THE COMPOSITION OF THE BASIC PROTEINS OF ECHINODERM SPERM

D. HAMER

Cancer Research Laboratories, The Medical School, Birmingham 15, England

Protein with strongly basic properties has been found in the nuclei of most cells in association with desoxyribonucleic acid. In the sperm of some species of fish these proteins are simple in composition (protamines), containing only 7 or 8 amino acids and of these arginine accounts for about 90 per cent of the protein nitrogen (Hamer and Woodhouse, 1949; Felix, 1953). It has also been claimed that protamines are found in other animals, for example in fowl sperm (*Gallus domesticus*) (Fischer, 1954). However, in the sperm of cod (Stedman and Stedman, 1951) and mammals (Dallam and Thomas, 1953) proteins of histone type have been reported and these differ from protamines in having 16 amino acids. Arginine is again the principal component (20–30 per cent protein N) and, along with lysine and histidine, gives the protein its characteristic basic properties (Hamer, 1951, 1953a).

These basic proteins are believed to form some part of the structure of the chromosomes of the cells and so it is particularly interesting to know if they show species differences in composition. With this in view, basic proteins have been isolated from the spermatozoa of three echinoderms, the sea urchin, sand dollar and starfish, and the amino acid compositions determined. A similar protein isolated from the sperm of a mollusc, the squid, was also examined. The only previous chemical examination of the basic proteins of the sperm of invertebrates has been by Hultin (1947) and Hultin and Herne (1948). These workers reported that the compositions of sea urchin and mollusc basic sperm proteins were intermediate between protamines and histones, containing in all 11 amino acids. There was a considerable difference between the mollusc and sea urchin proteins while the basic proteins from different species of sea urchin were more similar in composition.

The results presented here show that in the species examined the sperm contain histone-type basic proteins and that there are significant species differences in composition. Two preliminary reports have included some of the results presented here (Hamer, 1953b, 1954).

Materials and Methods

Sperm from sea urchins (*Arbacia punctulata*) and sand dollars (*Echinarachnius parma*) were obtained by injection of 0.5 *M* KCl and the sperm collected in sea water. Ripe testes of starfish (*Asterias forbesi*) and squid (*Loligo pealii*) were removed, cut into small pieces and the sperm allowed to shed into sea water. The sperm were collected by centrifugation, washed first with sea water and then several times with a saline-citrate solution (0.14 *M* NaCl, 0.02 *M* citric acid).

Sperm nuclei so obtained were next extracted directly with 0.2 *M* HCl for 24 hours and then, after centrifuging, the extract was dialyzed against distilled water. All these operations were carried out in the cold. This extract now contained the protein or group of proteins classified as basic nuclear proteins (protamines and histones) since they are of nuclear origin, are extracted by acid, and remain soluble after removal of the acid by dialysis (Hamer, 1953a). It was found that the basic protein in the dialyzed solution could not be precipitated by addition of small amounts of ammonia (unlike mammalian histones) or sodium hydroxide and so picric acid was added and the protein picrate collected. This was then dissolved in acetone and any insoluble material centrifuged off. The solubility of basic protein picrates in acetone has previously been used for fractionation by

TABLE I

Composition of basic proteins

	Sea urchin*	Sea star	Sand dollar	Squid
Alanine	9.8	9.85	9.3	4.2
Ammonia	3.8	3.9	3.7	4.0
Arginine	28.0	23.8	34.1	47.7
Aspartic acid	2.7	2.9	1.9	3.4
Glutamic acid	3.9	4.0	3.0	3.7
Glycine	5.7	4.4	4.85	3.6
Histidine	2.1	2.9	1.0	2.0
Leucine	7.2	5.8	6.1	5.8
Lysine	17.1	19.0	13.9	8.2
Phenylalanine	1.0	0.9	0.9	1.0
Proline	4.4	4.25	3.4	2.6
Serine	4.0	3.8	5.3	4.8
Threonine	3.45	3.1	4.2	2.1
Tyrosine	1.1	1.1	1.1	1.9
Valine	3.7	3.9	4.2	2.8
Methionine	1.0	1.4	x	x

Amino acid nitrogen expressed as percentage of protein nitrogen.

* Cystine and tryptophane estimation were carried out only on the sea urchin basic protein. Neither was present in significant amount (less than 0.1 and 0.06%, respectively). The nitrogen content of this protein sulphate was 14.4 per cent.

x Not estimated.

Rasmussen (1934) and Hultin (1947). Sulphuric acid was added dropwise until precipitation of the protein sulphate was complete. The sulphate was dissolved in a small amount of water and re-precipitated by addition of acetone, to remove any remaining picric acid. After washing again with acetone the white protein sulphate was dried *in vacuo*. It may be noted here that similar techniques were applied for the preparation of basic fractions from echinoderm eggs following direct extraction of the eggs with acid. Small amounts of proteins were obtained but these were insufficient for quantitative analysis.

