PARTIAL AMINO ACID SEQUENCE OF TWO NEW ARGININE–SERINE RICH HISTONES FROM MALE GONADS OF THE SEA URCHIN (*PARECHINUS ANGULOSUS*)

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1. Introduction

It has frequently been postulated that histone fractions F2al and F3 are evolutionarily extremely stable proteins [1–3]. In contrast histones F2b, F2a2 and F1 show considerable variations among the organisms from which they have been isolated [4–7]. The demonstration of these variations was based mainly on the differences in electrophoretic mobility without any characterization of the protein fraction. Early reports also indicated that calf thymus F2b might be heterogeneous [8–9].

As part of a program to study the evolutionary relations of the variable histones among widely divergent species, the histones present in male sera urchin gonads have been fractionated and characterized. Among these histones was a new arginine-serine rich protein which on polyacrylamide electrophoresis behaved like a protein with a molecular weight of approximately 15 000 daltons and with proline as the N-terminal amino acid. In addition, this histone behaved like F2b during Johns' [10] selective extraction.

In this communication evidence will be given that this histone fraction contains two closely related species. The cyanogen bromide fragments (CNBr) reveal that the two histones contain an identical C-terminal section and a different but closely related N-terminal section. Sequence studies revealed that the C-terminal amino acid sequence of the molecules is very closely related to calf thymus F2b [11]. The N-terminal regions of both proteins consist of closely related, repetitive sequences which are only vaguely similar to the corresponding section of calf thymus F2b. The N-terminal sequences in fact show a strong sequential similarity to calf thymus F2al [1]. The results are discussed.

2. Experimental

Chromatin was isolated from ripe male sea urchin gonads by homogenizing the tissue in 0.15 M NaCl–0.015 M sodium citrate (saline citrate) to remove soluble non-chromatin protein. This procedure was repeated twice, followed by homogenization in saline-citrate containing 0.2% Triton-X. The chromatin pellet was once again homogenized in saline-citrate and subsequently twice in cold (−15°C) 90% ethanol. In each case the homogenate was centrifuged at 4000 *g* for 10 min at 0°C. Crude histone fractions were obtained by the selective extraction of Johns [10] and further purified by column chromatography [12].

Gel electrophoresis, dansylation, cyanogen bromide (CNBr) cleavage and amino acid analysis were performed as previously reported [13] but using thioglycollic acid instead of phenol to prevent oxidative losses during protein hydrolysis.

Sequence analysis was performed on a Beckman 890 sequencer. Instead of Quadrol, dimethylaminopropyne buffer [14] was used in the Edman degradation. The protein and peptide programs provided by the manufacturer were modified to allow for the higher volatility of this buffer, i.e. several additions of buffer during the coupling reaction were made and the ethyl acetate wash was shortened.

PTH amino acids were identified by gas chromato-

Table 1
Amino acid composition of $F2b_{sea\ urchin}$ histones and CNBr cleavage fragments

	Heterogeneous $F2b_{sea\ urchin}$	Heterogeneous CNBr-1	Calf F2b**	$F2b\text{-}1_{sea\ urchin}$	CNBr-1 $F2b\text{-}2_{sea\ urchin}$	CNBr-2	Calf F2b**
Lys	11.00	13.51	(14)	16.46 (10)	14.55 (10)	5.13 (3)	(6)
His	1.46	1.27	(1)	1.15 (1)	1.26 (1)	1.55 (1)	(2)
Arg	17.14	19.80	(3)	18.39 (11)	22.41 (15)	11.42 (7)	(5)
Asp	3.60	2.30	(2)	1.48 (1)	1.47 (1)	6.23 (4)	(4)
Thr	7.44	4.89	(2)	5.20 (3)	2.94 (2)	10.77 (7)	(6)
Ser	9.53	9.79	(7)	9.38 (6)	10.58 (7)	9.76 (6)	(7)
Glu	6.60	5.36	(4)	6.72 (4)	3.13 (2)	10.62 (7)	(6)
Pro	5.84	7.43	(5)	7.76 (5)	9.27 (6)	2.33 (1–2)	(1)
Gly	9.09	12.66	(3)	13.76 (5)	12.84 (9)	5.08 (3)	(3)
Ala	7.21	6.76	(6)	4.49 (3)	6.78 (5)	11.18 (7)	(7)
Val	6.81	6.29	(5)	6.00 (4)	5.58 (4)	10.14 (6–7)	(4)
Met*	1.47	Present	–	Present (–	Present –	Absent –	–
Ile	3.26	3.81	(1)	3.81 (2)	3.82 (3)	2.64 (2)	(4)
Leu	4.49	2.64	(1)	1.67 (1)	1.50 (1)	7.73 (5)	(5)
Tyr	3.40	3.49	(3)	3.72 (2)	3.87 (3)	2.29 (1–2)	(2)
Phe	1.68	–	(0)	– (0)	– (0)	3.13 (2)	(2)
Basics / Acidics	2.90	4.51		4.39	9.31	1.07	
Residues / Molecule	134–138	80	57	61	69	62–65	64
Dansyl	Pro	Pro	Pro	Pro	Pro	Asp	Asp

