BIOSYNTHESIS OF HISTAMINE RIBOTIDE AND IMIDAZOLEACETATE RIBOTIDE [1]

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Karjala (1955) and Tabor and Hayaishi (1955) discovered independently the presence of imidazoleacetate riboside in the urine of rats previously injected with C^{14} labeled histamine or imidazoleacetate. On the other hand Alivisatos (1958) reported the biosynthesis of a histamine dinucleotide (a DPN molecule in which histamine replaces the nicotinamide moiety). The last compound was obtained when histamine and DPN were incubated with a partially purified beef spleen DPNase. These findings suggested that the search for ribotides which could be involved in the biosynthesis of the compounds cited above might be interesting, having in mind two reasons: (1) Ribosides commonly originate from ribotides through the action of phosphatases. (2) The occurrence of a histamine ribotide could open the possibility for another biosynthetic pathway for the histamine dinucleotide, not involving the exchange reaction and DPNase, but histamine ribotide and ATP. If this proved to be true, the pathway would be similar to that of the biosynthesis of DPN that involves nicotinamide ribotide and ATP (Kornberg, 1950).

This report deals with the biosynthesis and partial characterization of the ribotides of histamine and imidazoleacetate. Both compounds seem to be formed through reactions involving 5-phosphoribosylpyrophosphate (PRPP), a known intermediate in the

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biosynthesis of ribotides, including those containing imidazole rings (Kornberg, Lieberman and Simms, 1955). The provisional equations for the biosynthesis we will report are:

$$\text{Histamine} + \text{PRPP} \xrightarrow{-\text{PP}} \text{HRP (Histamine Ribotide)}$$

$$\text{Imidazoleacetate} + \text{PRPP} \xrightarrow{-\text{PP}} \text{IARP (Imidazoleacetate Ribotide)}$$

METHODS

The enzyme source was the supernatant obtained by centrifugation (20,000 g, 10 min.) of a 5% aqueous homogenate of mucosa of dog small intestine. The incubation mixtures contained the substances indicated in TABLE I. After 4 hrs. of incubation at 37ºC the extent of formation of the ribotides of histamine and imidazoleacetate was measured after applying the incubation mixture onto a column of DOWEX-50 H^+ (200-400 mesh, X 12) of 2 ml of bed volume per ml of incubation mixture. The effluent obtained with 0.1 N HCl contains both ribotides. These are sharply separated from free imidazoleacetate (2 N HCl) and histamine (6 N HCl) as shown in Fig. 1(A). The preliminary characterization of the ribotides was achieved by adding a large excess of acid-washed Norite to the 0.1 N HCl effluent (1 ml of sedimented Norite per 40 ml of effluent). After thorough washing, the sedimented Norite was eluted with 50% ethanol (pH = 5 to 6). Very little optical density at 260 mμ comes off the Norite under these conditions. This small U.V. absorption was removed by DOWEX-1 Cl^- (200-400 mesh, X 2) chromatography of the Norite eluate. Histamine and imidazoleacetate ribotides were promptly eluted with 0.005 N HCl. Practically all the radioactivity and no U.V. absorption come off in the first three resin bed volumes. The pooled eluates were evaporated in a dessicator and dissolved in a small volume of cold water. The sample thus obtained was used for phosphorus determination, imidazole ring content (Tabor, 1957) and radioactivity measurements before and

