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Fish Muscle Riboside Hydrolases

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During an investigation concerning the cause and control of Maillard browning in heated flesh materials it was found that free ribose, in concentrations which occasionally exceeded 0·1 % of the wet weight, appeared when muscles of certain fishes were stored at 0° or at higher temperatures. This property was not exhibited by all fish to the same extent, but the muscles of those which were active formed ribose from added ribonucleic acid, adenosine triphosphate, ribonucleotides, ribosides and ribose 5-phosphate (Tarr, 1953, 1954*a*). The total pentose content of the muscles of certain marine fishes examined has ranged from 0·1 to 0·3 %, but it is not as yet known whether this occurs as free nucleic acid, as nucleic acid loosely combined with protein as in nucleotropomyosin (Hamoir, 1951*a*, *b*) or in some other form. Since nucleosides have not been observed to be present in appreciable amounts in muscles of fish which have been frozen in liquid nitrogen while still alive, but are often present in relatively high concentrations in such tissue *post mortem*, it would seem probable that they arise by enzymic degradation of nucleic acid. This suggests that fish muscles contain a ribonuclease and other enzymes which hydrolyse ribonucleic acid to its constituent mononucleosides.

The formation of free ribose from these mononucleosides could occur by their direct hydrolysis by riboside hydrolase enzymes, or by nucleoside phosphorolysis followed by hydrolysis of the ribose 1-phosphate formed. Riboside hydrolase enzymes have not often been described, phosphorylative mechanisms apparently occurring with greater frequency (Laskowski, 1951). However, Carter (1951) isolated a specific non-phosphorolytic uridine nucleosidase from yeast, and Lampen & Wang (1952) obtained both purine and pyrimidine hydrolytic nucleosidases from *Escherichia coli*. Ribose was not formed from either ribose 1-phosphate or ribose 5-phosphate by these two last-named preparations. The present experiments, which have already been described briefly (Tarr, 1954*a*), have shown that fish muscles contain non-phosphorolytic nucleosidases.

EXPERIMENTAL

Materials. The nucleosides, ribose, barium ribose 5-phosphate and yeast ribose nucleic acid were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, or from Schwarz Laboratories, Mount Vernon, New York. Cation exchange resin XE 64, a finely ground form of IRC 50, was purchased from Rohm and Haas Co., Philadelphia, Pennsylvania. The resin was washed and suspended in 0·2 M sodium phosphate buffer as described by Hirs, Moore & Stein (1953).

Paper electrophoresis. The technique used was practically identical with that described by Kunkel & Tiselius (1951). Enzyme preparations were freeze-dried before dissolving in 0·1 M veronal buffer (pH 8·6) for application in amounts not exceeding 0·05 ml. to Munktell 20S paper. The conditions used were a 16 hr. development at about 3° and 6 v/cm. The papers were stained with bromophenol blue and eluted into 0·01 N-NaOH as outlined by the above investigators.

Paper chromatography. Ribose was determined quantitatively by paper chromatography (Tarr, 1954*b*). Five separate 10 μl. amounts of reaction mixture were applied to Whatman no. 1 papers. The papers were developed 16 hr. at 20–22° with ethyl acetate–acetic acid–water solvent (3:1:3, by vol.) (Jermyn & Isherwood, 1949) and sprayed with aniline hydrogen phthalate reagent (Partridge, 1949). The above solvent separates D-ribose from other pentose sugars (Hochster & Watson, 1954), and it was found that the pentose formed from ribosides by the enzymes to be described had an R_F value identical with that of D-ribose. Nucleosides and their corresponding purine and pyrimidine bases were determined by quantitative paper chromatography (Paladini & Leloir, 1952). Five 5 μl. amounts were applied to Whatman no. 1 papers, which were developed for 3 hr. at 37° with freshly prepared ethyl acetate–acetic acid–water (3:1:1·75, by vol.) solvent. The R_F values were not

absolutely consistent with this system, but rarely varied more than ±0·02 unit. The following R_F values were found: adenosine 0·6, adenine 0·68, guanosine 0·38, guanine 0·45, inosine 0·40, hypoxanthine 0·53, xanthosine 0·39, xanthine 0·50, cytidine 0·40, cytosine 0·51, uridine 0·56 and uracil 0·62. With guanine, xanthosine and xanthine, only a few micrograms could be applied to the paper, since overloading and consequent streaking tended to occur. Quantitative determinations were not made in these instances.

