ISOLATION OF CYTIDINE 5'-DIPHOSPHATE PARATOSE FROM SALMONELLA PARATYPHI A

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Received March 2, 1964

Paratose (3,6-dideoxy-D-glucose) is an important constituent of the antigenic lipopolysaccharide of S. paratyphi A (Davies et al., 1958). A sugar nucleotide, isolated from a rough strain of this organism, has been identified by the data presented in this communication as CDP-paratose.

A culture was grown in Pennassay medium (Difco) and harvested at the end of the log phase of growth. Five hundred grams of packed cells were boiled for 10 minutes in 2.3 liters of 70% ethanol. The suspension was filtered and the filtrate concentrated in vacuo to 500 ml. After centrifugation to remove insoluble material, the extract was separated into two fractions by passage through a column of Sephadex G-25. The low molecular weight fraction which contained approximately 1.8 mmoles of nucleotide (assuming an ϵ_{260} of 10,000) and 32 μmoles of dideoxyhexose (Cynkin and Ashwell, 1960), was adsorbed on a column of Dowex-1 formate. A gradient of 0 to 1.6 M ammonium formate was used to elute nucleotides, and at 0.5 M ammonium formate an ultraviolet-absorbing peak was eluted which exhibited a positive test for dideoxyhexose. Following adsorption and elution from charcoal, paper chromatography of this fraction in ethanol:1 M ammonium acetate, 7.5:3 (solvent I) revealed a major ultraviolet-absorbing band with an R_{CMP} of 2.1 along with several minor components. All of the dideoxyhexose was associated with the heavy band which was eluted and rechromatographed in ethanol:ammonium acetate, pH 3.8, 7.5:3, where it had an R_{CMP} of 1.1. The isolated material exhibited

ultraviolet absorption spectra characteristic for a cytidine derivative as shown in Table I. Calculated spectrophotometrically as cytidine, 24 μmoles of nucleotide were obtained. An analysis of the nucleotide is given in Table II.

TABLE I. Ultraviolet absorption spectra characteristics of CDP-paratose

Compound	pH 2			pH 7		
	λ_{max}	250/260	280/260	λ_{max}	250/260	280/260
CMP [1]	280	.45	2.06	271	.86	.94
CDP-Paratose	280	.43	2.1	271	.85	.91

[1] Pabst Laboratories Circular OR-7 (1955).

TABLE II. Analysis of CDP-paratose

Test	moles/mole of cytidine
Total phosphorus [1]	2.1
Acid-labile phosphorus [1]	0.9
Dideoxyhexose [2], as ascarylose	0.9
Reducing value [3], as ascarylose	0.1
Reducing value [3], as ascarylose after hydrolysis at pH 2 for 10 min. at 100°	1.1

[1] Ames and Dubin, 1960.

[2] Cynkin and Ashwell, 1960. Prior hydrolysis of the nucleotide is unnecessary for maximum color development because of the lability of CDP-paratose under the conditions of assay. cf. Heath and Elbein, 1962.

[3] Park and Johnson, 1949.

Hydrolysis of the material at pH 2 for 10 minutes liberated a neutral sugar and an ultraviolet-absorbing compound which chromatographed with cytidine diphosphate in solvent I. The liberated sugar chromatographed

as a single spot with paratose in pyridine:ethyl acetate:water, 1:3.6:1.15, propanol:ethyl acetate:water, 7:1:2, and isoamyl acetate:acetic acid:water, 3:3:1. These solvents distinguish paratose from the other naturally occurring 3,6-dideoxyhexoses, tyvelose, abequose, ascarylose, and colitose. Hydrolysis of the nucleotide in 1 N H_2SO_4 for 10 minutes at 100°, or treatment with nucleotide pyrophosphatase resulted in the formation of an ultraviolet-absorbing compound with the chromatographic properties of cytidine 5'-phosphate using isobutyric acid:1 M NH_4OH, 10:6, or 5 M ammonium acetate:saturated sodium borate:ethanol, 20:80:220. These solvents distinguish cytidine 5'-phosphate from its 2'- and 3'-isomers as well as from dCMP.

From these data it is probable that the isolated nucleotide is CDP-paratose in which paratose is linked glycosidically to the terminal phosphate of cytidine 5'-diphosphate. Other nucleotide-linked dideoxyhexoses that have been isolated include GDP-colitose (Heath, 1960), CDP-tyvelose, and CDP-abequose (Nikaido and Jokura, 1962), while CDP--ascarylose has been synthesized enzymatically (Matsuhashi *et al.*, 1964). Extracts from *S. paratyphi A* form CDP-D-glucose from CTP and D-glucose-1-phosphate (Ginsburg, O'Brien, and Hall, 1962), and can convert CDP-D-glucose to CDP-4-keto-6-deoxy-D-glucose (Ginsburg, unpublished). In view of the fact that GDP-4-keto-6-deoxy-D-mannose and CDP-4-keto-6-deoxy-D-glucose are intermediates in the formation of GDP-colitose (Elbein, 1963), and CDP-ascarylose (Matsuhashi *et al.*, 1964), respectively, it is probable that CDP-4-keto-6-deoxy-D-glucose gives rise to CDP-paratose. This conversion, however, remains to be demonstrated.

REFERENCES

Ames, B. N., and Dubin, D. T., J. Biol. Chem., 222, 5 (1960).
Cynkin, M. A., and Ashwell, G., Nature, 186, 155 (1960).
Davies, D. A. L., Staub, A. M., Fromme, J., Luderitz, O., and Westphal, O., Nature, 181, 822 (1958).

Elbein, A. D., Federation Proc., 22, 1819 (1963).
Ginsburg, V., O'Brien, P. J., and Hall, C., Biochem. Biophys. Research Communs., 7, 1 (1962).
Heath, E. C., Biochim. et Biophys. Acta, 39, 377 (1960).
Heath, E. C., and Elbein, A. D., Proc. Natl. Acad. Sci. U. S., 48, 1209 (1962).
Matsuhashi, S., Matsuhashi, M., Brown, J. G., and Strominger, J. L., Biochem. Biophys. Research Communs., 15, 60 (1964).
Nikaido, H., and Jokura, K., Biochem. Biophys. Research Communs., 6, 304 (1961).
Park, J. T., and Johnson, M. J., J. Biol. Chem., 181, 149 (1949).