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DETERMINATION OF ACTIVE HYDROGENS OF GUANOSINE AND YEAST RIBONUCLEIC ACID WITH DEUTERIUM OXIDE

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Microdetermination of active hydrogen with deuterium oxide was proposed in 1936 by R.J. Williams (*1*) and first applied to hydroxyproline and urea. It consists merely in dissolving the substances to be analysed in deuterium oxide, evaporating to dryness and determining the increase in weight due to the replacement of active hydrogens by deuterium. Bonhoeffer and Brown, (*2*) Klar, (*3*) and Williams (*1*) showed that the hydrogens of -OH, $-NH_2$ and -NH radicals in organic compounds were exchanged by simply dissolving them in deuterium oxide, while hydrogen of $-CH_3$, $-C_2H_5$ and $-C_6H_5$ was not exchanged at all. Since the principle is so simple and the manipulation involves nothing beyond drying and weighing, and the action is so mild, the authors have applied this method to the nucleic acid chemistry. After the applicability of this method was first checked on a nucleoside, guanosine, the authors used it to determine active hydrogens of yeast ribonucleic acid. The experiment showed that the number of active hydrogen of guanosine was 6, corresponding to the theoretical number, while the sodium nucleate was found to have 11–12 active hydrogens for every four phosphate groups.

EXPERIMENTAL

Experiment with Guanosine: First of all, two moles of crystal water of pure guanosine were previously expelled by heating at 110° in a pressure of 5 mm.Hg. Then 55.592 and 33.635 mg. of guanosine in small weighing bottles were dissolved in 1.0 ml. of 99.5 per cent deuterium oxide by heating, and then carefully evaporated and dried to constant weight in a pressure of 5 mm.Hg. at 110°; it was weighed again. (Table I).

The guanosine which underwent the above treatment was dissolved again in 1.0 ml. of 99.5 per cent deuterium oxide, evaporated and dried to constant weight and weighed again as above mentioned. But no increase was found. Therefore, the ex-

TABLE I

Number of Active Hydrogen of Guanosine

Guanosine	Increase after dissolving in deuterium oxide	Theoretical increase for one hydrogen	Number of active hydrogen
mg. 55.592,2	*mg.* 1.170	*mg.* 0.198,0	5.90 (≒6)
33.635,0	0.708	0.119,5	5.92 (≒6)

change of active hydrogens of guanosine seemed to accomplish by dissolving it in 99.5 per cent deuterium oxide only once.

Experiment with Yeast Nucleic Acid: Yeast ribonucleic acid employed was prepared from yeast by the method of Baumann and purified according to Makino (*4*) and has the following analytical data: N, 14.47%, P, 8.60%, therefore P:N=1:1.682. The acid was neutralized with *N*/10-NaOH using phenolphthaleine as indicator and evaporated and dried to constant weight at 110° in a pressure of 5 mm.Hg. 96.580 and 99.001 mg. of this sample were weighed out. Each sample was dissolved into 0.5 ml. of deuterium oxide and was carefully evaporated and dried to constant weight in a pressure of 5 mm.Hg. at 110° for about 18 hours. Weighing was repeated and the increase in weight was determined (Table II).

TABLE II

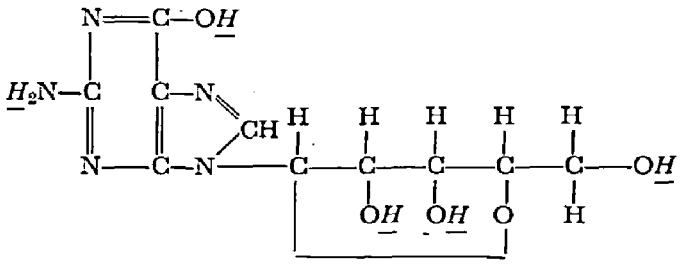
Number of Active Hydrogen of Yeast Ribonucleic Acid

Sodium salt of yeast nucleic acid	Increase after dissolving in deutrium oxide	Theoretical increase for one hydrogen	Number of active hydrogen per four phosphate groups
mg. 96.580	*mg.* 0.802,0	*mg.* 0.070,8	11.33
99.001	0.869,0	0.072,6	11.98
		Average	11.65

The sample which underwent the above treatment was dissolved again in 0.5 ml. of 99.5 per cent deuterium oxide. It was then evaporated and dried to constant weight and weighed again after treating as above. However no increase in weight was found. It seems that the exchange of active hydrogens of the sodium salt was completed by dissolving it in deuterium oxide only once.

DISCUSSION

The structure of guanosine is to be shown by the following Formula I.



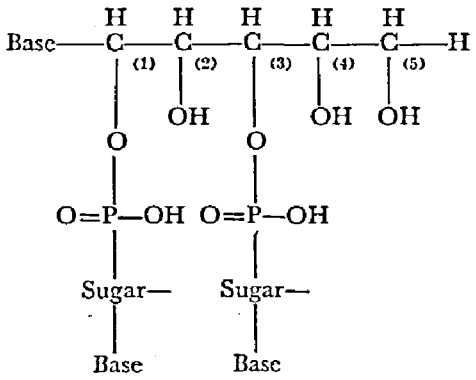
FORMULA I

Theoretically number of its active hydrogens is 6 which are underlined in the above formula, and our experimental data coincide with it as shown in the above table (Table I).

In certain yeast ribonucleic acid (especially, of Merck) the ratio of four bases (adenine, guanine, cytosine and uracil) contained in them is equimolecular (Bacher and Allen, (*5*) Makino and Uchida (*6*). Levene and Simms (*7*) showed that the amino- and hydroxyl-groups of adenine, cytosine, guanine and uracil exist in free state. Levene and Jacobs (*8*), and Falconer, Gulland, Hobday and Jackson (*9*) indicated that their amino-groups can be determined by Van Slyke's method. Bredereck, Koethning and Lehmann (*10*) prepared a desaminated nucleic acid without decomposing its internucleotides union. So it seems that the amino- and hydroxyl-groups have nothing to do with the internucleotidal esterlinkage. On the other hand our samples of yeast ribonucleic acids (especially, that of Merck) have a molecular weight corresponding to a tetranucleotide (*11*) and show to be four basic and when it was decomposed into four nucleotides by alkali an increase of 4 acidic groups are afresh found (*12*). According to the cyclic formula the active hydrogens of its tetrasodium salt are 12 while our experimental results indicated that the number is 11–12 as shown in the above table (Table II).

As the addendum to the above experiment we intended to study the attitude of yeast nucleic acid toward periodate oxidation in order to examine whether the possiblity exists or not that a inter-mononucleotide ester linkage takes place between a hydroxylgroup of position (1) of one of mononucleotides and a phosphoric acid group of the other

monounucleotide and accordingly hydroxyl-groups of position (4) and (5) of its sugar are free as Formula 2.



FORMULA 2

But the experimental results indicated that no consumption of oxygen was found when periodate was added to the solution of sodium salt of yeast ribonucleic acid.

SUMMARY

The authors determined active hydrogens of guanosine and yeast nucleic acid by the method proposed by Roger J. Williams which consists in dissolving the substances in deuterium oxide, evaporating to dryness and determining the increase in weight due to the replacement of active hydrogen by deuterium. The experiment showed that the number of active hydrogen of guanosine was 6, corresponding to the theoretical number, while the tetrasodium salt of the yeast nucleic acid was found to have 11–12 active hydrogens.

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