* After CNBr cleavage Met was analysed as homoserine lactone. Amino acids are expressed as mole percent of all amino acids recovered and most likely number of residues per molecule (in parentheses). Hydrolyses were performed in 5.7 N HCl–1% thioglycollic acid at 110°C for 24 hr. No corrections for hydrolytic losses have been made. Tryptophan was found to be absent as determined spectrophotometrically.

** From sequence data of corresponding sections (Iwai et al. [10]).

graphy [15] and/or amino acid analysis after hydrolysis of the PTH derivatives in 5.7 N HCl–1% thioglycollic acid for 24 hr at 130°C [16]. Amino acid analyses were performed on a Beckman 116 analyser modified to allow the detection of 3 nmoles amino acid.

3. Results and discussion

The selective extraction procedure of Johns [10] was routinely used to obtain crude histone fractions. Fractions F2a1, F2a2 and F3 were extracted in 80% ethanol–0.25 N HCl. They were each purified by column chromatography [12]. Amino acid analysis and partial sequencing revealed that they were all closely similar to the corresponding fractions from calf thymus. Subsequent extraction of the chromatin in 0.25 N HCl gave two major proteins. Elution of this fraction with 0.01 N HCl: 0.05 N NaCl from a Biogel P-60 column produced one peak with an elution volume corresponding to that of calf thymus fraction F1 and a second peak with that of F2b. Acrylamide gel electrophoresis of protein from the second peak revealed two bands. The amino acid analysis (table 1; column 2) showed considerable differences from calf thymus F2b. This supposed F2b fraction was therefore subjected to a more intensive investigation.

Since amino acid analysis gave integral ratios for most of the amino acids (table 1; column 2), it was initially assumed that the electrophoretic hetero-

geneity was due to amino acid modification. Accordingly the protein was cleaved by CNBr and the resulting peptides separated by elution with 0.01 N HCl from a 2.5 cm × 100 cm Sephadex G-100 column. Only two peptides were recovered whereas three were expected.

In calf thymus F2b only two amino acids separate the two methionine residues [11]. If an analogous situation would pertain to the sea urchin, then the third peptide is expected to be small and was not recovered. The amino acid compositions of the two peptides are given in table 1; column 3 and 7. The presence of homoserine lactone in CNBr-1 allowed it to be positioned at the amino end and the absence of homoserine lactone in CNBr-2 allowed it to be positioned at the carboxyl end. Comparison with the amino acid content from the analogous sections of calf thymus F2b indicates a close identity at the carboxyl end but large differences at the amino end. The amino terminal amino acids of both peptides are identical to those from calf thymus F2b.

The C-terminal fragment was subjected to automatic Edman degradation resulting in the positioning of the first nineteen amino acids (fig. 1). It can be seen that there are two amino acid changes as compared to calf F2b and that both can be explained by single base changes. On the basis of this homology and in spite of the obvious differences at the amino end of the protein, it was classified as histone $F2b_{sea\ urchin}$.

Peptide CNBr-1 was then sequenced by automatic Edman degradation. In spite of the apparent integral values obtained from amino acid analysis, it immediately became obvious that CNBr-1 was heterogeneous. This peptide mixture was therefore subjected to chromatography by gradient elution (400 ml 50 mM Na-acetate pH 4.5 → 400 ml 50 mM Na-acetate pH 4.5; 1.0 M NaCl) on a carboxymethyl cellulose column previously equilibrated in 50 mM Na-acetate pH 4.5 and separated into two components ($F2b\text{-}1_{sea\ urchin}$ and $F2b\text{-}2_{sea\ urchin}$). The amino acid composition of the two peptides is given in table 1; columns 5 and 6. Proline was shown to be the end group in both cases. Though there are some resemblances to the corresponding region of calf thymus F2b, the most striking difference exists in the increased proportion of arginine and glycine.

