PARTIAL AMINO ACID SEQUENCE OF HISTONE H1 FROM SPERM OF THE SEA URCHIN, *PARECHINUS ANGULOSUS*

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1. Introduction

In contrast to the conserved sequences of histones H4 and H3 [1], H1 histone has been reported to vary considerably in its structure, even within the organisms from which it has been isolated [2–4]. In continuation of our studies of sea urchin sperm histones, histone H1 has been purified and peptides prepared by cyanogen bromide and *N*-bromosuccinimide cleavage. These peptides have been aligned and partially sequenced. The carboxyl half of the molecule is intensely basic while the central region contains most of the hydrophobic residues. The amino terminal region is also basic and contains many of the proline residues. The distribution is therefore very similar to that found in other H1 histones that have been partially sequenced [1–3]. In this paper sea urchin H1 is compared to rabbit thymus histone H1 Fraction-3 [3], trout histone H1 [1,4] and chicken histone H5 [5,6] in terms of the amino acid distribution and partial sequences.

2. Experimental

Sea urchins were dissected and only ripe male gonads were collected. The tissue was placed on a double layer of muslin and the sperm were filtered out by washing with 0.15 M NaCl while the tissue was gently stirred with a glass rod. The cells were pelleted by centrifugation at 12 000 *g* for 10 min and then frozen in small batches (about 30 g) in liquid nitrogen and stored at −15°C until used.

To isolate histone H1, the sperm were unfrozen by resuspending in seven volumes of 0.15 M NaCl. The cells were broken in a Dounce homogenizer with a tight plunger, and the mixture centrifuged at 6000 *g* for 10 min. This process was repeated six or seven times. The final precipitate was then suspended in double distilled water (5 ml per g sperm) and allowed to swell for 1 h. With constant stirring, an equal volume of 10% perchloric acid (w/v) was added and the mixture sitrred for another hour [7]. After centrifugation the supernatant was extensively dialysed against deionized water and then freeze dried. All operations were done at + 4°C.

Gel electrophoresis was done according to Panyim and Chalkley [8]. N-terminal and C-terminal group determination with dansyl-HCl and carboxypeptidase respectively, cyanogen bromide (CN) cleavage, *N*-bromosuccinimide (NBS) cleavage and amino acid analysis were done as previously reported [9] but using thioglycollic acid instead of phenol to prevent oxidative losses during protein hydrolysis. The presence of dibromodienone spirolactone arising from tyrosine in the *N*-bromosuccinimide cleavage was monitored at 260 nm.

Automatic sequence analysis, identification of the PTH-amino acid derivatives, their quantitation and assignments to positions in the sequence was done as described previously [10] except that 0.1 mg dithioerythritol was added to each tube in the sequenator fraction collector to facilitate serine quantitation.

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3. Results and discussion

The portein extracted by 5% perchloric acid gave only a single band on acrylamide gel electrophoresis and on dansylation a blocked N-terminus. Amino acid analysis showed that the protein was very rich in both lysine and alanine residues (table 1) and was similar in many respects to both, mammalian H1 histones [1–3] and chicken erythrocyte H5 [5,6]. This sea urchin histone H1 has four methionine residues whereas of the mammalian H1 histones only a minor subfraction contains methionine [11] and histone H5 has only one methionine residue (table 2). Sea urchin histone H1 was therefore cleaved by cyanogen bromide and the freeze-dried peptides were partly separated into 5 fractions on a Sephadex G-100 column (fig.1). Fraction CN-0 eluted at the void volume and its amino acid composition was the same as the uncleaved protein. Fractions CN-I and CN-II on dansylation each contained only one N-terminal amino acid and each polypeptide gave a single band on acrylamide gel electrophoresis. CN-III, a mixture of peptides, was subsequently separated into fractions CN-IIIa–CN-IIIf (fig.2). Except for CN-IIIa the peptides were homogeneous on criteria of amino acid analysis and end group analysis by dansylation. The sum of the amino acids in CN-I, CN-II, CN-IIIb, CN-IIIc and CN-IIIf (table 2) accounts, within experimental error, for the amino acid composition of the uncleaved protein. CN-IIIc, CN-IIId and CN-IIIe represent each a hexapeptide of identical amino acid composition with alanine as the N- and homoserine as the C-terminal group. The differences revealed on the ion exchange column are expected because of the equilibrium between the C-terminal homoserine and its lactone [12] and a possible partial rearrangement at the aspartyl residue [12].