Chromatographic separation of ribose 1-phosphate and ribose 5-phosphate from ribose was carried out on Whatman no. 1 paper with *n*-butanol–acetic acid–water (1:1:0·5, by vol.) solvent. The enzyme reaction mixtures containing the pentose phosphate esters were applied in 5 μl. amounts to Whatman no. 1 paper, and were separated by ascending development with the above solvent for 8 hr. at 0°, at which temperature ribose 1-phosphate was not hydrolysed. With this system ribose 5-phosphate and ribose 1-phosphate had rather similar R_F values (0·31 and 0·37 respectively) and could be readily distinguished from ribose (R_F 0·5). The chromatograms were dried for 3 min. at 105° to remove the solvent, sprayed with aniline hydrogen phthalate reagent (Partridge, 1949) containing 0·5 ml. concentrated HCl/100 ml., and heated as usual.

Protein nitrogen. The method of Kingsley (1939) was used and was checked by micro-Kjeldahl determinations.

Enzyme preparation

Lingcod (*Ophiodon elongatus*) or rock cod (*Sebastodes* sp.) muscle from fish in rigor mortis was used. Muscle tissue which had been stored at −20° for several months also yielded satisfactory preparations. All procedures were conducted at about 0°. In a typical experiment 1·6 kg. of muscle were blended with 4·8 l. water and 204 g. of NaCl to bring the suspension to approximately 0·6 M with respect to this salt. N-HCl (240 ml.) was added slowly with rapid stirring to adjust the mixture to pH 4·6. The mixture on centrifuging at 2500 *g* yielded 4·7 l. of supernatant liquid. This was fractionated with $(NH_4)_2SO_4$ at pH 7·0. The fraction which precipitated between 0·4 and 0·6 saturation was collected by centrifuging, dissolved in water and dialysed for 2 days against repeated changes of demineralized water to yield 1·6 l. of crude riboside hydrolase containing 1 mg./ml. of protein N. Further purification was effected by treatment with cation-exchange resin XE 64 as follows.

The crude enzyme (90 ml.) was mixed with 450 ml. of wet packed XE 64 resin adjusted to pH 7·3, and the mixture kept for 30 min. at 0° with occasional stirring. The supernatant liquid was decanted, and the resin washed with fifteen successive 100 ml. portions of water by decantation. The enzyme preparation was eluted from the resin (about 450 ml.) by stirring in 66 g. NaCl (equivalent to about 2·5 M) gradually, and pouring the suspension on to a Whatman no. 4 conical filter. The resin was treated with 30 ml. of 2·5 M-NaCl and the surface then pressed firmly with a flattened glass rod to remove as much free liquid as possible. The eluate, after dialysing 18 hr. at 0° against several changes of demineralized water, measured 168 ml. and had 0·016 mg. protein N/ml. Analyses of the resin supernatants, washings and eluates in this and similar preparations showed that at least 95 % of the protein N initially present in the crude enzyme used could be accounted for. These resin eluates have been referred to as purified enzyme preparations. Neither crude nor purified enzyme preparations form ribose in absence of added nucleoside.

