CONVERSION OF CYTIDINE 5'-PHOSPHATE TO DEOXYCYTIDINE 5'-PHOSPHATE IN CELL-FREE MAMMALIAN EXTRACTS[1]

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The observation of Rose and Schweigert (1953) that uniformly C^{14}-labeled cytidine was utilized by the rat for DNA-dCMP[2] synthesis with an unchanged cytosine/sugar C^{14} ratio, provided the first direct evidence for reduction of ribose to 2-deoxyribose without cleavage of the pyrimidine-ribosyl bond. Recently, reports have appeared by Reichard and Rutberg (1960), using E. Coli, and Moore and Hurlbert (1960), using Novikoff hepatoma, demonstrating a conversion of CMP to dCMP in cell free extracts with the suggestion that TPNH is involved in the reduction of C-2'. We have independently observed the same reduction in extracts of Ehrlich ascites cells and calf thymus but have found a requirement for DPNH rather than TPNH. We wish to emphasize that conclusions concerning the identity of the reducing agent with mammalian extracts must await purification of the enzyme.

Experimental

Cytidine-U-C^{14} and cytidine-2-C^{14} were purchased (as was tritiated CMP) from Schwarz Laboratories and converted to CMP by incubation with phenyl phosphate and a carrot phosphotransferase (Tunis and Chargaff, 1956). CMP was purified before use by chromatography on Dowex-50, eluting with 0.02 M formic acid. Ascites cells were suspended in 2 volumes of 0.05 M phosphate or Tris buffer, pH 7.4; 3.5 g of 200 micron glass beads were added per ml of packed cells; and the suspension was shaken for 20 sec. in a Nossal vibrator.

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[2]The following abbreviations are used: DNA, deoxyribonucleic acid; CMP, cytidine 5'-phosphate; dCMP, deoxycytidine 5'-phosphate; DPNH, reduced diphosphpyridine nucleotide; TPNH, reduced triphosphpyridine nucleotide; ATP, adenosine triphosphate.

The glass was filtered off and whole cells removed by centrifugation at 1200 g for 5 min. Thymus was homogenized in the same buffers in a Servall Omni-mix for 1.5 min. and centrifuged. A clear solution was obtained after centrifugation at 105,000 x g for 1 hour.

After incubation at 37° with labeled substrate, dCMP carrier was added, and the protein precipitated with 0.5 M perchloric acid. The extract was kept at 100° for 20 min. and then chromatographed on a column of 2 per cent crosslinked Dowex-1-bicarbonate (1.5 cm x 0.8 cm^2), eluting with 0.003 M $Na_2B_4O_7$ in 0.06 M NH_4HCO_3. The dCMP fraction was purified by rechromatography on Dowex-50 (10 cm x 0.8 cm^2), eluting with 0.02 M formic acid. Aliquots were dried and counted in a windowless flow counter. Enzyme units are expressed as mμmoles dCMP formed per hour, calculated as 1000 times the ratio of total counts in dCMP to counts/min/μmole in CMP.

Results

In surveying a number of tissues it was found that essentially no conversion of CMP to dCMP could be detected with homogenates unless a reduced pyridine nucleotide was added to the incubation mixture. As indicated in Table I, TPNH was partially effective, but maximum activity was

Table I

Reduced Pyridine Nucleotide Requirement for dCMP Formation

Cofactor	A	B	C	D	E
	mμmoles dCMP/hr/100 mg protein				
None	0.1	0.15	0.2	9.9	7.4
TPNH	0.7	1.7	0.5	10.8	4.4
DPNH	3.5	6.0	2.9	55.6	34.0

Incubation mixtures contained 0.0004 M CMP, 0.005 M ATP, 0.010 M $MgCl_2$, and 0.0005 M TPNH or DPNH.
A: Thymus homogenate, CMP-2-C^{14}. B: Ascites homogenate, CMP-2-C^{14}.
C: Thymus homogenate, CMP-H^3. D: Soluble extract of stored frozen thymus, CMP-H^3. E: Soluble extract of fresh thymus, CMP-H^3.

obtained with DPNH. The activity with TPNH was not increased by addition of a TPNH regenerating system. When the clear supernatant solutions were used as in D and E, some activity was observed in the absence of reduced pyridine nucleotides, but only DPNH and not TPNH resulted in stimulation.

The increased activity observed with clear extracts was correlated with the removal of phosphatases in the particulate fraction. In all cases, ATP and Mg^{++} were required for activity. Oxidized pyridine nucleotides and sulfhydryl compounds (glutathione, BAL, dihydrolipoic acid) were ineffective.

The production of dCMP was found to be a linear function of time with test intervals from 15 min. to 2 hrs. A linear relation with enzyme concentration has not been observed, and in the presence of large amounts of extract, dCMP yields were strongly depressed.

That dCMP was formed from CMP without cleavage of the ribosyl bond was shown by using CMP-U-C^{14}. Counts were made on the isolated dCMP and on the cytosine obtained from it by hydrolysis with concentrated perchloric acid. When the precursor CMP contained 50 per cent of its C^{14} as cytosine, the recovered dCMP yielded 45 per cent as cytosine. These are identical within experimental error.

The reduction of CMP to dCMP was first observed in calf thymus extracts in the spring of 1959. During the summer the observed activity of such extracts decreased to zero. Active extracts were again obtained in the spring of 1960, but once more fell off to zero during the course of the summer. Since the average age of the calves being slaughtered increased during this period, we obtained thymus from 2 to 4 week old calves and found it fully active while tissues from the local slaughter house were completely inactive.

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