SEQUENCE OF THE CYSTEINE-CONTAINING PORTION OF HISTONE F2al FROM THE SEA URCHIN *PARECHINUS ANGULOSUS*

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1. Introduction

Histone F2al has been shown to be evolutionarily a very stable protein showing only two conservative changes in organisms as widely separated on the evolutionary scale as calf and pea [1]. The F2al from echinoderms, in contrast to both calf and pea, has been shown to contain cysteine [2]. We have isolated F2al from the sea urchin and placed in sequence 52 of the 102 amino acids. Cysteine was found to replace threonine at position 73.

2. Experimental

Chromatin was isolated from mature male sea urchin gonads by homogenizing the tissue in 0.15 M NaCl–0.15 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% Trition-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 [3] and further purified by column chromatography on Biogel P-60 using 0.05 M NaCl in 0.01 N HCl as the eluant [4]. Gel electrophoresis, cyanogen bromide (CNBr) cleavage, N-bromosuccinimide (NBS) cleavage, and amino acid analysis were performed as previously reported [5, 6] but using thioglycollic acid instead of phenol to prevent oxidative losses during protein hydrolysis. Dansylation was performed by the method of Gray [7] using 4 hr hydrolysis at 105°C. Cleavage at aspartic residues was performed by refluxing 1 mg protein per ml 0.03 N HCl solution for 12 hr [8].

S-carboxymethylation was performed by reducing a protein solution (10 mg/ml in 8 M urea, 0.1 M Tris–HCl, pH 8.6) with a 25-fold molar excess of dithiothreitol (DTT). The mixture was stirred under nitrogen for 15 min, sealed and incubated for a total of 3 hr. A 2-fold excess (over all sulfydryl groups in the mixture) of iodoacetate was added and the mixture was stirred for 15 min in the dark. The reaction was terminated by adding excess DTT and extensive dialysis was performed in the dark.

Sequence analysis was performed on a Beckman 890 sequencer. Instead of Quadrol, dimethylaminopropyne buffer [9] 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 chromatography [10] 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 [11]. Amino acid analysis were performed on a Beckman 116 analyser modified to allow the detection of 3 nmoles amino acid.

3. Results and discussion

On acrylamide gel electrophoresis the purified F2al from sea urchin (*P. angulosus*) migrated as a

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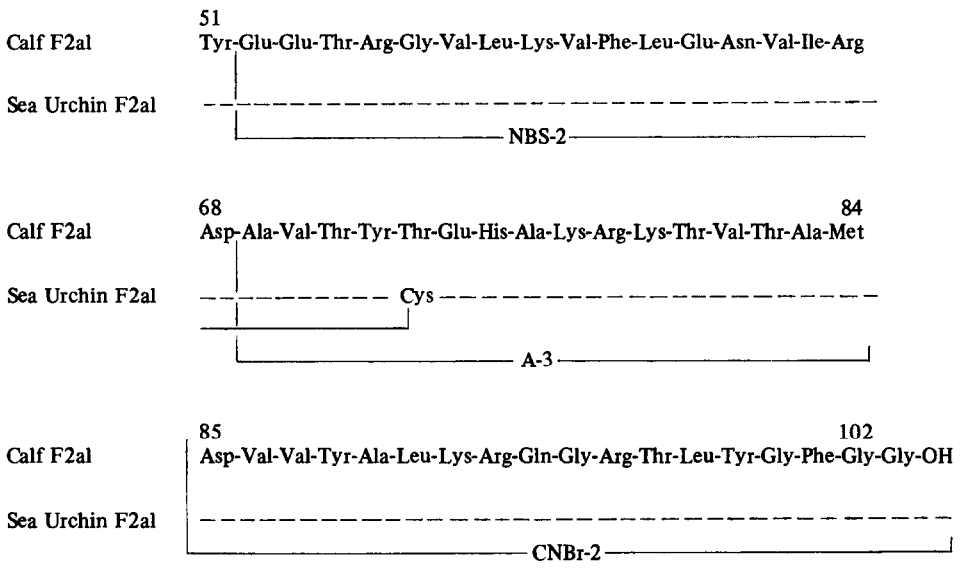

Fig. 1. Comparison of the sequence of the cysteine containing portion of sea urchin F2al with the corresponding portion of calf F2al [1].

single band with identical mobility to the non-acetylated form of F2al from calf. Gels on which the protein was not reduced with DTT showed the presence of a slow moving band (dimer formed by oxidation of cysteines) as previously noted in other echinoderms [2]. Amino acid analysis of S-carboxymethylated F2al from sea urchin indicated the presence of one mole per cent of S-carboxymethyl cysteine.