Experimental conditions. Unless otherwise stated, the nucleosides were used in 0·0133 M final concentration (equivalent to 2 mg./ml. of ribose), and were heated to effect solution where necessary. Xanthosine solutions were adjusted to pH 7. Enzyme reactions were carried out in small stoppered tubes containing 0·1 ml. each of nucleoside and buffer solutions and 0·2 ml. of enzyme preparation. The sodium phosphate and sodium acetate buffers used were 0·2 M with respect to the cations, and sodium veronal buffer 0·1 M with respect to diethylbarbituric acid.

RESULTS

Recovery and relative activities of enzyme preparations. The relative activity and recovery obtained with such preparations could not be assessed very accurately for there were considerable variations in their power to hydrolyse different ribosides. The calculations were also somewhat complicated by the facts that whole muscle contains undetermined amounts of ribosides, and its riboside hydrolase activity did not appear to be noticeably affected by pH, as is the case with the enzyme preparations. Thus with whole lingcod muscle, ribose formation with and without added inosine was found to be similar at natural pH (6·6) or at pH 5·7. The data given in Table 1 show that one purified preparation hydrolysed guanosine over 1500 times as actively on a protein basis as did whole muscle, but that the purification procedures, especially that involving the ion-exchange resin, resulted in very poor recoveries.

Electrophoresis experiments. Paper electrophoresis showed that crude riboside hydrolase preparations yielded two (and occasionally three) protein zones, while purified preparations yielded only a single zone which did not move appreciably from the point of application. This suggested that riboside hydrolase activity may be associated with a protein which does not migrate appreciably by either electrophoresis or electro-osmosis under the experimental conditions. However, although inosine was hydrolysed actively by preparations which had been applied to filter papers moistened with 0·1 M sodium veronal buffer pH 8·6, even after these had been stored several days at 0°, the enzyme activity was rapidly lost on the application of an electric potential (4–6 v/cm.). The reason for this loss of activity has not yet been established.

pH–*Activity relationship.* Crude preparations hydrolysed the four purine ribosides and cytidine, but not uridine, ribonucleic acid or ribonucleotides, at pH 5·6. On the other hand, at pH 8·6 inosine was the only riboside which was hydrolysed appreciably, though some crude preparations, which contained an active adenosine deaminase, also formed ribose from this substrate. The results obtained in a typical experiment are shown in Table 2.

Table 1. *Comparative activity and recovery of riboside hydrolases*

Blended whole muscle (3 g.) at natural pH 6·6 + nucleoside solution or water (1 ml.) formed the following amounts of ribose in 1 hr. at 37°: control, 220; guanosine, 290 and inosine, 620 μg./g. (1 g. ≃ 26 mg. protein N). The crude enzyme (0·5 mg. protein N/ml. final concentration) formed 1700 μg./ml. of ribose from guanosine and 1000 μg./ml. from inosine in 3 hr. at 37° in 0·1 M acetate buffer pH 5·0. The freeze-dried purified preparation (0·04 mg. protein N/ml.) formed 1070 μg./ml. of ribose from guanosine and 1060 μg./ml. from inosine in 3 hr. at 37° in 0·1 M acetate buffer pH 5·5, while the other purified preparation (0·005 mg. protein N/ml.) formed 520 μg./ml. of ribose from guanosine in 6 hr. under similar conditions. Neither crude nor purified enzyme preparations formed ribose in absence of added nucleosides.

Preparation studied	Ribose (μg.) formed in 1 hr. at 37° by 1 mg. of protein N*		Ribose (mg.) formed by 100 g. of original muscle or by the quantity of enzyme recovered from that amount of muscle†	
	Inosine	Guanosine	Inosine	Guanosine
Muscle	23·8	11·1	38·8	82·9
Crude enzyme	666 (28)	1134 (102)	28·4 (73)	16·6 (20)
Purified enzyme (freeze-dried)	8830 (370)	8930 (805)	4·2 (10·8)	4·12 (4·97)
Purified enzyme (not freeze-dried)	—	17 400 (1582)	—	4·70 (5·67)

* The figures in parentheses refer to the increase in activity in terms of that of the whole muscle.

