Studies on Glucose-6-phosphatase of Aquatic Animals.—II. The Enzyme Activities in Fish Tissues*

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The existence of glucose-6-phosphatase [D-glucose-6-phosphate phosphohydrolase, EC, 3.1.3.9] has been reported in liver, kidney, and intestinal mucosa of terrestrial animals by numerous investigators¹⁻⁴⁾. Though the enzyme was recently found in the hepatopancreas of carp²⁾ and the liver of rainbow trout⁵⁾, no systematic study on the distribution of the enzyme in fishes has been reported.

In the previous paper⁶⁾, the enzymatic properties of hepatic G6Pase in flying fish and yellow-tail were reported. The present paper deals with the levels of G6Pase activity in various fishes.

Experimental

Fishes. Fishes as described below were used: flying fish, *Cypselurus heterurus döderleini*; barracuda, *Sphyræna schlegeli*; yellow-tail, *Seriola quinqueradiata*; white croaker, *Argyrosomus argentatus*; sea bream, *Chrysophrys major*; sea bass, *Lateolabrax japonicus*; carp, *Cyprinus carpio*; brook trout, *Salvelinus fontinalis*; ayu, *Plecoglossus altivelis*; eel, *Anguilla japonica*.

The marine fishes were caught in Wakasa Bay and used within or soon after the period of *rigor mortis* (Sea bass caught by angling was living). Carp, brook trout, and eel were cultivated fishes, and ayu was caught with a gill-net. These fresh-water fishes were living. The experiments were done in the period from September to October, 1965.

Preparation of enzyme solution. Liver, kidney, heart, digestive tract, pyloric caecum, dark meat, and ordinary meat were carefully removed from fish body, weighed, and placed on ice. Digestive tract was cutted lengthwise and washed with deionized water. Each of the organs was homogenized with 5~20 volumes of cold 0.25 M sucrose containing 0.001 M EDTA (pH 7.5) for 5 minutes. After the homogenate was centrifuged at 150×g for 10 minutes, the supernatant solution was used for G6Pase assay.

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Abbreviations used: G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; Pi, inorganic phosphate; EDTA, ethylenediamine tetraacetate.

Estimation of enzyme activity. G6Pase activity was measured as described in the previous paper⁶⁾. The amount of the enzyme which causes the release of 1 μ mole of Pi from G6P for 10 minutes at 40°C and pH 6.0 is defined as 1 unit of G6Pase.

Table 1. Glucose-6-phosphatase activities in various organs of flying fish.

Organ	Activity (units)*		
	per mg protein	per g organ	per organ
Liver	1.09	167	575
	0.88	154	610
	1.03	174	635
Kidney	0.26	18.5	5.6
	0.28	21.3	9.6
	0.11	10.2	5.6
Heart	0	0	0
	0.01	1.6	0.4
	0.05	4.8	1.7
Digestive tract	0.09	11.0	22.6
	0.08	8.1	21.5
	0.12	8.2	25.8
Dark meat	0.04	2.3	
	0.13	6.5	
	0.11	5.0	
Ordinary meat	0	0	
	0	0	
	±	±	

* The amount of enzyme which causes the release of 1 μ mole of Pi from G6P for 10 minutes at 40°C and pH 6.0 is defined as 1 enzyme unit.

Reaction mixture contained, in a final volume of 1.4 ml, 6 μ moles of G6P, 1 μ mole of EDTA, 30 μ moles of veronal buffer, and 0.2 ml of enzyme preparation.

Table 2. Glucose-6-phosphatase activities in various organs of barracuda.

Organ	Activity (units)		
	per mg protein	per g organ	per organ
Liver	1.34	178	552
	1.26	131	303
	1.39	186	409
Kidney	0.19	18.5	9.8
	0.19	22.6	15.8
Digestive tract	0.40	12.0	20.4
	0.11	5.2	6.8
Pyloric caecum	0.20	30.1	47.9
	0.07	9.0	17.1
	0.14	12.3	24.6
Dark meat	0.23	16.0	
	0.03	4.1	

Assay conditions are as in Table 1.

Results and Discussion

G6Pase activities in various organs of fish. Activities of G6Pase in the organs of flying fish and barracuda were compared. Values for G6Pase activity were calculated per mg protein, per g organ, and per whole organ. The results are given in Tables 1 and 2. From the results, it was evident that the highest level of the enzyme activity was found in the liver, and that most of the enzyme was distributed in the liver. In the case of fish, feeding on lower carbohydrate food compared with domestic animals, glucose seems to be formed mainly in the liver by the catalytic action of hepatic G6Pase from G6P, hydrolysate of glycogen and intermediate of gluconeogenesis, and to be transported into other tissues and used for energy source.

