Short Communications

A new guanosine nucleotide from brewer's yeast*

Application of an improved method for the separation of GDPM**, UDPAG and UDPG[1] on anion-exchange columns has revealed (Fig. 1) the presence of a hitherto unknown guanosine-3'-phosphate derivative (GMPX) in alcoholic extracts of brewer's yeast.

The ultraviolet absorption spectrum of GMPX corresponds to that of guanosine, and shows the same changes with pH. Moreover, guanine could be identified by paper chromatography and by its spectrum after hydrolysing GMPX in 1 *N* acid during 60 min at 100° C.

Analysis indicated the presence of only one phosphate group per molecule of guanosine.

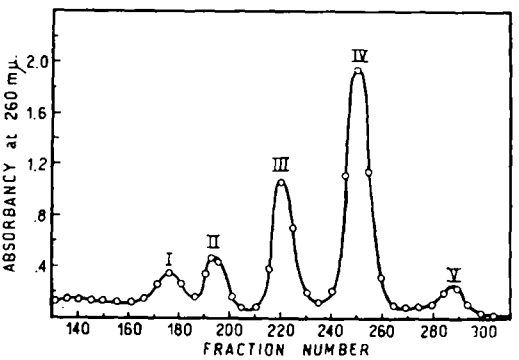

Fig. 1. Elution diagram of an alcoholic extract of brewer's yeast. The alcoholic extract (containing about 600 μmoles calculated as uridine from the absorbancy at 260 mμ) was applied directly through a Dowex-1 X 4 (200-400 mesh) column (1 × 100 cm) in the chloride form. Elution was carried out at constant pH, with a sodium chloride gradient (initial concentration, 0.01 *N* HCl, 0.02 *N* NaCl to final concentration, 0.01 *N* HCl, 0.1 *N* NaCl). The flow rate was 0.4 ml/min and 12 ml fractions were collected. Peak I, GMPX; peak II, GDPM; peak III, UDPAG; peak IV, UDPG; peak V, uridine derivative. Peak I was missing in a similar elution diagram from baker's yeast.

Both the rates of colour development with orcinol[2] and of hydrolysis of the phosphate group were the same for GMPX and guanosine-3'-phosphate and different from those of guanosine-5'-phosphate. Guanosine-3'-phosphate was identified as a breakdown product of GMPX, after acid hydrolysis by paper chromatography in three different solvents (Table I). The small amount of GMP-2', which was also formed (see Table I), can be ascribed to the isomerization catalyzed

TABLE I

PAPER CHROMATOGRAPHY OF NUCLEOTIDES

Substance	$R_{Adenosine}$ *values of ultraviolet-absorbing spots*		
	Ethanol-ammonium acetate[7]		*Ammonium sulphate-isopropanol-water*[8]
	pH 7.5	*pH 3.8*	
GMPX	0.67	0.70	
GDPM	0.26	0.17	
UDPG	0.44	0.38	
UDPAG	0.55	0.49	
GMPX heated 5 min pH 2, at 100° C	0.19	0.50	2.70-3.27 §
GMP-3'	0.19	0.49	2.70
GMP-5'	0.14	0.35	3.75
GMP-2'			3.25

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** The following abbreviations are used: GDPM for guanosine diphosphate mannose; UDPAG for uridine diphosphate acetylglucosamine; UDPG for uridine diphosphate glucose; GMP-2', GMP-3' and GMP-5' for the 2'-, 3'- and 5'-monophosphates of guanosine.

TABLE II

PAPER CHROMATOGRAPHY OF PRODUCTS OF ACID HYDROLYSIS OF GMPX

Solvent, phenol–water[5].

Substance	R_{Ribose} of reducing spots	R_{Ribose} of ultraviolet-absorbing spots
GMPX		1.33
GMPX heated 5 min pH 2, at 100° C	0.88	1.12
GMP-3′		1.10
Glucose	0.60	
Mannose	0.72	

by acid[8]. The presence of a cyclic 2′-3′-phosphate is unlikely since in that case the hydrolysis mixture should contain roughly equal amounts of the 2′ and 3′-phosphates. The position of the phosphate group in the guanylic acid was further confirmed by treatment with a specific 3′-nucleotidase, from germinating Rye grass[4]. The guanylic acid obtained from GMPX was hydrolysed by this enzyme, yielding inorganic phosphate at practically the same rate as GMP-3′, while GMP-2′ and GMP-5′ were not attacked.

The identity of the X group has not been determined. When a sample of GMPX was hydrolysed at pH 2 during 5 min at 100° C, and submitted without further treatment to paper chromatography (Table II) with phenol–water as solvent[5], a single spot, corresponding to GMP-3′, was observed under ultraviolet light. However, another spot was found after treatment with alkaline silver nitrate which reveals reducing substances[6] (see Table II).

Paper electrophoresis of GMPX, before and after hydrolysis, showed (Fig. 2) that on treatment with acid, a secondary phosphoric acid group appeared.

This behaviour on acid hydrolysis, together with the ultraviolet absorption spectra, suggest that the residue X is unlikely to be directly attached to the purine ring. It appears that GMPX is a derivate of guanosine-3′-phosphate in which a group X is joined to the phosphate residue. This linkage is very labile since GMPX is completely hydrolysed to GMP-3′ at pH 2 during 5 min at 100° C.

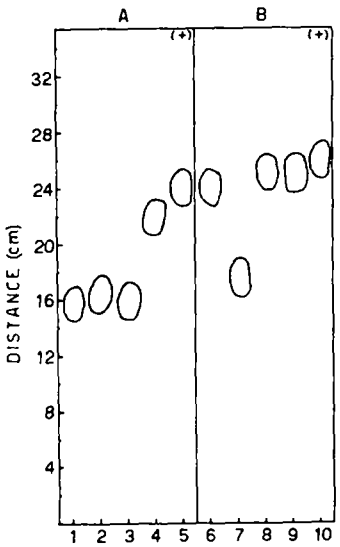

Fig. 2. Paper ionophoresis with Whatman No. 1 paper, of GMPX before and after hydrolysis. A. Ionophoresis at 500 volts in 0.05 *M* ammonium acetate buffer pH 3.8 for 4 h; B. Ionophoresis at 500 volts in 0.05 *M* boric acid–sodium borate buffer, pH 8.1 for 4 h. 1, 6, GMP-3′; 2, 7, GMPX; 3, 8 GMPX heated 5 min pH 2 at 100° C; 4, 9, GDPM; 5, 10, UDPG.

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1 H. G. PONTIS, E. CABIB AND L. F. LELOIR (in preparation).
2 H. G. ALBAUM AND W. W. UMBREIT, *J. Biol. Chem.*, 167 (1947) 369.
3 J. X. KHYM AND W. E. COHN, *J. Am. Chem. Soc.*, 76 (1954) 1818.
4 L. SHUSTER AND N. O. KAPLAN, *J. Biol. Chem.*, 201 (1953) 535.
5 S. M. PARTRIDGE, *Biochem. J.*, 42 (1948) 238.
6 W. E. TREVELYAN, D. P. PROCTER AND J. S. HARRISON, *Nature*, 166 (1950) 444.
7 A. C. PALADINI AND L. F. LELOIR, *Biochem. J.*, 51 (1952) 426.
8 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 49 (1951) 401.

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