Isolation of a Cytidylic Acid-Glucose-Peptide Nucleotide from Smut Chlamydospores

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Various nucleotide-peptide compounds have been described within the past few years (Michelson 1963). Bergkvist (1958) described the isolation of a cytidylic acid-peptide complex from Polyporus squamosus, from which acidic hydrolysis yielded cytidine-2' and 3' phosphates. No cytidine-5-phosphate sugar peptide complex has been isolated from a natural source. The only cytidine-5-phosphate sugar nucleotide known to occur naturally is CMP-5'-sialic acid which was isolated from Escherichia coli (Comb et al 1959).

In the course of a study of the nucleotides in the alcholic extract of the chlamydospores of loose smut of oats Ustilago avenae (Pers.) Rostr. we have isolated a new cytidylic acid glucose-peptide nucleotide (CMPX). The purpose of this report is to present experimental data about this substance.

CMPX was isolated from 75% ethanolic extract of Ustilago avenae chlamydospores. The alcholic extract was evaporated to dryness under vacuum and the water soluble material including the nucleotides was applied to a Dowex-1 anion exchange column (chloride form) (Mori et al 1960).

The elution of the nucleotides was performed with increasing concentrations of dilute HCl or a mixture of HCl and NaCl. Among the different components eluted from the column which will

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be presented later, we obtained the substance CMPX which was eluted before AMP

The fraction containing CMPX was concentrated by adsorption on acetic acid washed charcoal and eluted with ammoniacal ethanol solution. This eluate was freed from ammonia by bubbling nitrogen through it at $0^{\circ}C$, then freeze dried. The amount of CMPX obtained was about 0.30 u mol per gram fresh weight (calculated as cytidine, The ultraviolet adsorption spectra of CMPX at pH 1, 7, and 12, was similar to authentic CMP-5' (fig. 1).

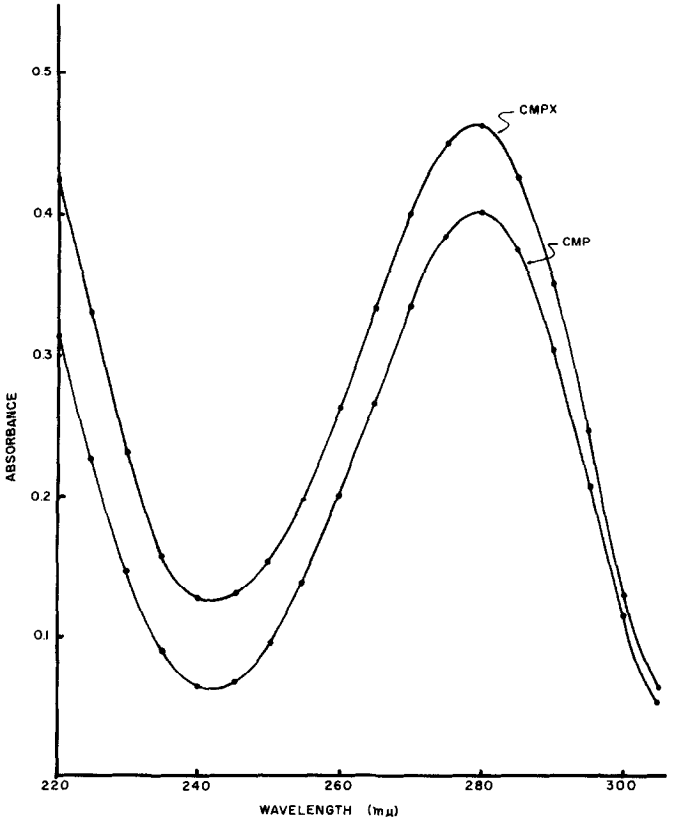


Fig. 1. Ultraviolet absorption spectra of CMP and CMPX at pH 1 (0.1 N HCl)

The substance was subjected to paper chromatography on Whatman No. 1 filter paper prewashed with 2N acetic acid using the solvent system of Paladini and Leloir (1952). The chromatogram was inspected with a short wave length ultraviolet lamp and CMPX was located as a single spot which moved faster than authentic CMP-5' in this solvent system (table 1).

