molecule[17]. In invertebrate muscles where the amount of myosin is usually less, tropomyosin A occurs in the free, unbound form. The difference between vertebrate and invertebrate muscles is thus a quantitative rather than a qualitative one.

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Received March 14th, 1958

Preliminary Notes

An aminosugar nucleotide from *Carcinus maenas*

It has been demonstrated that uridine pyrophosphate (UPP) derivatives can act as glycosyl donors for the enzymic synthesis of both disaccharides and polysaccharides[1,2]. (For recent reviews see refs [3,4].) GLASER AND BROWN[5] have shown that the incorporation of ^{14}C-labelled N-acetylglucosamine into chitin by cell-free extracts of *Neurospora crassa* involves ^{14}C-labelled uridine-pyrophosphate-N-acetylglucosamine (UPPAG) as an intermediate. Attempts are now being made to decide whether uridine nucleotides are also concerned in the biosynthesis of chitin by arthropods. The present communication reports the isolation of what appears to be a new UPP derivative from the hypodermis of the shore crab, *Carcinus maenas*.

A crude extract was obtained from hypodermis of 20 crabs (supplied by the Marine Biological Laboratory, Plymouth) in the following way. The fresh tissue was transferred immediately after removal to 100 ml of icewater, and was rapidly minced in a Waring blendor. The minced tissue was boiled for 1 min, filtered through muslin and the residue re-extracted with a further 50 ml of boiling water. The combined filtrates were stirred with unground acid-washed[6] moist Nuchar C (about 40 g) until the liquid became colourless. The filtered charcoal was then washed with water (150 ml) and the nucleotides were extracted repeatedly with 50% aqueous ethanol containing 1% (v/v) 0.88 *N* ammonia until the extracts become pale straw coloured. The combined extracts, concentrated under reduced pressure (temp. 35°) to about 100 ml were applied to a Dowex-1 × 2 (Cl^-) column (200–400 mesh, 20 cm × 0.64 cm^2). The nucleotides were separated in 10-ml fractions by stepwise elution at room temperature at a flow rate not exceeding 40 ml/h.

The following elutants were used: I, 0.01 *N* HCl containing 0.01 *N* NaCl; II, 0.01 *N* HCl containing 0.03 *M* NaCl; III, 0.01 *N* HCl containing 0.06 *M* NaCl; IV, 0.01 *N* HCl containing 0.10 *M* NaCl; V, 0.01 *N* HCl containing 0.20 *M* NaCl.

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With elutant IV, a nucleotide fraction was obtained which after hydrolysis gave positive tests for N-acetylaminosugar. The material did not give a "direct" Ehrlich reaction. In repeat experiments, this fraction was found to be removed from the column slowly by prolonged elution with III.

From 300 μmoles of crude nucleotides (calculated from the extinction at 260 mμ assuming a value of 10 for the millimolar extinction coefficient) applied to the column, 3.5 μmoles of the above aminosugar-containing nucleotide was obtained. The remainder of the u.v.-absorbing material was comprised of fractions giving adenine- and guanine-type spectra. Aminosugars were absent from these fractions.

The nucleotide possessed a typical uridine u.v. spectrum in acidic and alkaline solutions, and the absorption was abolished in acidic conditions by addition of bromine. Hydrolysis of the nucleotide (designated UPPX) in 0.01 *N* HCl at 100° for 3.5 min yielded uridine pyrophosphate, identified chromatographically in ammonium acetate–ethanol[7] by comparison with authentic material. Under more vigorous hydrolytic conditions (0.1 *N* HCl, at 100° for 10 min) UPPX gave uridine monophosphate, which was identified as the 5'-isomer by paper chromatography (in ammonium acetate–ethanol[7] and isopropanol–ammonium sulphate[8]) and by paper electrophoresis in borate buffer (pH 8, 1 *M*, 8.8 V/cm for 6 h).

Determinations of the total and "acid-labile" (1 *N* HCl at 100° for 20 min) phosphate esters using a modification[9] of Berenblum and Chain's method indicated the presence of two ester phosphates, one of which was acid-labile. Quantitative estimations of N-acetylaminosugar content (a modification[10] of the Morgan and Elson method) of UPPX after acidic hydrolysis (0.01 or 0.1 *N* HCl) revealed the presence of not more than one mole of N-acetylaminosugar per mole of uridine. The spectrum of the chromagen was closely similar to that given by authentic N-acetylglucosamine. The latter was also demonstrated chromatographically in hydrolysates of UPPX. Using a number of solvent systems, chromatographic examination of further hydrolysates (2 *N* HCl for 6 h at 100°) revealed an aminosugar resembling glucosamine and this was also indicated by the formation of arabinose in the STOFFYN AND JEANLOZ[11] method.

There was no evidence of other ninhydrin-positive components even after hydrolysis of UPPX for 18 h by 6 *N* HCl at 100°.

The composition of UPPX can be summarised as follows: Uridine (1.00 mole), total phosphate (1.97), acid-labile phosphate (0.99), N-acetyl aminosugar (0.82).

It is clear that the *Carcinus* nucleotide, UPPX, closely resembles UPPAG but is distinguished from it by being more strongly retained by Dowex-1 and by its consistently higher $R_{\text{Adenosine}}$ value[7,12] (UPPX, 0.66–0.74; UPPAG, 0.55–0.58) in ammonium acetate–ethanol. Though this solvent system is known to be temperature sensitive, UPPX in all experiments was found to be separable from UPPAG. Similarly, it was confirmed that UPPAG added to Dowex-1 (Cl^-) columns was completely and sharply removed by elutant III.

In view of these properties, it is suggested that UPPX differs from UPPAG in the aminosugar moiety, perhaps by the presence of some labile substituent group(s). The substance is clearly different from the nucleotides described by PARK[13].

At least one compound having properties closely similar to UPPX has been found in the hypodermis of the spider crab (*Maia squinado*). The lobster (*Homarus vulgaris*) was found to contain both UPPAG and UPPX.

The authors thank the Trustees of the Christopher Welch Scholarships and the Medical Research Council for their awards to one of them (M. R. L.).

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Received March 6th, 1958