† The figures in parentheses denote the actual percentage of the initial riboside hydrolase activity of the muscle which could be accounted for in given enzyme preparations.

Table 2. *Hydrolysis of ribosides at different* pH *values by a crude enzyme preparation*

Conditions: 0·46 mg./ml. protein N; 0·1 M phosphate buffer pH 5·6 or 0·05 M veronal buffer pH 8·6; incubation temperature 37°.

	Ribose (μg./ml.)			
	Initial pH 5·6		Initial pH 8·6	
	1 hr.	2 hr.	1 hr.	2 hr.
Adenosine	490	820	50	110
Guanosine	800	1130	50	50
Inosine	520	850	380	1020
Xanthosine	230	180	0	0
Cytidine	160	175	0	0

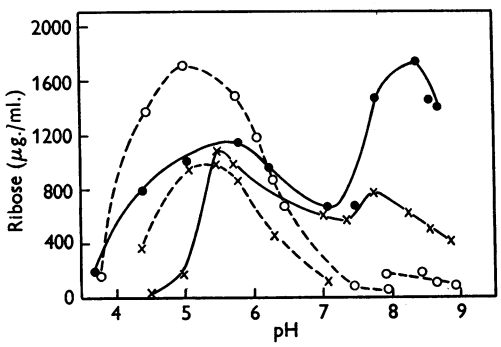

Fig. 1. pH/Activity curves of riboside hydrolase preparations with guanosine and inosine as substrates. Crude enzyme, inosine (●—●); purified enzyme, inosine (×—×); crude enzyme, guanosine (○- - -○); purified enzyme, guanosine (×----×). Crude enzyme, not freeze-dried, 0·5 mg. protein N/ml.; purified enzyme, freeze-dried, 0·04 mg. protein N/ml. Incubation period 3 hr. at 37°. Buffers: pH 3·7–5·5, 0·1 M acetate; pH 5·5–7·8, 0·1 M phosphate; pH 7·8–9·0, 0·05 M veronal.

Further experiments with crude and purified preparations confirmed and extended these initial findings. Fig. 1 shows the pH/activity curves which were obtained when crude and purified enzyme preparations hydrolysed guanosine and inosine. Guanosine was hydrolysed most actively at pH 5·0 by the crude preparations, and at pH 5·5 by the purified enzyme preparations. No hydrolysis of this riboside occurred at pH values above 7 with the purified enzyme, though the crude enzyme exhibited slight activity in this region. With inosine as substrate both the crude and purified preparations exhibited two pH optima, one in the acid and the other in the alkaline region. This indicated that the purified riboside hydrolase preparations possessed two distinct enzymes, one which hydrolysed inosine with a pH optimum of about 8, and another which hydrolysed purine ribosides and cytidine and having a pH optimum of about 5·5. With purified enzyme preparations hydrolysis proceeded at approximately the same rate in phosphate or acetate buffers at pH 5·5, and in veronal or phosphate buffers at pH 8·0, and it therefore was concluded that the type of buffer had no important effect on the pH–activity relationship.

Substrate specificity. The substrate specificity of purified enzyme preparations was studied at both acid and alkaline pH optima. Lingcod muscle itself hydrolyses uridine (Tarr, 1954*b*), and since neither crude nor purified enzyme preparations from this source do this, it would seem likely that the muscle possesses a uridine hydrolase which is distinct from the present enzymes. The results of an experiment in which single ribosides and mixtures of ribosides were hydrolysed with a purified enzyme preparation at pH 5·5 are given in Table 3. They

Table 3. *Ribose formation from single ribonucleosides and from mixtures containing equal amounts of two ribonucleosides at* pH 5·5

Protein N 0·033 mg./ml.; 0·1 M acetate buffer pH 5·5; 3 hr. incubation at 37°.

