Sulfated Macromolecules in Early Embryos of *Limnaea*

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An attempt has been made to partially characterize the substance responsible for the rhythmic incorporation of ^{35}S (inorganic sulfate) in the course of the cell cycle in early *Limnaea* embryos. This trichloroacetic acid (TCA) insoluble substance is partly pronase sensitive, and dissociable into two fractions after treatment with NaCl. One of these remains TCA-insoluble while the other is TCA soluble but precipitable by cetyl pyridinium chloride. Thus, unlike in some other higher organisms, the major part of the inorganic sulfate is incorporated here into a fraction which is not a simple mucopolysaccharide, but is more likely to be a protein-mucopolysaccharide complex, rather like the chondromucoprotein of chick-embryo cartilage.

This note describes the partial chemical characterization of the TCA-insoluble substance containing sulfur which is known to show a marked metabolic rhythm during mitosis in Limnaea eggs [1].

The TCA-insoluble substance was acid-hydrolysed and two-dimensional paper chromatograms (with *n*-butanol : acetic acid. water = 4 : 1 : 1 and water saturated phenol) were developed. Acid hydrolysis releases inorganic sulphate which remains at the origin. Chromatograms developed on X-ray plates show this spot at the origin and another "hot" spot. There is no hot spot corresponding to cystein. The counts in the precipitate and in the supernatant of of pronase treated and untreated parts were measured. (Under these conditions the carrier bovine serum albumin is not precipitated after pronase digestion, thus showing the effective nature of the digestion.) Thus it was seen that the substance is partly pronase sensitive. The TCA-insoluble substance was dissolved in formate buffer (or 1 N NaOH) and NaCl (final concentration 2 M) or directly in 4 M NaCl. This procedure dissociates the substance, a part of the count is rendered TCA-soluble. The major part of these TCA-soluble counts is precipitable by cetyl-pyridinium chloride (3 – 10%). This suggests that the substance is likely to be of the acid-mucopolysaccharide type.

It thus seems that in *Limnaea* eggs inorganic sulfate is incorporated into a substance that is not a simple mucopolysaccharide, but is probably a protein-MPS complex. This is, interestingly, rather like the chondromucoprotein of chick-embryo eartilage [2] and unlike sulphated mucopolysaccharides of organisms like sea-urchin [3, 4] or Fucus [5].

Addendum. 1. Hyaluronidase sensitivity of the substance has been tested (D. Ghosal, in preparation). 2. It has been shown by paper electrophoresis that while the whole molecule remains immovable, the presumed MPS molecule is fast moving (faster than heparin).

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