The protein was also cleaved at its two tyrosine residues with *N*-bromosuccinimide and the resulting peptides were separated with 0.01 N HCl on a Sephadex G-100 column (25 × 900 mm). Only two peptides instead of the three expected were recovered, namely NBS-II and NBS-III. Within the limits of the accuracy of the amino acid determination NBS-III corresponds to the total of CN-IIIb, CN-II,

Table 1
Amino acid compositions of H1 and H5 histones from various species

	Chicken H5 [5] (mol %)	Sea Urchin H1[a] (mol %)	Calf H1 [10] (mol %)
Asp (A)	1.5	1.8	2.7
Thr	3.0	1.9	5.5
Ser	11.9	6.0	6.5
Glu (A)	3.8	2.3	4.2
Pro	7.8	7.3	10.1
Gly	5.0	4.2	6.9
Ala	15.9	24.9	23.3
Val	4.2	3.7	4.9
Met	0.4	1.8	0.0
Ile	3.0	1.0	0.9
Leu	4.3	2.2	4.1
Tyr	1.5	0.9	0.6
Phe	0.5	0.4	0.6
Lys (B)	24.6	29.5	27.8
His (B)	1.5	1.0	0.0
Arg (B)	11.0	11.0	2.0
Acidics	5.3	4.1	6.9
Basics	37.1	41.5	29.8
B/A	7.0	10.1	4.3
Lys/Arg	2.2	2.7	13.9

[a] Uncorrected for destruction of amino acids and incomplete hydrolysis.

Table 2
Amino acid composition* of H1 histone from sperm of the sea urchin (*Parechinus angulosus*) and of peptides derived by cleavage with cyanogen bromide and *N*-bromosuccinimide

	Uncleaved protein		NBS-III		CN-IIIb		CN-II		CN-IIIf		NBS-II		CN-IIIc		CN-I	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Asp	3.92	4	0	0	0	0	0	0	0	0	2.80	3	1.23	1	2.04	2
Thr	4.35	4	1.86	2	.81	1	1.00	1	0	0	3.03	3	0	0	2.45	2
Ser	13.37	13	9.05	9	0	0	5.26	5	2.80	3	5.39	5	0	0	4.74	5
Glx	5.14	5	4.05	4	.93	1	2.27	2	1.08	1	1.05	1	0	0	1.00	1
Pro	16.30	16	9.76	10	0	0	8.04	8	0	0	10.21	10	0	0	8.91	9
Gly	9.49	10	2.09	2	0	0	1.17	1	1.00	1	7.55	8	0	0	7.23	7
Ala	55.75	56	13.19	13	3.16	3	7.64	8	2.21	2	48.54	49	.92	1	43.63	44
Val	8.25	8	2.10	2	1.00	1	1.14	1	0	0	6.57	7	.85	1	5.66	6
Met	3.96	4	0[c]		0[e]	1	0[e]	1	0[e]	1	0[c]	0	0[e]	1	0	0
Ile	2.18	2	1.99	2	.88	1	0	0	.95	1	0	0	0	0	0	0
Leu	4.82	5	1.05	1	0	0	.97	1	0	0	3.56	4	0	0	3.53	4
Tyr	2.09	2	0	1[d]	0	0	0	0	.98	1	0	0	1.18	1	0	0
Phe	0.99	1	0	0	0	0	0	0	0	0	0.91	1	0	0	0.9	1
Lys	65.92	66	13.69	14	0	0	8.22	8	4.40	4	53.60	54	0	0	51.52	52
His	2.17	2	0[c]		0	0	1.00	1	0	0	0[c]		0	0	0.98	1
Arg	24.94	25	10.13	10	0	0	7.95	8	1.10	1	15.73	16	1.22	1	14.25	14
Total		223		70		8		45		15		161		6		150

[a] Number of residues.
[b] Nearest integral.
[c] Oxidized by treatment.
[d] Detected as spirolactone
[e] On amino acid analysis:homoserine lactone present
* No corrections for losses due to destruction or to incompleteness of hydrolysis have been made.