Substrate	Ribose concentration in nucleosides (mg./ml.)	Ribose formed (μg./ml.)	Theoretical amount of ribose assuming that both ribosides were hydrolysed independently (μg./ml.)
Adenosine	2	490	—
Adenosine	4	620	—
Inosine	2	470	—
Inosine	4	580	—
Guanosine	2	600	—
Guanosine	4	790	—
Xanthosine	2	150	—
Xanthosine	4	160	—
Cytidine	2	100	—
Cytidine	4	160	—
Uridine	2	0	—
Uridine	4	0	—
Adenosine + inosine	4	570	960
Adenosine + guanosine	4	510	1090
Adenosine + xanthosine	4	510	640
Adenosine + cytidine	4	505	590
Adenosine + uridine	4	360	490
Inosine + guanosine	4	570	1070
Inosine + xanthosine	4	500	620
Inosine + cytidine	4	500	570
Inosine + uridine	4	280	470
Guanosine + xanthosine	4	520	750
Guanosine + cytidine	4	660	700
Guanosine + uridine	4	560	600
Xanthosine + cytidine	4	180	250
Xanthosine + uridine	4	170	150
Cytidine + uridine	4	50	100

Table 4. *Ribose formation from single ribonucleosides and from mixtures containing equal amounts of two ribonucleosides at* pH 8·6

Protein N 0·063 mg./ml.; 0·1 M veronal buffer pH 8·6; 4 hr. incubation at 37°.

Substrate	Ribose concentration in nucleoside(s) (mg./ml.)	Ribose formed (μg./ml.)
Adenosine	2	0
Adenosine	4	0
Inosine	2	260
Inosine	4	350
Guanosine	2	0
Xanthosine	2	0
Cytidine	2	0
Uridine	2	0
Inosine + adenosine	4	< 25
Inosine + guanosine	4	255
Inosine + xanthosine	4	275
Inosine + cytidine	4	260
Inosine + uridine	4	245

indicate that nucleoside hydrolysis in the acid pH range is probably due to a single enzyme since in no instance did a mixture of two different nucleosides yield the amount of ribose which would be expected if separate enzymes were involved. In fact, in almost every instance the amount of ribose formed from a mixture of two ribonucleosides was slightly less than that formed from any single riboside at the 4 mg./g. ribose equivalent level. The existence of this competitive effect, and lack of an additive one, would lend support to the assumption that the enzyme which has a pH optimum of about 5·5 is a non-specific purine and pyrimidine nucleoside hydrolase.

The substrate specificity of the enzyme which had a pH optimum of about 8·0 was quite different. It hydrolysed only inosine, and this hydrolysis was almost completely inhibited by adenosine, and not by other purine ribosides (Table 4). It is therefore a specific inosine hydrolase.

Temperature–activity relationship. Fish muscle acid riboside hydrolase is active over a wide range of temperatures. The action was comparatively slow between 0 and 20°, but increased markedly above 20° as is shown in Table 5. At 50 and 55° hydrolysis was very rapid but the enzyme soon became inactive.

Proof that direct hydrolysis of ribosides occurs. Purified riboside hydrolase was incubated with various ribosides at pH 5·5, and the simultaneous appearance of both free ribose and the corresponding bases was determined qualitatively and, in several

instances, quantitatively by paper chromatography. The qualitative tests showed that hydrolysis of a given ribonucleoside was accompanied by simultaneous formation of free ribose and the corresponding purine or pyrimidine base. Table 6 shows that the amount of ribose which appeared on hydrolysis was, in those instances tested, about equivalent to that which was calculated from the quantity of purine or pyrimidine formed.

