An additional sugar component of ribonucleic acids

Although several additional purine and pyrimidine constituents of RNA have recently been described[1-6], no sugar component other than D-ribose has been reported. A study of the alkaline hydrolysis products of RNA has led us to the discovery of small amounts of an additional sugar component in some ribonucleic acids.

From the mechanism of alkaline hydrolysis of RNA, involving phosphoryl migration between the 2′ and 3′ hydroxyl groups[7], all the internucleotide linkages should be labile in alkali and yield a mixture of ribonucleoside 2′- and 3′-phosphates. We find that many ribonucleic acids on hydrolysis in 1 *N* KOH at 30° (or 37°) yield in addition to mononucleotides, a small proportion (less than 5 %) of dinucleotides which are stable to further alkaline treatment under these conditions. These are found in hydrolysates of RNA prepared from several sources, including wheat embryo, leaves of *Beta vulgaris* and *Nicotiana glutinosa*, rat-liver microsomes, and the "soluble" RNA fraction[8] from rat liver. They appear to be absent from alkaline hydrolysates of RNA from turnip-yellow-mosaic virus and *Aerobacter aerogenes*. Some evidence for the presence of unidentified polynucleotides in alkaline hydrolysates of yeast RNA has previously been reported by K. C. SMITH AND ALLEN[9].

To isolate the dinucleotides the alkaline hydrolysate was first run on paper chromatograms in *iso*propanol–water–ammonia[10], where the mononucleotides separate into two bands, one containing guanylic acid and a faster moving band of adenylic, cytidylic and uridylic acids. The material from the guanylic acid band was subjected to paper electrophoresis in 0.05 *M* phosphate, pH 2.1 where in addition to guanylic acid a number of u.v.-absorbing bands separated (Table I). One of these (band 5) was identified as the additional nucleotide described by COHN[3] and DAVIS AND ALLEN[4].

TABLE I

PARTIAL SEPARATION OF DINUCLEOTIDES FROM AN ALKALINE HYDROLYSATE OF WHEAT-EMBRYO RNA

Paper electrophoresis in 0.05 *M* phosphate, pH 2.1, of the material from the guanylic position on the paper chromatogram in *iso*propanol–water–ammonia[9].

Band	*Mobility (cm/h at 20 V/cm)*	*Components*
1	1.3	CpCp, CpAp, ApCp, ApAp
2	3.7	Guanylic acid*
3	5.4	UpCp, CpUp, UpAp, ApUp
4	6.4	GpUp, UpGp
5	7.1	Additional mononucleotide[3,4]
6	9.0	UpUp

* This band has not been carefully examined and would be expected to contain the dinucleotides GpC, CpG, ApG, GpA and GpG if these were present. The latter has been tentatively identified from rat-liver soluble RNA.

Bands 1, 3, 4 and 6 (Table I), however, contained substances with the properties of dinucleotides. On treatment with prostate phosphomonoesterase[11] these were converted to substances with the chromatographic behaviour and electrophoretic mobilities similar to those of dinucleoside monophosphates. The material in band 3, for example, after treatment with phosphomonoesterase separated on paper electrophoresis in 0.05 *M* ammonium formate, pH 3.5, into four substances, identified as the dinucleoside monophosphates UpC, CpU, UpA and ApU, derived from the corresponding dinucleotides by removal of the terminal phosphate group. Other dinucleotides identified from the bands in electrophoretic separation at pH 2.1 were ApCp, CpAp,

Abbreviations: RNA, ribonucleic acid; A, G, C, and U, nucleoside residues of adenine, guanine, cytosine and uracil respectively (for abbreviations of dinucleotides, see [10,11]).

CpCp, ApAp, GpUp, UpGp and UpUp (Table I). In addition, the dinucleoside monophosphate GpA was isolated, using ion-exchange chromatography on Dowex-1 formate, from an alkaline hydrolysate of wheat-embryo RNA which had been treated with prostatic phosphomonoesterase.

The alkali-stable dinucleotides all appear to have similar structures. The dinucleoside monophosphates derived by phosphomonoesterase treatment are each split by the diesterase and 5'-nucleotidase in crude Russell-viper venom to yield two nucleosides in approx. equal molar proportions. One of these is a riboside but the other has properties differing both from the ribosides and deoxyribosides. The unusual nucleosides of adenine, guanine, cytosine and uracil have been isolated. They have u.v.-absorption spectra at pH 1 and pH 13 similar to those of the corresponding ribosides but differ from ribosides in the following properties. (a) Their electrophoretic mobilities in 0.05 *M* borate, pH 9.2, are approximately those of the corresponding deoxyribosides showing they do not form complexes with borate. (b) On paper chromatography in *n*-butanol–water–formic acid[12] and *iso*propanol–water–ammonia the nucleosides have R_F-values greater than those of both the corresponding ribosides and deoxyribosides.