The analytical methods used were those previously applied to thymus histones (Hamer, 1951, 1953a). The protein was hydrolyzed for 18 hours with 6 N HCl under reflux. After repeated evaporation to remove acid, aliquots were analyzed by the starch column chromatographic technique of Stein and Moore (1949).

Separation of leucine and isoleucine was not carried out and these results are reported together as leucine. The identity of all components was confirmed separately by paper chromatography. Methionine, cystine and tryptophane were tested for independently when sufficient material was available by the following methods: methionine by the nitroprusside reaction (Horn, Jones and Blum, 1946); tryptophane using p-dimethylaminobenzaldehyde in sulphuric acid on unhydrolyzed protein (Spies and Chambers, 1949); cystine by Shinohara's modification of the Folin-Winterstein reaction (Block and Bolling, 1951). Nitrogen was determined by a micro-Kjeldahl method and phosphorus by Holman's (1943) colorimetric method after perchloric acid combustion. Recoveries of protein nitrogen in terms of amino acid nitrogen and ammonia ranged from 95–99 per cent.

Results

The results of the amino acid analyses are given in the table. It will be seen that all the basic proteins examined have the general type of composition found in the histone group. In each case, the basic amino acids arginine, lysine, and histidine together account for about 50 per cent of the protein nitrogen. Only small amounts of the aromatic amino acids tyrosine and phenylalanine are present and, in the case of the sea urchin specimen, analysis showed there was no significant amount of tryptophane or cystine. Glutamic and aspartic acids represent 7–8 per cent of the whole. Compared with histones from mammalian cells, the echinoderm sperm histones contain appreciably more alanine and lysine but less leucine, phenylalanine and tyrosine. The protein from the squid sperm contains considerably more arginine than is found in the specimens from sea urchin and starfish by colorimetric estimation though this was not sufficient to be detected chromatographically on the starch columns.

From estimations of the nitrogen and phosphorus contents of sea urchin sperm nuclei before and after extraction with acid it was estimated that about 27 per cent of the dry weight of the nuclei was nucleic acid, 21 per cent histone extractable by acid and the remaining 52 per cent acid-insoluble protein. The nature of this protein has not been investigated though in the case of mammalian tissues it has been found to be similar in composition to the basic protein but to contain, in addition, measurable amounts of cystine and tryptophane.

Discussion

The results presented above differ from those of Hultin and Herne (1948) in that 16 amino acids were found in the basic proteins of all the species examined whereas only 11 had previously been reported for related species (*e.g., Arbacia lixula, Brissopsis lyrifera*). It would seem unlikely that this is due to a species difference but is more likely to arise from differences in analytical methods. For qualitative analysis Hultin and Herne carried out a preliminary fractionation of the hydrolysates on carbon and other adsorbents. Possibly the small amounts of tyrosine, phenylalanine, and methionine present were lost in this treatment but the failure to detect glutamic acid and aspartic acid is difficult to account for.

Significant species differences were found between the three echinoderms examined. Considerable variations occur in the lysine and arginine contents while there are minor variations in the amounts of other amino acids, for example

leucine and glycine. The molluscs, as represented by the squid specimen, show more considerable changes in composition but the basic protein is still of the histone-type. At the moment there seems no rational way of predicting whether the sperm nuclei of a particular species will contain a protamine or a histone as the basic component of the desoxyribonucleo-protein complex. A number of fish and fowl sperm contain protamines while the sperm of some mammals and invertebrates contain histones. At present there are insufficient data to justify a claim that protamines occur widely in sperm nuclei (Fischer, 1954).

The results quoted for the relative amounts of histone, nucleic acid and acid-insoluble material are in agreement with those obtained by Bernstein and Mazia (1953) using sperm nucleoprotein of the sea urchin *Strongylocentrotus purpuratus*. There is certainly a considerable amount of protein associated with the nucleic acid in the sperm nuclei which is not "histone" in the sense that it is not readily extracted by acid. This has also been found in studies on mammalian somatic cells, and in discussing the possible arrangement of these fractions it has been suggested that the histone may be held by simple ionic forces while at least part of the acid-insoluble protein is held by chemical links to the nucleic acid (Hamer, 1953a, 1954). The work of Barton (1952a, 1952b) on the fractionation of invertebrate sperm with acid and nucleases lends some support to such a structural arrangement.

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Summary

1. Basic proteins have been extracted from sperm of the sea urchin, sand dollar and starfish, and also from the squid. The amino acid composition of these proteins has been determined.

2. All the basic proteins were of histone type and were similar in general properties and composition to mammalian histones.

3. There are significant species differences among the echinoderms examined, particularly in the arginine and lysine contents. The basic protein of squid sperm contains much more arginine and less lysine than the echinoderm specimens.

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