A purified freeze-dried riboside hydrolase preparation (0·06 mg. protein N/ml.) was incubated 3 hr. at 37° in 0·05M veronal buffer pH 8·6 or in 0·1M acetate buffer pH 5·5 containing 2 mg./ml. of ribose 1-phosphate or ribose 5-phosphate, or 4 mg./ml. of inosine. The reaction mixtures were analysed for ribose and for the ribose phosphates by paper chromatography. The preparation hydrolysed inosine at pH 5·5 (2200 μg./ml. of ribose) and at pH 8·6 (590 μg./ml. of ribose). However, no ribose could be detected when amounts of reaction mixtures equivalent to 20 μg. of ribose 1-phosphate or ribose 5-phosphate were chromatographed at 0° as described above. Since the reagent used to spray the chromatogram allows the detection of 0·25 μg. of ribose, it was concluded that purified riboside hydrolase does not hydrolyse either of the ribose phosphate esters. This indicates that the enzyme is a true hydrolase and that phosphorolysis of the ribosides is not involved. The fact that purified preparations which have been thoroughly dialysed against demineralized water hydrolyse ribosides equally well in acetate or phosphate buffer of the same pH values also indicates that these enzymes are strictly nucleoside hydrolases.

Isolation of D-*ribose and xanthine.* Guanosine (500 mg.) was incubated at 37° with 40 ml. of crude riboside hydrolase containing 1·5 mg. protein N/ml. The mixture was adjusted to pH 5·0 with 0·2N-HCl and was stirred gently, the pH being kept between 5·0 and 5·5 by regular addition of HCl. After 10 hr. the pH did not rise appreciably, and quantitative chromatographic analysis indicated that approximately the theoretical amount of ribose was present (265 mg.). The mixture was brought to pH 4·6, chilled to 0°, and the precipitate of protein and purine base removed by centrifuging at 0° and 14000 *g*. The precipitate was suspended in 20 ml. of water and the centrifuging repeated. The clear supernatant fluids were pooled, freeze-dried, and the dry residue extracted by shaking mechanically with four successive 25 ml. portions of 99% ethanol. The clear filtrate was dried *in vacuo* over H_2SO_4 and ribose diphenylhydrazone prepared from the residue by the method of Mandl & Neuberg (1952). Crude ribose diphenylhydrazone (364 mg., m.p. 142°) was obtained in 65% of the theoretical yield. The crude material was recrystallized from hot chloroform and its characteristics were compared with those of similarly crystallized ribose diphenylhydrazone prepared from an authentic sample of D-ribose. (Mixed m.p. and m.p. of authentic and unknown 137–138°; C, 64·66; H, 6·50. Calc. for $C_{17}H_{20}N_2O_4$: C, 64·54, H, 6·37%. The following values were found for $[\alpha]_D^{23}$ in methanol.

Table 5. *Temperature–activity relationships of a purified riboside hydrolase preparation*

0·044 mg. protein N/ml.; 0·05M phosphate buffer pH 5·6.

Temp. (°)	Ribose (μg./ml.)					
	Guanosine			Inosine		
	1 hr.	2 hr.	4 hr.	1 hr.	2 hr.	4 hr.
20	50	—	120	60	—	140
25	140	—	300	185	—	200
30	145	—	500	180	—	310
37	180	460	1120	—	230	580
45	270	610	920	—	330	570
50	310	560	—	—	400	—
55	500	505	—	220	505	—

Table 6. *Hydrolysis of ribosides with simultaneous liberation of purine or pyrimidine base and of ribose*

Ribosides 0·0266M, purified enzyme 0·1 mg. protein N/ml., 0·1M acetate buffer pH 5·5, 37°.

Substrate	Time (hr.)	Corresponding base found (μg./10 μl.)	Ribose	
			Found (μg./10 μl.)	Calc. (μg./10 μl.)
Adenosine	1	6·97	8·00	7·75
	2	9·53	12·68	10·6
	4	23·7	30·4	26·3
Inosine	1	3·68	4·24	4·05
	2	14·8	15·2	16·3
Xanthosine	2	8·2	7·10	8·09
Cytidine	2	7·7	8·00	11·4

Table 7. *Stability of freeze-dried riboside hydrolase*

Enzyme corresponded to 2 ml. purified preparation. To this was added 0·2 ml. inosine (equiv. to 4 mg./ml. ribose) and 0·2 ml. 0·2M acetate buffer pH 5·5. 2 hr. incubation at 37°.