CMPX was extracted from the paper with 0.1 N HCl for 24 hours at room temperature and hydrolyzed (1N HCl at 100°C for 1 hour). The hydrolyzate was subjected to paper chromatography and CMP-5' was identified by its R_f against an authentic sample and also by its ultraviolet absorption spectrum. Mild acidic hydrolysis of CMPX (0.1 N HCl for 15 min. at 100°C) on the other hand revealed the presence of a reducing sugar. When the hydrolyzate was chromatographed on Whatman No. 1 filter paper in two solvent systems, n-butanol-acetic acid-water (63-10-27) and pyridine-butanol water (4:6:3), the sugar was found to have the same R_f as authentic glucose (table 1).

CMPX was also hydrolyzed in 6 N HCl for 20 hours at 100°C in a sealed tube followed by evaporation to dryness. The residue was dissolved in a few drops of distilled water and the evaporation repeated three times to get rid of the HCl. The hydrolyzate was chromatographed on Whatman No. 1 filter paper in two dimensions using phenol-water in the first dimension and n-butanol-acetic acid-water (63-10-27) in the second. The chromatogram was then developed with an acetone solution of ninhydrin revealing the presence of at least 9 amino acids. Seven of the amino acids were found to have identical R_f's to aspartic acid, glutamic acid, serine, threonine, glycine, alanine, and lysine. The glutamic acid spot was more intense than the other amino acids indicating its presence in higher concentrations than the other amino acids.

Total phosphorus analysis of CMPX was performed according to

TABLE 1 ANALYTICAL DATA OF CMPX ISOLATED FROM SMUT CHLAMYDOSPORES

	Total Phosphorus[a] (mols/mole base)[b]	Ninhydrin reaction of U.V. absorbing spot.	RCMP (21 hrs run)[c] nucleotides Ethanol: ammonium acetate (pH 7.5)	$R_{Glucose}$ reducing sugar[d] n-Butanol, acetic acid, water (63:10:27 v/v)	Pyridine, butanol, Water, (6:4:3 v/v)
CMPX	0.96	+ve	1.54	----	----
" hydrolyzed (0.1N HCl at 100° for 15 min).		+ve	1.43	1.00	1.00
" hydrolyzed (1 N HCl at 100° for 1 hour).		-ve	1.00	1.00	1.00
CMP-5'		-ve	1.00	----	----
CMP-2' and 3'		-ve	1.13	----	----
Glucose				1.00	1.00

a according to the method of Bartlett (1959)

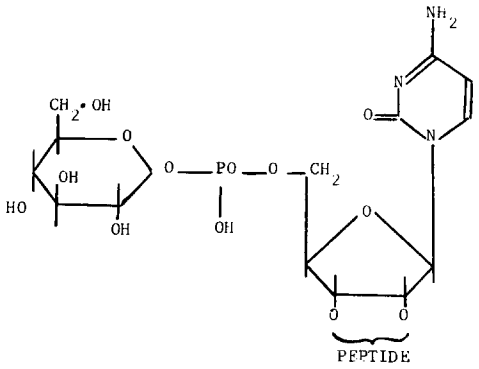
b calculated from molar absorbancy of cytidine (13.0×10^3) Dorough and Seaton (1954)

c solvent after Paladini and Leloir (1952

d $AgNO_3$ was used to locate sugars (Trevelyan et al 1950)

the method of Bartlett (1959) and revealed the presence of one mole of acid stable phosphorus per mole of base (table 1).

The ease with which CMPX was hydrolyzed in acid suggests that glucose is directly attached to the phosphate of CMP-5'. The peptide moiety is probably attached to the ribose. This is indicated by the appearance of a U.V. absorbing ninhydrin labile spot with a lower R_{cmp} value than CMPX and higher than CMP, after glucose has been removed by mild acidic hydrolysis (table 1). The following structure was proposed for CMPX on the basis of the evidence presented.



The biological importance of CMPX, is not known at the moment. It is of interest to point out that several uridine-sugar-peptide nucleotides have been identified in some species of bacteria (Salton 1964). These nucleotides were reported to be involved in the cell wall biosynthesis (Strominger 1960). The importance of CMPX in a similar mechanism in the smut chlamydospores is also possible.

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