Isolation of Cytidylyl-(5'→3')-adenosine-5' Phosphate (pApC), Adenylyl-(5'→3')-cytidine-5' Phosphate (pCpA), Uridylyl-(5'→3')-adenosine-5' Phosphate (pApU) and Adenylyl-(5'→3')-uridine-5' Phosphate (pUpA) from Embryos of the Copepod *Euchaeta japonica* Marukawa

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It has recently been shown that adenylyl-(5'→3')-adenosine-5' phosphate (pApA) is the major acid soluble nucleotide of the embryos of the marine calanoid copepod *Euchaeta japonica* Marukawa (Hepner and Smith, 1967). The present communication reports the isolation, from the same organism, of the di-ribonucleotides pApC, pCpA, pApU and pUpA.

The acid soluble nucleotides from 5 gm of embryos (numbering approximately 50,000) were extracted and then fractionated on a column of diethylaminoethyl cellulose (45 cm x 1.2 cm) using a linearly increasing concentration of ammonium bicarbonate, pH 8.0, to elute the nucleotides (Oikawa and Smith, 1966). The elution profile is shown in

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Figure 1. The major component, pApA, was eluted by 0.12 M ammonium bicarbonate in peak 9 (Hepner and Smith, 1967). Peak 7, eluted by 0.11 M ammonium bicarbonate, contained a nucleotide with an absorption maximum in water at 262 mμ. After rechromatography on diethylaminoethyl cellulose, the nucleotide was homogeneous as judged by paper chromatography in isobutyric acid: M ammonium hydroxide (5:3) and ammonium sulfate:0.1 M phosphate, pH 6.8:n-propanol (30 gm:50ml:1 ml).

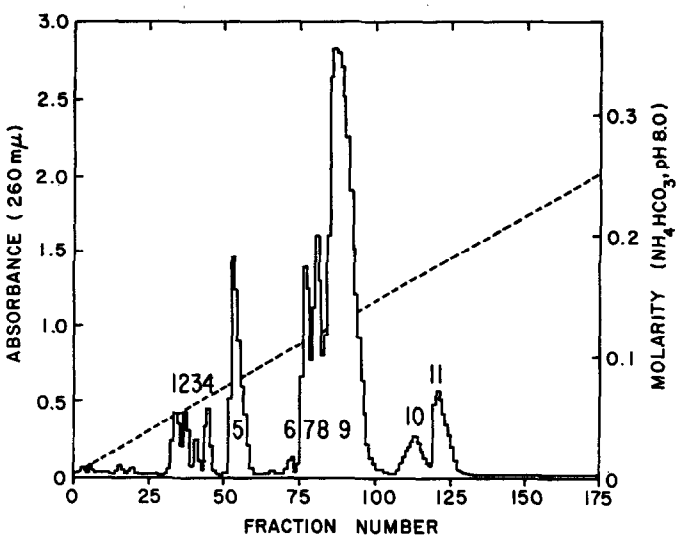
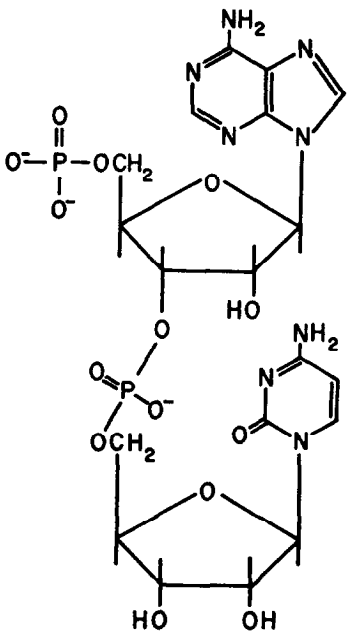