When the amino acid content was compared to that of calf F2al, the presence of cysteine and the reduction of threonine residues from seven to six were the only differences noted. Dansylation indicated that like the other F2al's previously studied the N-terminal amino acid is blocked. A dimer indicating the presence of cysteine in F2al was also found in the star fish *Marthasterias glacialis* and the primitive sea urchin *Echinolampas crassa.* Cysteine could not be demonstrated after the carboxymethylation procedure and amino acid analysis were performed on the F2al's from chicken (*Gallus domesticus*), shark (*Poroderma africanum*), mollusc (*Patella granatina*), and cycad (*Encephalartos caffer*).

The finding that F2al from sea urchin contains one less threonine and an additional cysteine residue when compared to the corresponding calf thymus histone, indicated that a threonine residue may have been replaced by cysteine (two base changes required). In order to locate the precise position of this change, sea urchin F2al was chemically cleaved wiht the aim to bring all threonine positions within easy reach of automated sequencing.

After CNBr cleavage of CM F2al at the single methionine, the two expected peptides were recovered. The small peptide (CNBr-2) was separated from the large one (CNBr-1) by elution with 0.01 N HCl from a Sephadex G-100 column. Peptide CNBr-2 was sequenced and found to be identical to the C-terminal 18 amino acids of calf F2al which included one threonine (fig. 1). The CNBr cleavage was incomplete (approximately 50–70% in various runs) so CNBr-1 was separated from the uncleaved protein by repeated runs on Sephadex G-100 columns. Purified CNBr-1 had a blocked N-terminal amino acid and amino acid analysis showed it contained the remaining 84 amino acids of the protein. Homoserine lactone indicated the presence of methionine. Five threonines and one S-carboxymethyl cysteine were present.

Peptide CNBr-1 which contains two tyrosine residues was therefore subjected to NBS cleavage. The resulting peptides were isolated by chromatography on G-100, G-50 or G-25 as needed. A peptide

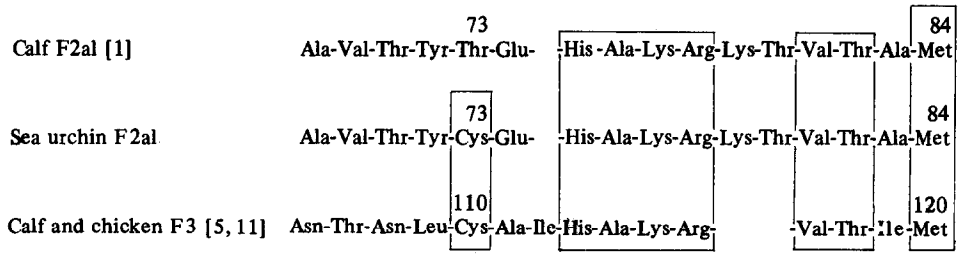

Fig. 2. Comparison of similar amino acid sequences in F3 and F2al.

was isolated (NBS-2) with an amino acid sequence which corresponded exactly to positions 52 to 71 of calf (fig. 1). This included two threonine residues. Another large peptide (NBS-1) with a blocked N-terminal end group proved on amino acid analysis to have the expected residues if compared to the 50 N-terminal amino acids of calf F2al including one residue of threonine [1]. The NBS reagent was noted to destroy S-carboxymethyl cysteine, cysteic acid and cysteine so another cleavage method was applied in an attempt to locate cysteine.

Aspartic acid cleavage was therefore performed on CNBr-1 which contains four aspartic acid or asparagine residues. A small peptide (A-3) with an alanine amino end group and containing S-carboxymethyl cysteine and homoserine lactone was isolated. This peptide was found to correspond to positions 68–84 of calf F2al, but with cysteine at position 73. The 4 N-terminal amino acids overlap the C-terminal region of NBS-2 (fig. 1). Thus 52 of the 102 residues have been placed in sequence.

Peptide A-3 has a similar sequence to a region of F3 histones [5, 12]. The present investigation has extended this similarity further toward the N-terminal end of F3 histones to include the cysteine residue (fig. 2).

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