RESISTANCE TO ACID HYDROLYSIS OF N-RIBOSYL DERIVATIVES OF VARIOUS SUBSTITUTED IMIDAZOLES AND PYRIMIDINE IMIDAZOLES (PURINES)*

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Diphosphopyridine nucleotide (DPN) reacts in the presence of a soluble, purified beef spleen DPN-ase (Alivisatos and Woolley, 1956) with 5-amino-4-carboxamido-imidazole (Alivisatos and Woolley, 1955) or histamine (Alivisatos, 1958) to yield displacement products in which the nicotinamide moiety of DPN is irreversibly replaced by the imidazole (imidazolysis; Alivisatos, 1959). The N-ribosyl bond of the product of the former reaction, the diphospho-(5-amino-4-carboxamido-imidazole) nucleotide, is identical to the N-ribosyl bond present in the well known precursor of purines, (5-amino-4-carboxamido-imidazole)-ribonucleotide (Alivisatos et al, 1958). l-Histidine is almost unreactive in this system, but with the aid of 2(ring)C^{14}-labelled histidine, the corresponding dinucleotide was isolated in very small quantities and its properties were studied (LaMantia and Alivisatos, 1959).

In a survey of the reactivity of other imidazoles in this system, a number of new dinucleotides were prepared (see table I). An interesting finding was that imidazoles bearing a carboxyl in the side chain attached to the 4 (or, 5) position of the nucleus (e.g., histidine, imidazoleacetic acid) are extremely unreactive. This observation might further contribute to the

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TABLE I

ANALYTICAL DATA AND pK' VALUES FOR THE PROTONATION OF THE IMIDAZOLE NUCLEUS OF CERTAIN DINUCLEOTIDES

Compound	μmoles Ribose per 2 μatoms Esterified Phosphorus*		pK' values**
	Orcinol Method	Periodate Consumption	
1. Diphospho-imidazole Nucleotide	1.04	2.10	6.4
2. Diphospho-histamine Nucleotide	1.14	1.80	5.1
3. Diphospho-acetylhistamine Nucleotide	1.14	2.25	6.5
4. [Diphospho-benzimidazole Nucleotide]	1.05	2.07	(5.7)
5. Diphospho-histidine Nucleotide	1.34	2.19	5.1
6. [Diphospho-(imidazoleacetic acid) Nucleotide]	----	----	---
7. Diphospho-(5-amino-4-carboxamido-imidazole) Nucleotide	1.92	1.85	(3.8)
8. Inosinic-adenylic pyrophosphate	2.05	2.03	---
9. Diphospho-pyridine Nucleotide	2.08	2.04	---
10. 5'-Adenylic Acid	1.90	1.80	---

The isolation of compounds in brackets was not yet accomplished. However, analytical data in the bibliography for benzimidazole ribonucleoside (Weissbach et al, 1959) agree with analytical data obtained from DPN-ase reaction-mixtures (Alivisatos, LaMantia and Matijevitch, unpublished observations), and the resistance to acid hydrolysis of the imidazole-ribose bond in the imidazoleacetic acid ribonucleoside was already established (Tabor and Hayaishi, 1955).

* Determined as the difference between total (King, 1932) and inorganic (Lowry and Lopez, 1946) phosphorus. For ribose determinations, see text.

** Determined electrophoretically (Alivisatos et al, in press). pK'-values in parentheses are not to be regarded as definite.

knowledge (Alivisatos, 1959) of the nature of the active site(s) of the mammalian DPN-ase.

It was also observed that the N-ribosyl bond of these dinucleotides is very resistant to acid hydrolysis, with the notable exception of the respective bonds in diphospho-(5-amino-4-carboxamido-imidazole) nucleotide and in certain dinucleotides bearing pyrimidine-imidazoles (purines). Thus, ribose determinations were carried out by two alternative procedures: One (Mejbaum, 1939) involves preliminary hydrolysis of the N-ribosyl bond in 6N HCl for 20 minutes at 100°C. The other (periodate consumption; Dixon and Lipkin, 1954) is independent of such a hydrolytic step. Results gathered in table I show that the N-ribosyl bond of pyridine and purine derivatives (i.e., in DPN and inosinic adenylic pyrophosphate) and the N-ribosyl bond of 5-amino-4-carboxamido-imidazole are all hydrolyzable under the conditions of the Mejbaum test. In contrast to this the N-ribosyl bond of all other imidazole derivatives is practically non-hydrolyzable under the above conditions.

Although the behaviour of the N-ribosyl bond in a few of these compounds under the above mentioned conditions of acid hydrolysis was already known, lack of similar knowledge for various substituted imidazoles prevented a systematic study of the reasons for such differences (Kenner, 1957). It is obviou from the present studies that the inherent stability of the N-ribosyl bond of imidazole derivatives (No. 1-6, table I) is diminished upon addition of an amino-group on position 5 of the nucleus (e.g.,No. 7, table I). The N-ribosyl bond of purine derivatives (No. 8 and 9, table I) behaves similarly to the bond in the 5-amino-4-carboxamido-imidazole dinucleotide (No. 7, table I).

This relative instability to acid hydrolysis is most probably due to protonation of either the amino-group in the 5-position of the imidazole or a nitrogen in the pyrimidine moiety of the purine (possibly the purine nitrogen number 3) in the respective ribonucleosides. This protonation would occur in addition to the protonation of the amidine nitrogen of the imidazole nucleus

in ribonucleosides of imidazoles and of purines*. The combined inductive effects would lead to increased positive charge at the amidine nitrogen that participates in the N-ribosyl bond. The ensuing situation would be similar to that of the oxidized form of DPN. The permanent polarization of the N-ribosyl bond in the latter compound affords an explanation of its relative lability to acid hydrolysis (see, also, Alivisatos,1959). Experiments with a number of other imidazoles, especially 4(5)-methyl-imidazole, 4(5)-methyl-5(4)-nitroimidazole and 4(5)-methyl-5(4)-amino-imidazole are now in progress. It is hoped that knowledge gained in this field might further our understanding of the mechanism of enzymic hydrolysis of DPN and transfer reactions catalyzed by the DPN-ase.

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* Conjugation with the pyrimidine ring changes the imidazole character so drastically that purines in many ways are not recognizable as imidazoles (Todd,1957;Barnard and Stein,1958). However, there is very little doubt that at the hydrogen ion concentrations employed in our experiments (i.e., about 6N HCl) the amidine nitrogen of the imidazole nucleus in ribonucleosides of imidazoles (see table I) and of purines (Albert and Brown, 1959) would be protonated. This would be true even when a powerful electron sink is present in the 5 position of the imidazole, as in the 4(5)-nitroimidazole (pK' for the protonation of the free compound, about 0.5) or the 4(5)-methyl-5(4)-nitroimidazole (pK' for the protonation of the free compound about 1.0). (Alivisatos and Lukacs, unpublished data).

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