Storage conditions		Ribose formed from inosine
Days	Temp. (°)	(μg./ml.)
1	−20	1180
1	0	1190
1	20	1050
18	−20	1110
18	0	1130
18	37	1035

Authentic: initial, −3·81°; 24 hr., −7·27°; 48 hr., −3·1°. Unknown: initial, −5·37°; 24 hr., −7·70°; 48 hr., −3·8°.)

The residue from the ethanol extraction was shaken several hours at room temperature with 10% NH_4OH (v/v) and the insoluble residue removed by centrifuging. The supernatant liquid was evaporated *in vacuo* over H_2SO_4 to yield 280 mg. of a dry, white residue, which had an absorption spectrum in the ultraviolet region similar to that of xanthine. The purity was only 55% as calculated from the molar extinction coefficient in 0·1N-NaOH at 285 mμ. This represented 57% of the theoretical amount, assuming both complete hydrolysis of the guanosine and deamination of the guanine formed. The crude material was stirred for 1 hr. with 25 ml. N-HCl, filtered with suction and the filtrate adjusted to pH 7 with 2·5N-NaOH. The white crystalline material which formed on standing overnight at 0° was collected on a sintered glass filter, washed with water, and dried over P_2O_5 *in vacuo* (yield 120 mg.). This material, when dissolved in 0·1N-NaOH, had an ultraviolet extinction coefficient at 285 mμ. characteristic of xanthine. $\epsilon^{10^{-4}}_{calc.}$ 0·99, found 0·92 (93%) (Stimson & Reuter, 1943, Hotchkiss, 1948). The following absorption ratios were found: 290/280 = 0·87, 250/260 = 1·08, 280/250 = 1·96; literature values for xanthine 0·91, 1·12 and 2·04 respectively (Hotchkiss, 1948).

Stability of freeze-dried preparations. A purified preparation which had been dialysed 18 hr. against demineralized water and which was not quite free from sodium chloride was freeze-dried in 2 ml. portions in small vials which were sealed and stored at four different temperatures. The results of activity tests (Table 7) indicate that freeze-dried preparations are fairly stable for short periods. No prolonged storage tests have yet been made. Tests indicate that the presence of a trace of NaCl in these preparations improves their stability.

SUMMARY

1. A method of preparation of crude riboside hydrolase enzymes from muscles of certain marine fishes is described. The muscle is extracted with cold 0·5–0·6M-NaCl or KCl at pH 4·6 and the extract fractionated with $(NH_4)_2SO_4$ at pH 7, the fraction obtained between 0·4 and 0·6 saturation possessing most of the activity.

2. Crude preparations were purified by stirring with Amberlite cation-exchange resin XE 64 at pH values slightly above 7, eluting with NaCl and dialysing.

3. Crude preparations contained two (and occasionally three) proteins as judged by zone electrophoresis while purified preparations showed a single protein zone.

4. The purest preparation obtained hydrolysed guanosine over 1500 times as actively as did whole muscle on a protein N basis.

5. Purified preparations contained two distinct enzymes, a non-specific riboside hydrolase with a pH optimum of about 5·5 which hydrolysed purine ribosides and cytidine, and a specific inosine hydrolase with a pH optimum of about 8·0. These enzymes were not separated. Neither ribose 1-phosphate nor ribose 5-phosphate was hydrolysed, and nucleoside hydrolysis proceeded in the absence of added inorganic phosphate.

6. D-Ribose diphenylhydrazone and xanthine were isolated from a reaction mixture of guanosine and crude riboside hydrolase.

7. The enzyme preparations withstood freeze-drying and were active even at 55°.

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