The two fractions were then subjected to automatic Edman degradation. Thirty-seven residues of $F2b\text{-}1_{sea\ urchin}$ and forty-one residues of $F2b\text{-}2_{sea\ urchin}$ were positioned (fig. 2). Both can be arranged as related, repeating sequences of five amino acids. The simplest way of deriving one sequence from the other is by postulating two base changes. One base change would result in a replacement of arginine in $F2b\text{-}2_{sea\ urchin}$ by threonine in $F2b\text{-}1_{sea\ urchin}$ and the other base change would result in a replacement of arginine in $F2b\text{-}1_{sea\ urchin}$ by glycine in $F2b\text{-}2_{sea\ urchin}$ Subsequent to these base changes the sequences of five amino acids in both proteins may

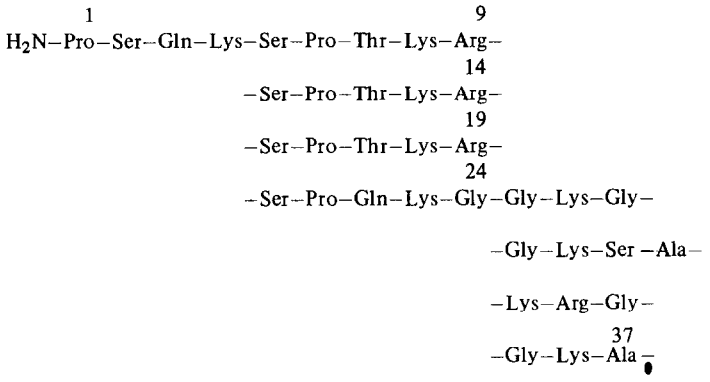
	1								10
Sea Urchin	H_2N–Asn–	Ser–	Phe–	Val–	Asn–	Asp–	Val–	Phe–	Glu–Arg–
Calf*						–Ile –			

	11							19
Sea Urchin	Ile–	Ala–	Gly–	Glu–	Ala–	Ser–	Arg–	Leu–Thr–
Calf*								–Ala –

*Iwai et al. [8]

Fig. 1. Amino acid sequence data of CNBr-2 peptide from heterogeneous F2b histones.

F2b–$1_{\text{sea urchin}}$



F2b–$2_{\text{sea urchin}}$

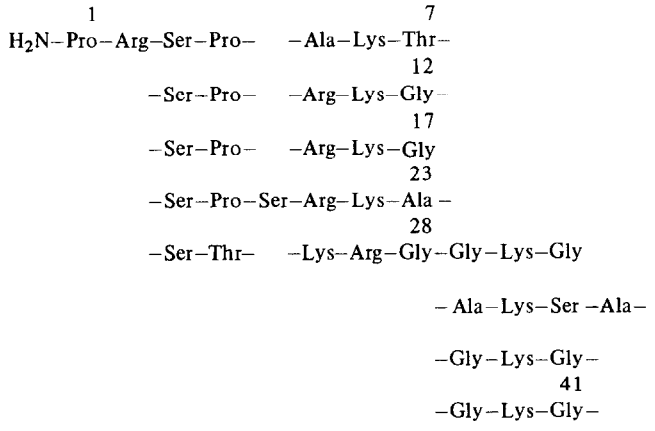


Fig. 2. Amino acid sequence data of the two subfractions (F2b–$1_{\text{sea urchin}}$ and F2b–$2_{\text{sea urchin}}$) of CNBr–1.

have duplicated several times and further limited mutation occurred. An insertion of a serine residue at position 20 of F2b-$2_{\text{sea urchin}}$ must also be postulated.

If the sequence F2b-$2_{\text{sea urchin}}$ is compared to the sequences of calf thymus histones, it can be seen that, from the amino end, the sequence is initially homologous to calf thymus F2b but later switches to a homology with calf thymus F2al (fig. 3). At the carboxyl end of the two sea urchin F2b the sequence is almost identical again to calf F2b. Since overlapping peptides have not yet been sequenced, the change back to homology with calf F2b cannot be stated with certainty.