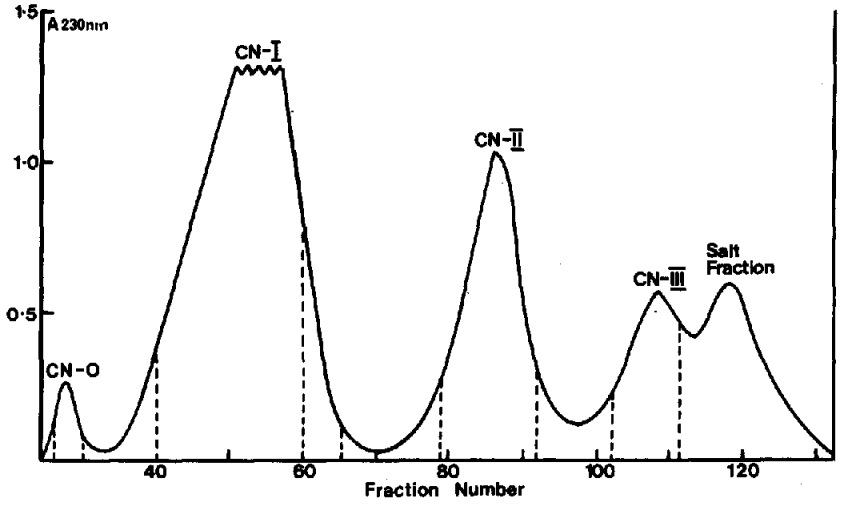


Fig.1. Elution with 0.01 N HCl of 242 mg of cyanogen bromide cleavage peptides of sea urchin H1-histone from a Sephadex G-100 column (50 × 1000 mm). Fraction size: 10 ml. Fraction CN-I, CN-II and CN-III have been isolated as indicated.

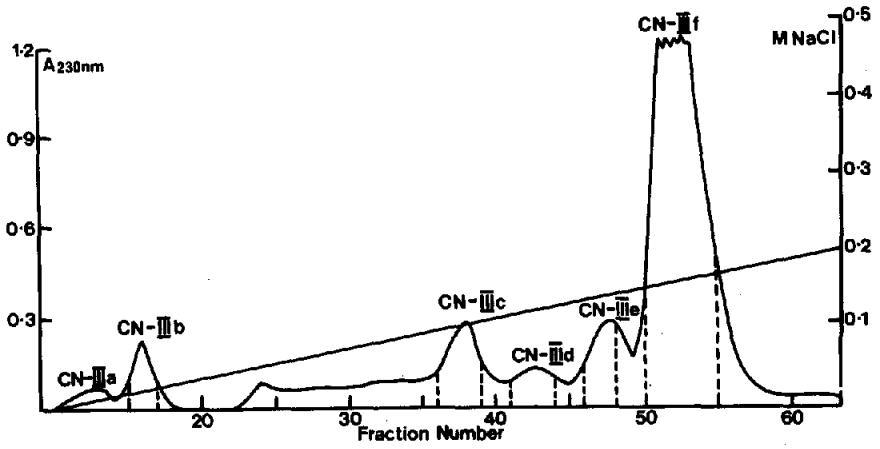


Fig.2. Separation of CN-III peptides on a carboxymethyl cellulose column (15 × 300 mm) by a linear sodium chloride gradient in 0.05 M sodium acetate buffer at pH 4.5. Fractions were combined for further analysis as indicated.

