

The Use of 2,4-Dinitro-1-fluorobenzene in the Separation and Identification of Amino Acids from Sea Water

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Difficulties in the separation of amino acids and peptides from sea water arise from the high salt content, as previously mentioned by many authors. Recently a few reports have appeared describing the separation and identification of amino acids from hydrolysates of surface and deep-sea water^{1,2} and free amino acids from unhydrolysed sea water,³ but the methods employed have the disadvantage that a large sample is needed and that they are rather laborious and time-consuming.

The use of 2,4-dinitro-1-fluorobenzene (DNFB) was described by Sanger⁴ in his work on free amino groups in insulin and by Porter and Sanger⁵ on free amino groups in haemoglobins. This reagent has subsequently been widely used for isolation and characterization of amino acids and peptides from biological fluids and tissue extracts.

Work done in this laboratory has shown that it is possible to extract and identify amino acids in sea water as their dinitrophenyl derivatives after reaction with DNFB.

Outline of the procedure. A five liter sample of sea water was filtered through H. A. Millipore filter (0.45 μ) and adjusted to pH 9.3. After addition of 5 ml DNFB dissolved in 100 ml acetone, the sample was shaken at room temperature in total darkness for 4 h. The excess DNFB was extracted with 3 \times 200 ml peroxide-free ether. The solution was then acidified with hydrochloric acid to pH 2.4 and the ether soluble DNP-amino acids extracted with 6 \times 200 ml portions of peroxide free ether. The combined ether extract was washed three times with 0.1 N hydrochloric acid and dried with anhydrous sodium sulphate. The ether extract was then evaporated to a small volume and transferred to a cold finger sublimation apparatus as designed by Mills,⁶ where most of the dinitrophenol was sublimed off *in vacuo*. The residue was taken up in a measured volume of acetone for chromatography.

The 0.1 N hydrochloric acid washings of the ether extract were added to the ether extracted sample and this was then extracted with 4 \times 200 ml portions of butanol. The water saturated butanol extract was concentrated in a rotary vacuum evaporator and the salts which precipitated were filtered off. The salts were washed with a small volume of butanol, the butanol extracts combined, concentrated further and then diluted with acetone saturated with 6 N hydrochloric acid to give a solution suitable for chromatography.

An artificial sea water sample with 50 μ g of each of 19 amino acids was treated according to the same procedure.

Circular thin-layer chromatography as described by Stahl⁷, using glass plates measuring 20 \times 20 cm with an approximately 0.25 mm thick layer of Silicagel G nach Stahl,⁷ was employed for the separation of the DNP-amino acids. The extracts and the standards were applied as described for the circular paper chromatograms³. The cabinets consisted of an 18 cm diameter evaporating dish and a centrally placed wick of rolled paper held by a glass tube. The solvent system used was chloroform: benzylalcohol:acetic acid (70:30:3), which has the advantage that it completely separates the dinitrophenol and dinitroaniline from the DNP-amino acids⁷. The chromatograms were run for approximately 2 h and the solvent was removed with a stream of warm air. The chromatographic glass plate was then placed face downwards on a photographic paper (Kodak, Bromide WSG. 3S) made sensitive to UV-light by dipping in a bath of 1 % citric acid in 95 % ethanol⁸ for less than 2 min, and exposed to UV-light directly from above for about one second.

Care was taken throughout the procedure to minimize exposure to light.

Results. The ether extract from the sea water sample showed 12 bands with approximate $R_{\text{DNP-leu}}$ -values 0.26, 0.32, 0.38, 0.48, 0.55, 0.59, 0.64, 0.67, 0.70, 0.77, 0.81, 0.89 and 0.95. Standards having approximately the same $R_{\text{DNP-leu}}$ -values run on the same chromatogram were used in an overlapping technique on a second chromatogram, showing that DNP-glycine, DNP-threonine, DNP-valine and DNP-phenylalanine were present. From the starting sector and nearly out to the first distinct band with $R_{\text{DNP-leu}} = 0.26$ a broad wavy pattern disturbed the underlying bands. Another intense but narrow disturbing band occurred at $R_{\text{DNP-leu}} = 0.70$. It was possible to remove most of these two bands by continuing the sublimation after the

dinitrophenol was removed from the sample; chromatograms of the last fraction of that sublimate gave two bands with the same $R_{\text{DNP-leu}}$ -values as in the sample. The butanol extract from the sea-water showed three bands with $R_{\text{DNP-leu}}$ -values 0.08, 0.17 and 0.32, as yet not identified.

The ether extract of the artificial sea water sample showed 14 distinct bands and the butanol extract showed four bands on the chromatograms. These results are in good agreement with the statement of Smith⁹ regarding the solubilities of various DNP-amino acids in ether and butanol.

The results, while of a preliminary nature, indicate that DNFB can be used to identify amino acids in sea water samples. It may also be possible to study peptides and proteins in sea water with this reagent although their concentrations may require the use of a preliminary concentration step, such as the ferric hydroxide gel procedure described by Jeffrey and Hood¹⁰. Further studies using this method are currently being carried out in order to determine the amino acid patterns of sea water samples obtained at various localities at different times of the year.

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The Crystal Structure of Aberrant Otoliths

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The main constituent of cod otoliths, calcium carbonate, has been shown by Dannevig¹ and others² to be present in the form of aragonite.

We have investigated the nature of the aberrant forms which occasionally occur, since it was thought that this knowledge might be of use in studying the factors affecting the structure and growth of normal otoliths. There appear to be two main aberrant forms, which we have designated Type 1 and Type 2 (Fig. 1).

In the Type 1 modification the nacreous smooth-ribbed structure of the normal cod otolith is completely lost, being replaced by a translucent randomly-crystalline structure which does, however, retain the general overall shape of the normal otolith. The Type 2 modification partly retains the structure of the normal otolith, but a portion is covered by markedly crystalline accretions which may cause considerable distortion of the normal shape.

X-Ray powder analyses of whole Type 1 otoliths and the crystalline accretions from Type 2 otoliths showed that Type 1 otoliths contain a mixture of calcite and vaterite while the accretions from Type 2 contain calcite only (Fig. 2).

The occurrence of vaterite in the Type 1 aberration is interesting since this modification of calcium carbonate only appears to have been found once in plant or animal sources before³ namely in small amounts in human gallstones⁴.

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