Evidence for the structure of the dinucleotides was obtained using a mixture of UpGp and GpUp. Among the products of hydrolysis in 1 *N* HCl at 100° for 1 h were guanine, and uridine 2'- and 3'-phosphates, while acid hydrolysis after treatment with phosphomonoesterase gave guanine and uridine. (In each case an unidentified uracil derivative was also formed.) This suggested that the singly esterified phosphate, in the case of GpUp, was in the 2' or 3' position.

A mixture of GpU and UpG was subjected to the WHITFELD degradation procedure of periodate oxidation and subsequent alkali breakdown[13]. Two mononucleotides, one containing guanine and the other uracil, were obtained. On electrophoresis in 0.05 *M* borate, pH 9.2, they migrated with guanosine 3'-phosphate and uridine 3'-phosphate respectively and they were unaffected by the 5'-nucleotidase in Russell-viper venom. Treatment with prostate phosphomonoesterase converted them to substances having spectral, chromatographic and electrophoretic properties identical with the unusual guanine and uracil nucleosides described above. This is consistent with the structures G_xpU and U_xpG where "*x*" denotes the unusual nucleoside residue which would be linked through a phosphodiester linkage on the 3' hydroxyl to the 5' hydroxyl of the ribonucleoside residue.

Substances with the expected properties of the *x* nucleoside 5'-phosphates of adenine and uracil were isolated from snake-venom diesterase digests of the RNA of rat-liver microsomes. Treatment with venom containing 5'-nucleotidase converted these to the *x* nucleosides. In the intact RNA chains these nucleosides are thus linked by both 5' and 3' phosphoester bonds.

Since the *x* nucleosides do not form borate complexes they cannot possess the *cis* hydroxyl groups on the 2' and 3' hydroxyl groups of the ribosides. This suggested that the sugar might be a ribose substituted on the 2' position, particularly as a 2'-substituted riboside linked by 3', 5' phosphodiester bonds in a polyribonucleotide chain would be expected to yield a dinucleotide of the type G_xpUp on alkaline hydrolysis. While further work is in progress to establish the identity of the sugar component of the unusual nucleosides, this has been found to have properties similar to 2(or 3)-O-methyl ribose. The adenine *x* nucleoside has the same R_F values as

2'(or 3')-O-methyl adenosine on paper chromatography in *n*-butanol–water–formic acid and *n*-butanol–water–ammonia[12]. After hydrolysis in 1 *N* H_2SO_4 and paper chromatography in *n*-butanol–water–ammonia both the guanine *x* nucleoside and the monomethyl adenosine gave substances reacting with an aniline hydrogen phthalate sugar reagent[14] with identical R_F values.

Agricultural Research Council Virus Research Unit, Huntingdon Road, Cambridge (Great Britain) J. D. SMITH D. B. DUNN

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Received October 23rd, 1958

Uridine diphosphate-N-acetylglucosamine-4-epimerase from *Bacillus subtilus*

Uridine diphosphate-N-acetylgalactosamine has been isolated from liver[1] and oviduct[2]. We wish to report the enzymic formation of this compound from uridine diphosphate-N-acetylglucosamine in sonic extracts of *B. subtilus* (ATCC 9945), by a reaction similar to that catalyzed by uridine diphosphategalactose-4-epimerase[3,4]. This strain of *B. subtilus* is known to synthesize a polysaccharide which contains N-acetylgalactosamine[5].

The enzyme system in liver reported to form free N-acetylgalactosamine from UDPAG by an epimerase reaction[6] has been shown to form N-acetylmannosamine[7], and is not identical with the enzyme from *B. subtilus*.

In a typical experiment 20 μmoles UDPAG were incubated in 0.05 *M* tris-(hydroxymethyl)aminomethane buffer, 0.01 *M* $MgCl_2$, 0.001 *M* ethylenediaminetetraacetic acid, pH 8.0, with a *B. subtilus* sonic extract (20 mg protein) in a final vol. of 7 ml for 1 h at 37°. The reaction mixture was then adjusted to pH 2 and heated at 100° for 20 min to hydrolyze the nucleotide-bound sugar. The reaction mixture was neutralized, deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and deionized with Amberlite MB-2.

Abbreviation: UDPAG, uridine diphosphate-N-acetylglucosamine.