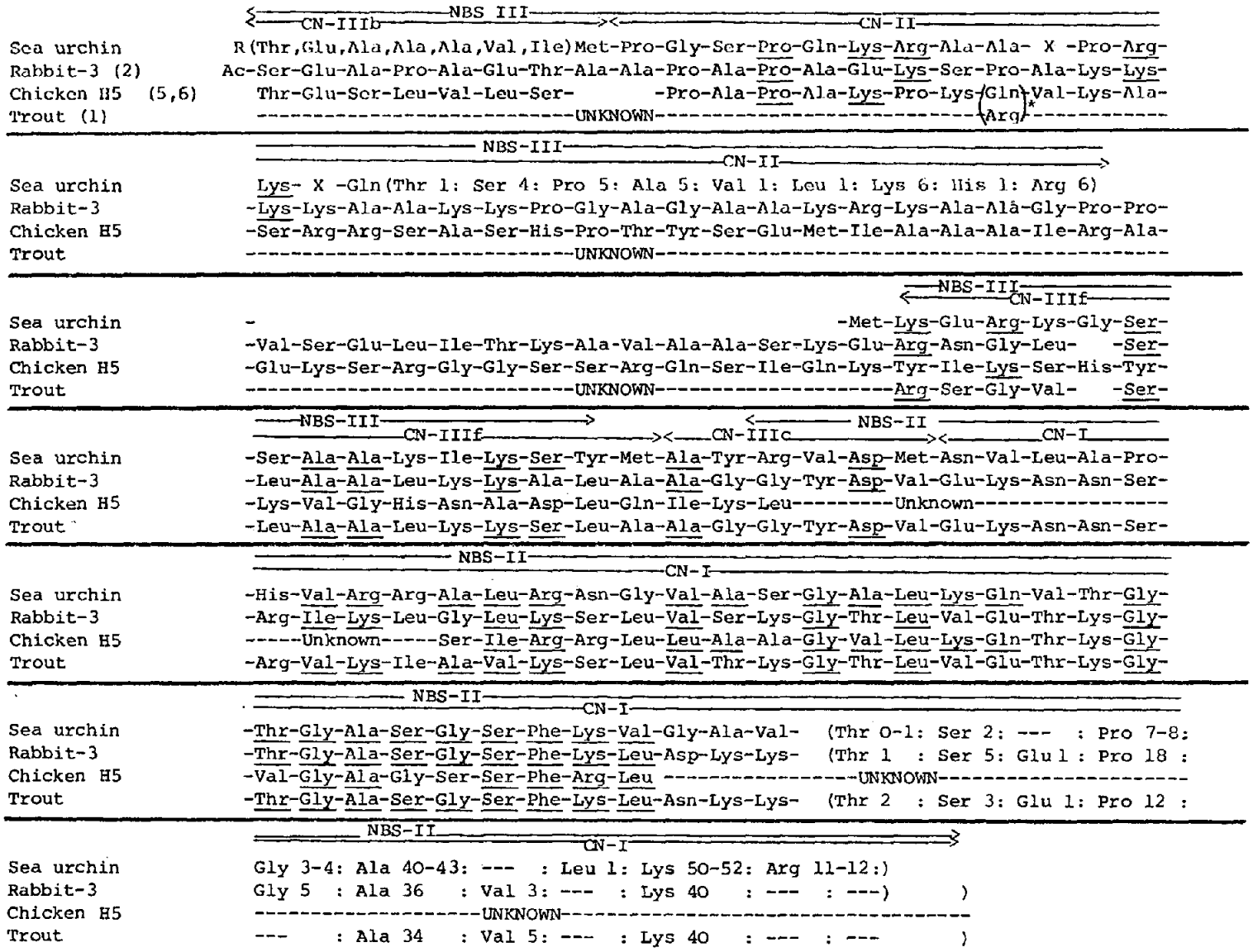
CN-IIIf if the destruction of methionine through the NBS treatment is taken into account (table 2), whereas NBS-II has a composition close to the sum of CN-I plus CN-IIIc (table 2). As to the methionine analysis of NBS-II the same applies as for NBS-III.

Peptides CN-I and CN-II have been partly, and CN-IIIf completely, sequenced. Peptide NBS-II has been partially sequenced to establish an overlap between CN-IIIc and CN-I. These sequences together with the knowledge of the specificity of the cleavages and that of the N- and C-terminal amino acids of the various peptides isolated and their composition allow the alignment of all fragments (fig.3). Two tyrosine residues occupy the centre of the molecule and are only separated by two amino acids. Cleavage with NBS has therefore resulted in addition to the two large peptides NBS-II and NBS-III also in a tripeptide which has not been recovered consisting of the N-terminal oxidation product of methionine followed by alanine and the C-terminal spirolactone generated from tyrosine by NBS. Also shown are comparisons to known regions of rabbit thymus-3 H1 [2,3], trout H1 [1,4] and chicken erythrocyte H5 [5,6]. In two positions in peptide CN-II assignments were not yet possible. These positions may be occupied by substituted serine residues which are probably destroyed through β-elimination during sequential degradation.

It is apparent that the general distribution of the various types of amino acids is similar in all four proteins but that there are many differences in detail. The amino terminal ends in all four proteins are blocked and the N-terminal regions are rich in proline residues and fairly basic. The central regions are all hydrophobic while the carboxyl terminal halves of each protein are all intensely basic. This is especially true for the sea urchin H1.

Though these general features of amino acid residue distribution are common, the details of amino acid compositions and the sequences (table 1, fig.3) are different. Both, histones H1 from sea urchin and H5 from chicken erythrocytes, have a high arginine content in comparison to H1 (rabbit thymus-3). Unlike other H1s and H5, the sea urchin histone H1 contains four methionine residues. All four proteins have a high content of lysine, proline, alanine and serine residues.

It is therefore probable that histone H1 (sea urchin) performs a similar role in the complete repression of transcription in sperm cells as is postulated for histone H5 (chicken erythrocytes) in shutting down the transcription in erythrocyte nuclei. As pointed out by Garel et al. [5] H1 and H5 histones have a common ancestry. The large number of amino acid changes suggest that these proteins are not subject to the same rigorous restrictions as are imposed on H4 or H3, whereas the similar distribution of amino acid categories indicates the performance of the same biological function in all species.



Note : Those amino acids which are identical or have similar properties to the residues in sea urchin F1 histone have been underlined. * Heterogeneous position in H5.

Fig.3. Comparison of amino acid sequences of sea urchin H1-, trout H1- and chicken erythrocyte H5-histones.

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