makes the assumptions that there is no randomization of isotope from C_1 to C_4, C_5 or C_6, and that the oxidation of C_1 of glucose via the hexose monophosphate pathway predominates over that of carbon C_2 and C_3, we can calculate[8] that 75 % of the CO_2 produced from glucose would come via this pathway. However, these assumptions are doubtful[5,8] and this percentage is thus considered as semi-quantitative[9].

The existence of an active pentose pathway in the thyroid tissue implies a supply of reduced triphosphopyridine nucleotide, which is, in fact, required in the deiodination of iodotyrosines[2,3] by a microsomal enzyme and may be the most efficient coenzyme for iodide binding to protein in the thyroid homogenate[10].

Since the hexose monophosphate pathway seems to be an active metabolic route for glucose in the adrenal gland[7] and in testis[11], the possibility of this being a common property of endocrine glands should be considered.

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Intracellular ribonuclease from *Bacillus subtilis*

NISHIMURA AND NOMURA previously reported that *Bacillus subtilis* accumulated large amounts of ribonucleases in a culture medium; one of these enzymes was highly purified[1,2]. The specificity, molecular weight and immunological nature of these extracellular enzymes have also been described[3].

The present report deals with the presence of an intracellular RNase in this organism which has quite different properties from the extracellular RNases. A lysozyme lysate was prepared from bacteria which had been harvested from the culture medium (70 h) by centrifugation, and washed 3 times with 0.05 *M* phosphate buffer, pH 7.3. The lysate showed a small amount of RNase activity, measured by a modi-

Abbreviations: RNA, ribonucleic acid; RNase, ribonuclease; EDTA, ethylenediaminetetracetic acid.

fication of the method of FRISH-NIGGEMEYER AND REDDI[4]. This activity was not inhibited at all by the addition of anti-extracellular RNases which had been shown substantially to inhibit extracellular RNases.

The RNase in the lysate was precipitated at pH 4, and completely inactivated by heating at 100° for 5 min. As shown in Fig. 1, the pH optimum of the enzyme was about 5.8 whereas that of the extracellular RNases were 7.5. The intracellular RNase was completely inhibited by the addition of $1.7 \cdot 10^{-3}$ *M* EDTA, although the same concentration of EDTA had no effect on extracellular RNases as indicated in Table I.

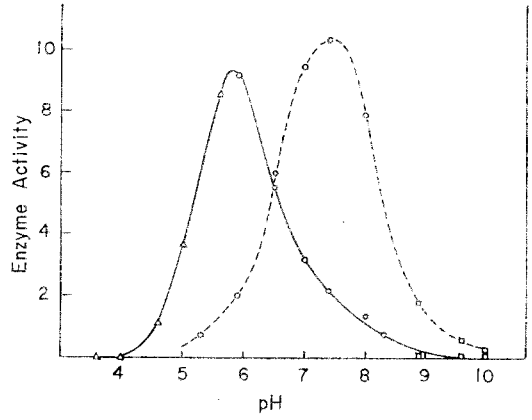

Fig. 1. pH-activity curves of intracellular and extracellular RNases. The final concentration of the various buffers was 0.067 *M*. △, Acetate; ○, phosphate; □, carbonate-bicarbonate; —, intracellular RNase; - - -, extracellular RNase (peak II).

TABLE I

EFFECT OF EDTA ON INTRACELLULAR AND EXTRACELLULAR RNASE

EDTA (M)	*Relative activity*		
	Extracellular RNase peak I	*Extracellular RNase peak II*	*Intracellular RNase*
$1.7 \cdot 10^{-2}$	—	100	0
$1.7 \cdot 10^{-3}$	100	100	0
$1.7 \cdot 10^{-4}$	100	100	58
0	100	100	100

Intracellular RNase was purified about 17 times by the following method: The lysate was centrifuged for 20 min at 27,000 × *g* and the supernatant fractionated with $(NH_4)_2SO_4$. The precipitate between 0.4 and 0.7 saturation at pH 5.6 was collected, dissolved in a small volume of water, and dialyzed overnight at 4°. 1 vol. 10% streptomycin was added at pH 7.3 to 10 vol. of this dialysate. After standing for 2 h, the precipitate formed was removed by centrifugation. At this point the recovery of the enzyme in the supernatant was about 70%. The enzyme was then precipitated at 0.7 saturation with $(NH_4)_2SO_4$. The precipitate was taken up in a small volume of water, dialyzed to remove salt and fractionated on the starch-electrophoresis apparatus (2 cm^2 × 40 cm). As shown in Fig. 2, the intracellular RNase could be separated from the phosphodiesterase which was in the original lysate.

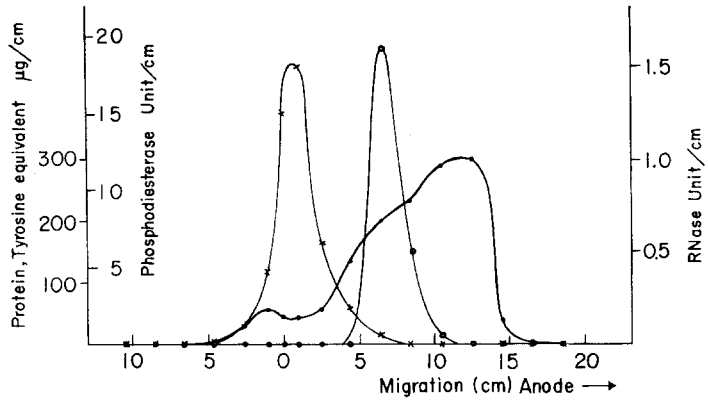

Fig. 2. Zone electrophoresis of intracellular RNase. The runs were made at 4° with a potential gradient of 7 V/cm for approximately 5.5 h. The starch was treated with 0.05 *M* tris(hydroxymethyl)aminomethane buffer, pH 8.3. The charged enzyme preparation contained protein, 2,700 μg (tyrosine equivalent); RNase, 5.0 units (the activity corresponding to 1 μg of crystalline pancreatic RNase at optimum pH was defined as one RNase unit), and 85 phosphodiesterase units determined in a reaction mixture which contained 1 μmole Ca[bis(*p*-nitrophenyl)phosphate]$_2$, 50 μmoles tris(hydroxymethyl)aminomethane, pH 8.3, and aliquot of the enzyme in 2 ml of water. The amount of enzyme which caused an absorbancy increment at 440 mμ of 0.05 in 1 h at 30° was defined as one enzyme unit. ○, RNase; ×, phosphodiesterase; ●, protein.

Purified RNase produced all four constituent mononucleotides from yeast RNA. Nucleosides or free bases could not be detected. All four cyclic mononucleotides could be isolated from the dialysate, when yeast RNA was hydrolyzed by this enzyme in cellulose tubing suspended in a large volume of water. The identification of these cyclic mononucleotides was made by the method of MARKHAM AND SMITH[5].

The total activity of extracellular alkaline RNases in the culture medium after 70-h incubation was about 500 times larger than that of intracellular acid RNase in the cells. On the other hand alkaline RNases which were detected in the cell lysate were less than 1 % of the intracellular acid RNase, although the neutralizing units for anti-extracellular RNases was much larger than a theoretical value calculated from the enzyme activity. All of the intracellular acid RNase was contained in the supernatant fraction, and could not be detected in cell debris. These facts indicated the presence and distribution of two types of RNases, *i.e.* alkaline and acid RNases, in this organism which were different from the two RNases described for mammalian tissues[6,7].

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