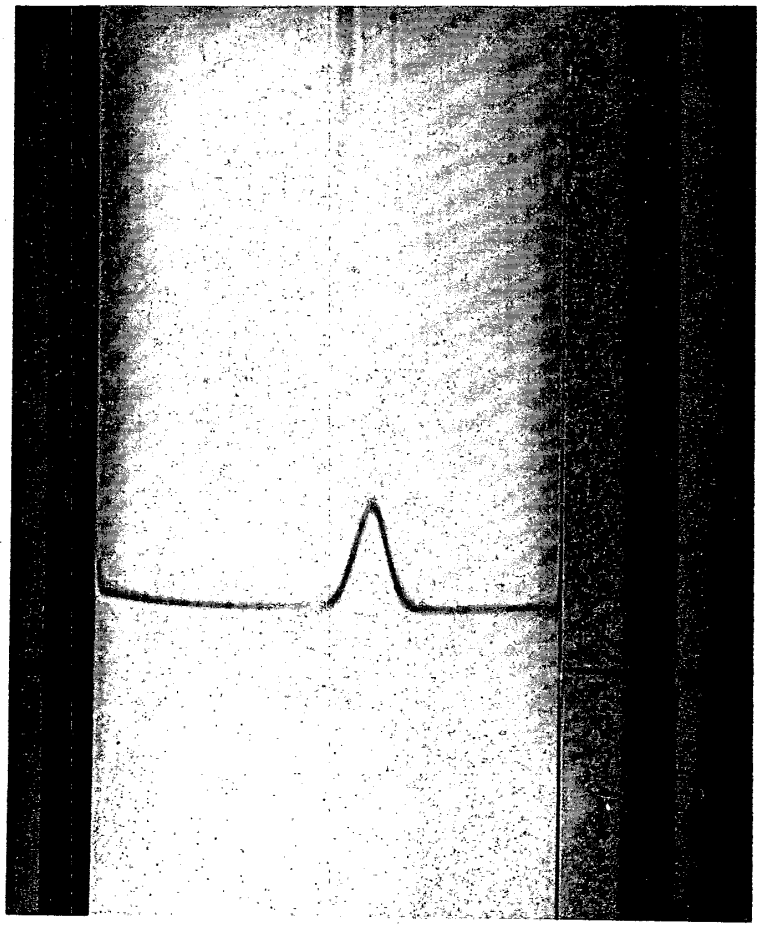


FIG. 5. Ultracentrifugal pattern of rainbow trout aldolase in phosphate buffer, pH 7.3, μ 0.15 at 60,000 r.p.m. after 48 min. (protein concentration 0.8%)

buffer, pH 7.3, μ0.15 at 60,000 r.p.m. (protein concentration 0.8%). During the ultracentrifugal sedimentation, rainbow trout aldolase showed a single peak (Fig. 5)

Discussion

It is said that the fish enzyme activity is generally lower than that of mammarian (8, 16). Shibata (8) found that the carp aldolase was equal to only about half of the full activity of rabbit aldolase, and Kwon and Olcott (9) also reported that the aldolase activity of tuna was about 60 per cent of the values of rabbit and rat. From these data it is conceivable that the content of enzyme in fish muscle is low or the enzyme is chemically unstable. We have noted that the crystalline aldolase from rainbow trout was reduced in activity because of regaining over 12 hours for preparation. While the rabbit aldolase prepared under the same conditions did not decrease its activity. On the supposition that the crystalline aldolase concerned is chemically homogeneous, we are now inclined to favor the view that fish aldolase is more labile than mammalian aldolase. As mentioned above, it is necessary to think about the purity of the crystalline aldolase. Baranowski and Niederland (7) reported that the crystalline myogen A prepared from rabbit muscle was not a single protein, though it was electrophoretically and ultracentrifugally homogeneous. It was proved that our crystalline aldolase did not contain glyceraldehyde-3-phosphate dehydrogenase by experiments using the staining method of Takeo (16) together with starch-gel electrophoresis. As already described, the starch-gel electrophoretic pattern of fish aldolase showed a diffuse or tailing zone in various buffer systems. In this case, however, it has not yet been decided whether this phenomenon depended upon the property of the enzyme or upon contamination from other proteins. As shown in Fig. 4, the electrophoretic pattern of the crude extract of enzyme had the same zone with crystalline aldolase. Therefore it is considered that the low activity of fish aldolase depends mainly upon the property native to the enzyme.

Acknowledgements

The authors wish to thank Dr. T. Kariya and Mrs. Y. Haga for assistance in obtaining various samples, Dr. K. Shimura and Mr. H. Imaide for helping in ultracentrifugal analysis, and Mr. M. Hishimoto for suggestion in carrying out electrophoresis.

References

1) Meyerhof, O. and Lohmann, K., *Biochem. Z.*, **271**, 89 (1934); 273, 413 (1934)
2) Herbert, D., Gordon, H., Subrahmanyan, V. and Green, D.E., *Biochem. J.* **34**, 1108 (1940)
3) Warburg, O. and Christian, W., *Biochem. Z.*, **314**, 149 (1943)
4) Taylor, J.F., Green, A.A. and Cori, G.T., *J. Biol. Chem.*, **173**, 591 (1948)

5) Engelhardt, W.A., *Yale J. Biol. and Med.*, **15**, 21 (1942–43)
6) Baranowski, T., *Z. Physiol. Chem.*, **260**, 43 (1939)
7) Baranowski, T. and Niederland, T.R., *J. Biol. Chem.*, **180**, 543 (1949)
8) Shibata, T., *Bull. Fac. Fish. Univ. Hokkaido*, **9**, 218 (1958) (in Japanese, with English summary)
9) Kwon, T. and Olcott, H., *Comp. Biochem. Biophys.*, **15**, 7 (1965)
10) Henrott, J.G., *Biochim. Biophys. Acta*, **39**, 103 (1960)
11) Brenner-Holzach, O. and Staehelin, M., *Helv. Physiol. Acta*, **11**, 212 (1953)
12) Aoki, K., "*Protein Nucleic acid Enzyme*", **9**(6), 474 (1964) (in Japanese, with English summary)
13) Honma, K. and Kojima, A., *SABCO J.*, **1**(1), 30 (1964) (in Japanese, with English summary)
14) Scopes, R.K., *Biochem. J.*, **107**, 139 (1968)
15) Roberts, E. and Tsuyuki, H., *Biochim. Biophys. Acta*, **73**, 673 (1963)
16) Takeo, K., *The Physico-Chemical Biology* **14**(1), 15 (1969) (in Japanese, with English summary)