The enzyme was also detected in the kidney, digestive tract, and pyloric caecum, but its specific activities in these organs were very low in comparison with those in the livers. In the dark meat, some hydrolysis of G6P was observed, while not in

Table 3. Hepatic glucose-6-phosphatase activities of various fishes.

Species	Activity (units)			Body weight (g)	Fork length (cm)
	per mg protein	per g liver	per 100 g body wt.		
Flying fish	1.09	167	268	215	27.8
	0.88	154	257	245	28.4
	1.03	174	254	250	27.8
Barracuda	1.34	178	193	285	27.0
	1.26	131	148	195	30.0
	1.39	186	182	210	31.0
Yellow-tail	1.25 (0.08)*	204	177	520	31.0
	1.46 (0.08)	218	232	510	31.0
	1.18 (0.06)	166	146	375	28.0
White croaker	0.67 (0.06)	79	100	175	21.0
	1.24 (0.12)	160	101	190	22.0
	1.34 (0.15)	157	162	170	21.0
Sea bream	1.15 (0.04)	201	190	205	22.0
	0.96 (0.09)	165	107	200	21.5
	0.97 (0.11)	135	88	200	20.0
Sea bass	1.54 (0.02)	183	112	490	37.0
	2.10 (0.05)	257	153	540	39.0
Carp**	0.50 (0.02)	61	147	390	22.5
	0.32 (0.14)	28	71	345	23.5
	0.65 (0.14)	68	140	415	25.5
Brook trout	0.47 (0.10)	53	35	105	18.0
	0.32 (0.05)	41	41	100	18.0
	0.34 (0.05)	48	57	690	35.0
	0.35 (0.12)	44	36	715	36.5
Ayu	0.14 (0.03)	24	59	42.0	18.5
	0.22 (0.07)	35	74	39.5	16.5
	0.58 (0.14)	80	54	44.0	17.5
Eel	1.78 (0.02)	219	210	150	46.0
	1.66 (0.02)	202	207	145	47.0
	1.97 (0.02)	242	250	140	42.5

Assay conditions are as in Table 1.

* Value in parentheses is activity on β -glycerophosphate substituted for G6P.

** Hepatopancreas was used.

heart tissue and ordinary meat. Therefore, it seems that dark meat have a physiological function as well as the internal organs.

Hepatic G6Pase activities of various fishes. Activities of hepatic G6Pase in various species of fishes were compared. Values for the activity were calculated per mg protein, per g liver, and per 100 g of body weight. The results are given in Table 3. In order to investigate how much non-specific phosphatases participated in the hydrolysis of G6P, β -glycerophosphate was used for substrate. Activity of Pi-liberating enzyme on β -glycerophosphate is shown in the parentheses in Table 3.

Firstly, it was recognized that the levels of the hepatic G6Pase activity per mg protein in the marine fishes were higher than those in fresh-water ones, that is, 1~1.5 units compared to 0.2~0.5 units for the latter, and also that the levels of the activity per 100 g of body weight in the former were 100~250 units compared to 50~100 units for the latter. Consequently, the levels of hepatic G6Pase activity in the marine fishes were higher than in fresh-water ones. However, the enzyme activity in eel was at the similar level to that in the marine fish. The breeding migration of eel may partially account for this result.

Then, the levels of hepatic G6Pase activity per 100 g of body weight in the migratory fishes, such as flying fish, yellow-tail, and eel, were generally higher than those in non-migratory ones. It is interesting to note that the levels of hepatic G6Pase activity in fishes may relate to the scale of migration, though the species and numbers of the fishes examined were not so much.

In addition, the hydrolysis of β -glycerophosphate in the livers was so low that the liberation of Pi from G6P seems to be performed by specific G6Pase.

Effect of temperature on G6Pase activity. Effect of temperature on hepatic

Fig. 1. Temperature-activity curves of hepatic G6Pase of fishes.

○—○, Flying fish; ●---●, Barracuda;
○-·-·○, Yellow-tail; ●····●, Brook trout.

G6Pase activity in some species of fishes was examined. As shown in Fig. 1, the optimum temperature was found at 40°C for flying fish, yellow-tail, and barracuda,

but at 35°C for brook trout. This may suggest that optimum temperature of hepatic G6Pase in a fish relates to the environmental temperature of the fish.

Summary

Levels of glucose-6-phosphatase (G6Pase) activity in various fishes were investigated, and the results obtained were as follows:

1. The highest level of G6Pase activity in fish organs was found in liver, and low levels in kidney, digestive tract, pyloric caecum, and dark meat. But the enzyme was not detected in heart tissue and ordinary meat.

2. In the tested condition, the livers of marine or migratory fishes showed higher G6Pase activity than those of fresh-water or non-migratory fishes, respectively.

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