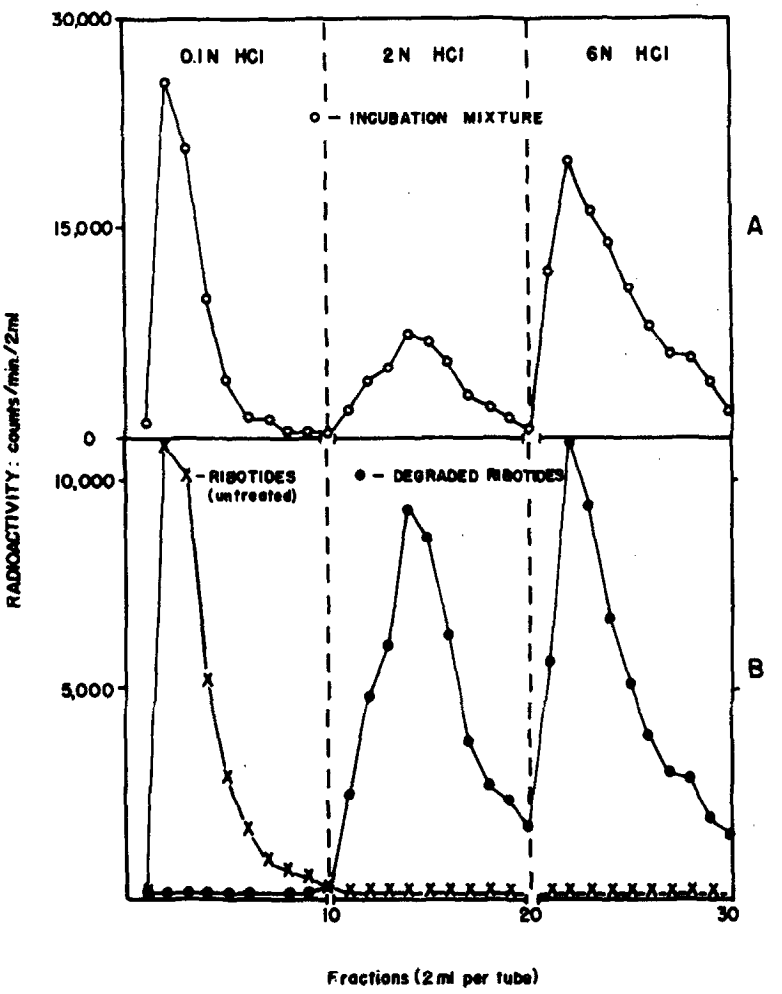

Fig. 1: Dowex 50 (H^+) Chromatography of the incubation mixture and of the acid hydrolysed ribotides

after acid hydrolysis. The hydrolysis was done in a sealed tube, in 0.1 N HCl, for 5 hrs. at 145-150°C. DOWEX-50 H^+ chromatography similar to that already described, was employed to study the products of the acid hydrolysis of the ribotides, as shown in Fig. 1(B). Chromatography of the unhydrolysed sample was run for comparison.

RESULTS AND DISCUSSION

The data in Fig. 1(A) indicate that about 35% of the histamine was converted to ribotides and 14% appears as free imidazoleacetate.

Furthermore both ribotides are formed in about the same amount as shown in Fig. 1(B); hydrolysis of the mixed ribotides yield essentially equal amounts of histamine and imidazoleacetate. Although both ribotides did not react with orcinol, which is in agreement with Tabor and Hayaishi's findings with imidazoleacetate riboside (1955), they do react with periodate.

The effects of PRPP, ATP, imidazoleacetate, Tris buffer and aminoguanidine, on the synthesis of the ribotides can be seen in TABLE I. It may be that the imidazoleacetate inhibition is due to the dilution of that C^{14} imidazoleacetate originating from the C^{14} histamine through histaminase action. Extracts of mucosa of small intestine are rich in histaminase, an enzyme strongly inhibited by aminoguanidine (Zeller et al, 1952). The effect of ATP on the ribotide synthesis remains to be

T A B L E I

Effect of some substances on the synthesis of the ribotides of histamine and imidazoleacetate.

The incubation mixture (1 ml) contains: Histamine 2-(ring) C^{14} dihydrochloride (1.3 µmoles, S.A. = 1.1×10^6 counts/min/µmole), PRPP (1.9 µmoles), ATP (5.0 µmoles), potassium phosphate buffer pH = 7.6 (125 µmoles), KF (50 µmoles) $MgCl_2$ (2.0 µmoles) and the enzyme (3 to 12 mg of protein). Aminoguanidine (0.01 µmole) and unlabeled imidazoleacetate (13 µmoles). The data express the results obtained after DOWEX-50 H^+ chromatography of 0.2 ml of the incubation mixture.

Incubation mixture	mµmoles of HRP and IARP	mµmoles of free imidazoleacetate
Complete	45.4	49.0
Complete - PRPP	19.6	67.6
Complete - ATP	4.6	85.9
Complete + Aminoguanidine	8.5	9.6
Complete + Imidazoleacetate	23.3	29.2
Complete - Phosphate + Tris	2.2	21.4

explained. There is enough PRPP at the end of the incubation time (about 1 μmole), so that the ATP effect does not seems to be due to the resynthesis of PRPP.

Our preliminary proposal of the identity of the compounds as HRP and IARP rest on the following data beyond the chromatographic results already shown: (1)Both compounds appear to form borate complexes and these are more firmly retained on DOWEX-1 (Khym and Cohn, 1953). (2) The two isolated compounds, as with other ribonucleotides, show little movement on Whatman 1 paper using n-propanol-NH_3 (Ames and Mitchell, 1952), while with the hydrolysed samples,all the radioactivity migrated with histamine a n d imidazoleacetate (Rf = 0.59 and 0.29, respectively). (3) A sample of the isolated compounds containing 0.66 μmole per ml of the ribotides (measured by radioactivity), was found to contain 0.76 μmole per ml of ribose (measured by periodate consumption, according to Dixon and Lipkin, 1954) and 0.80 μmole per ml of phosphate (Fiske and Subbarow, 1925). (4) Snake venom 5'-nucleotidase splits off the phosphate of both ribotides to the corresponding ribosides. The ribosides thus formed behave like free histamine and imidazoleacetate on DOWEX-50.

During our study of the effect of ATP on the reaction, Crowley (1960) showed that rabbit liver enzyme catalysed a similar reaction leading to the synthesis of imidazoleacetate ribotide. Our results, including the requirement for ATP, are in agreement with his findings.

It is difficult at present to visualise the importance of the histamine ribotide. It would be interesting to determine whether histamine ribotide is an intermediate in the synthesis of the histamine dinucleotide. With respect to a possible role of the histamine dinucleotide, it was found that nicotinamide and its analogs, known to be inhibitors of animal DPNase, inhibit the release of histamine in the anaphylactic reaction <u>in vitro</u> (Mota, Silva and Fernandes, 1960).

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