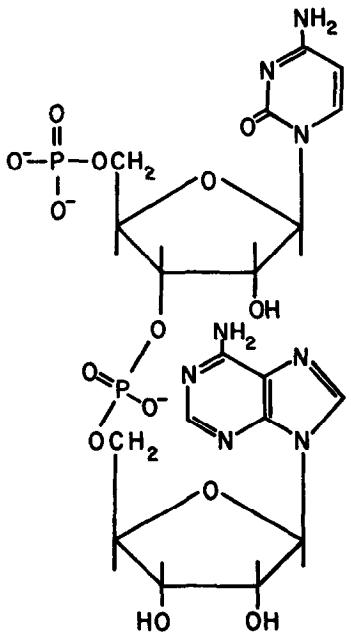
Figure 1. Separation of acid soluble nucleotides from *Euchaeta japonica* (5 gm) on a column of diethylaminoethyl cellulose (45 cm x 1.2 cm). Fractions (20 ml) were collected at 20 minute intervals. Peaks 1 to 5 contained ribonucleoside-5' and deoxyribonucleoside-5' phosphates. Peak 7 contained pApC (approx. 5 μmoles); peak 8, pCpA (approx. 5 μmoles); peak 9, pApU (approx. 1 μmole), pUpA (approx. 1 μmole) and pApA (approx. 25 μmoles; Hepner and Smith, 1967). Peak 11 contained guanosine-5' triphosphate. The contents of peaks 6 and 10 have not been characterized.

The ratio of phosphate liberated by *E. coli* alkaline phosphatase to total phosphate (Ames, 1966) to pentose (Ashwell, 1957) was 0.9:2.0:1.0. The nucleotide was rapidly degraded by phosphodiesterase I from *Crotalus adamanteus* venom (Razzell, 1963) to yield equimolar amounts of adenosine-5' phosphate and cytidine-5' phosphate. Degradation with sodium periodate in the presence of lysine (Neu and Heppel, 1964) yielded one equivalent of cytosine and one of adenosine-3',5' diphosphate. Similarly, ribonuclease T_2 (Uchida and Egami, 1966) yielded cytidine and adenosine-3',5' diphosphate in equivalent amounts. The product of the reaction of the nucleotide with *E. coli* alkaline phosphatase was chromatographically identical with cytidylyl-(5'→3')-adenosine. These data are consistent with the nucleotide being cytidylyl-(5'→3')-adenosine-5' phosphate (pApC); formula 1.



(1)

Peak 8, eluted by 0.115 M ammonium bicarbonate, was further purified by rechromatography on diethylaminoethyl cellulose and by paper chromatography in isobutyric acid : M ammonium hydroxide (5:3). This nucleotide was also degraded by phosphodiesterase I to equimolar amounts of adenosine-5 phosphate and cytidine-5' phosphate. Degradation with sodium periodate and lysine yielded equimolar amounts of adenine and a nucleotide with properties expected of cytidine-3',5' diphosphate. Ribonuclease T_2 yielded the same nucleotide and adenosine in equal amounts. *E. coli* alkaline phosphate yield a product chromatographically identical with adenylyl-(5'→3')-cytidine (CpA). These data are consistent with the nucleotide being adenylyl-(5'→3')-cytidine-5' phosphate (pCpA); formula 2.



(2)

The early fractions of peak 9 were rechromatographed as in the purification of pCpA to yield nucleotide material with an absorption maximum at 260 mμ in water. Degradation by phosphodiesterase I yielded equimolar amounts of adenosine-5' phosphate and uridine-5' phosphate. Ribonuclease T_2 yielded approximately equal amounts of uridine, adenosine, adenosine-3',5' phosphate and a nucleotide with properties expected of uridine-3',5' phosphate. These data are consistent with the nucleotide containing approximately equimolar amounts of adenylyl-(5'→3')-uridine-5' phosphate (pUpA) and uridylyl-(5'→3')-adenosine-5' phosphate (pApU).

The approximate amounts of the five dinucleotides present in 1 gm of *Euchaeta japonica* embryos are pApA, 5 μmoles; pApC, 1 μmole; pCpA, 1 μmole; pApU, 200 mμmoles; pUpA, 200 mμmoles. It is possible that present in the embryos are others of the 16 dinucleotides which can be formed from the 4 common ribonucleoside-5' phosphates. However, they have not been detected in this study and cannot be present in amounts greater than 50 mμmoles/gm.

The biosynthesis of the dinucleotides in *Euchaeta japonica* embryos and their biological function are not yet understood. Possible roles for this type of molecule lay in the storage of organic phosphate or as regulators of nucleic acid or protein synthesis (Hepner and Smith, 1967). In this connection it is of interest that the dinucleotide pUpC can promote the specific binding to ribosomes of serine transfer-RNA (Rottman and Nirenberg, 1966).

Acknowledgement

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