Since it has also been demonstrated that the arginine rich histones F2al, F2a2 and F3 have a common ancestry [2, 17], these two $F2b_{\text{sea urchin}}$ histones establish a link between the arginine-rich and the lysine-rich histones. This study at the same time shows that the primary structure of $F2b_{\text{sea urchin}}$ varies significantly from calf thymus histones. In contrast, sea urchin histones F3 and F2al are virtually

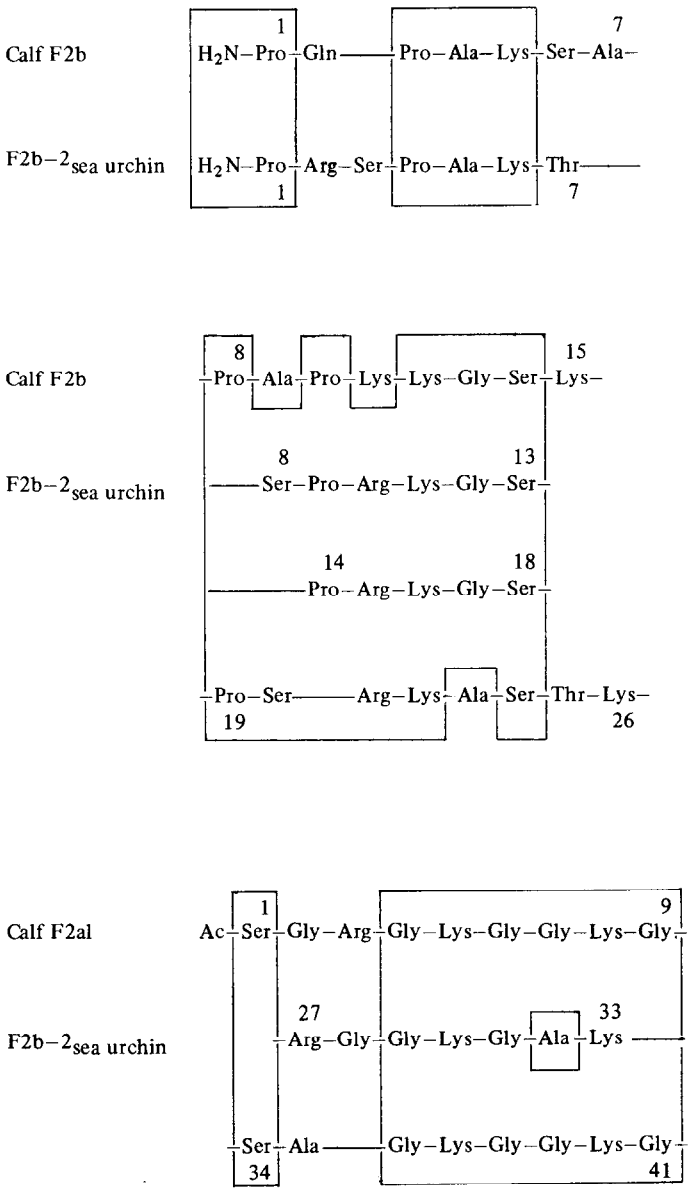


Fig. 3. Comparison of amino acid sequences of calf thymus histones and F2b–2sea urchin.

identical to the respective calf thymus histone in agreement with the conservative nature of these fractions [1–3, 6–7]. Further studies are in progress to complete the amino acid sequence of the two sea urchin F2b.

The partially established sequences in this paper raise questions of nomenclature. According to the new nomenclature proposed and accepted at the Gordon Research Conference on Nuclear Proteins, Chromatin Structure and Regulation (Beaver Dam, Wisc.,

July 3–7, 1972) cited by Hnilica [18], these proteins should be named RSG. RSG, however, gives no indication of the relationship of these proteins to both F2b and F2a1. In this case even the F nomenclature is unsatisfactory since this is based on elution paterns using chromatographic methods which have been superseded in the meantime by other techniques [12]. However, on the basis of the extensive homology with calf thymus F2b at the amino ends, these proteins have tentatively been designated as F2b-$1_{\text{sea urchin}}$ and F2b-$2_{\text{sea urchin}}$. When the complexity and diversity of histone structure has been further analysed a unified nomenclature may become more apparent.

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