THE COMPOSITION OF CYANOGEN BROMIDE CLEAVAGE PRODUCTS FROM HEN'S EGG PHOSVITIN

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1. Introduction

The isolation of two discrete phosphoproteins has been described by Clark [1]. Only the larger and more abundant phosphoprotein, designated S_1S, was found to contain methionine, one of these residues being present per molecular weight of 3.4×10^4. We report here on the cleavage of this phosphoprotein at the position of the methionyl residue. Two phosphopeptides which differ in molecular weight and amino acid composition have been isolated. Their position in the original polypeptide chain has been assigned.

2. Materials and methods

The isolation of phosvitin S_1S has been described by Clark [1].

Cyanogen bromide cleavage was based on a modification [2] of the method of Gross and Witkop [3]. The phosphopeptide mixture obtained was fractionated on Sephadex G-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) using the same three-column arrangement and conditions described by Clark [1]. The large peptide and other high molecular weight components were detectable in the column eluate by measurement of absorbance at 280 nm. The small peptide was detected by use of the *o*-tolidine test [4] on dried spots of eluate applied to filter paper.

Recovery of the large peptide and high molecular weight components was by dialysis and freeze-drying. The eluate containing the small peptide was freeze-dried without dialysis, taken up in the minimum amount of water and desalted on a column of Sephadex G-15, 150 cm long and 1.9 cm diameter Tolidine-positive eluate, which emerged as one band, was collected in several parts and freeze-dried. Since no significant differences in the amino acid composition of these parts were found, they were combined.

Analytical techniques, unless specified otherwise, are as reported by Clark [1]. Protein dansylation was performed according to the recommendations of Gros and Labouesse [5]. *C*-Terminal residue identification by tritium labelling was according to the aqueous one-step procedure of Matsuo et al. [6]. The product was then hydrolysed by the method used for amino acid analysis and chromatographed on a Beckman 120 amino acid analyser. The ninhydrin-developed eluate was passed into a fraction collector on emergence from the analyser colorimeter and aliquots (1 ml) from each amino acid peak were mixed with water (2 ml) and Triton X-100–toluene–POPOP–PPO scintillator solution (13 ml) of the tT 7:6 formula [7] before counting tritium activities in 20 ml glass vials on a Tri-Carb Scintillation Spectrometer, Model 574 (Packard Instruments Co., Illinois, USA). Tryptophan was detected in dried spots of protein solution on filter paper using Ehrlich's test [8].

3. Results

The elution pattern of the cyanogen bromide reaction product on Sephadex is given in fig. 1, together with the elution pattern of the starting material for comparison. Table 1 gives the relevant amino acid analysis data. Values for the starting material are derived from Clark [1], taking mean values of fractions S_{1b} and S_{1c} because these cuts, being narrower than the cut made to isolate fraction S_1S, should reflect the composition of a purer phosphoprotein.

Table 1
Composition of phosvitin S_1S and its cyanogen bromide cleavage products.

Amino acid	Phosvitin S_1S [1]		Large peptide $CNBrS_1$		Small peptide $CNBrS_2$	
	Residues/ 10^4 g*	Residues/ 3.5×10^4 g*	Residues/ 10^4 g	Residues/ 3.0×10^4 g*	Residues/ 10^4 g*	Residues/ 5×10^3 g*
Ser	35.6	125 (125)	37.0	111 (111)	27.6	13.8 (14)
Thr**	0.54	1.9 (2)	0.08	0.2 (0)	4.00	2.0 (2)
Arg	3.02	10.6 (10)	3.08	9.2 (9)	1.81	0.9 (1)
Lys	4.26	14.9 (15)	4.03	12.1 (12)	5.45	2.7 (3)
His	3.30	11.6 (12)	4.01	12.0 (12)	0	0 (0)
Asp	3.88	13.6 (13)	3.57	10.7 (11)	3.82	1.9 (2)
Glu	3.12	10.9 (12)	3.39	10.2 (10)	4.46	2.2 (2)
Gly	1.45	5.0 (5)	1.33	4.0 (4)	1.83	0.9 (1)
Ala	2.28	8.0 (8)	0.78	2.3 (3)	8.81	4.4 (5)
Val	0.84	2.9 (3)	0.89	2.7 (3)	0	0 (0)
Leu	0.82	2.9 (3)	0.89	2.7 (3)	0.08	0 (0)
Ile	0.52	1.8 (2)	0.49	1.5 (2)	0.07	0 (0)
Pro	0.91	3.2 (3)	0.08	0.2 (0)	5.61	2.8 (3)
Phe	0.30	1.1 (1)	0.03	0.1 (0)	1.70	0.9 (1)
Tyr	0.12	0.4 (1)	0.10	0.3 (1)	0	0 (0)
Met	0.25	0.9 (1)	0	0 (0)	0	0 (0)
Glcn	1.01	3.5 (3)	0.98	2.9 (3)	0	0 (0)
Pthr	0.90	3.2 (3)	0.12	0.4 (1)	3.57	1.8 (2)
Hsl†			0	0 (0)	2.12	1.1 (1)

* Weights are moisture-free, but include non-dialysable components such as bound carbohydrate, phosphate and counter-ion (sodium). Figures in parenthesis for phosvitin S_1S are the sum of integral values (in parenthesis) of peptides $CNBrS_1$ and $CNBrS_2$.

** Excluding phosphothreonine (Pthr).

† Homoserine and its lactone.

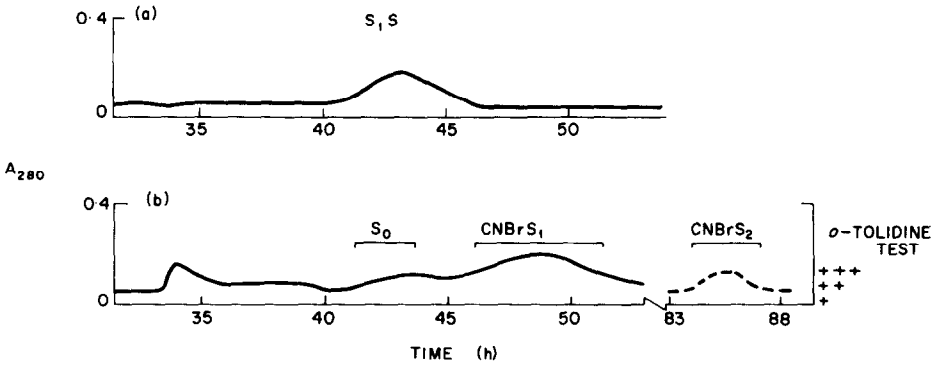

Fig. 1. Elution diagrams of phosvitin S_1S (a) and its cyanogen bromide reaction product (b) on Sephadex G-100. Conditions: column length, 450 cm; diam. 3.8 cm; buffer 0.3 M sodium acetate, pH 4.5; flow 40–45 ml/hr.

Table 2
C-Terminal residue identification: incorporation of tritium into the amino acids of phosvitin S_1S and its large cyanogen bromide peptide.

Amino acid	Percentage of total counts (less background)	
	Phosvitin S_1S	Peptide $CNBrS_1$
Trp	6	
Ser	0	0
Thr	4	1
Arg	10	3
Lys	0	4
His	6	10
Asp	32	23
Glu	15	13
Gly	0	5
Ala	0	1
Val	0	1
Leu	0	2
Ile	4	17
Pro	0	
Phe	0	0
Tyr	23	21
Met	0	

The Ehrlich test for tryptophan was positive for peptide $CNBrS_1$ (and phosvitin S_1S) only.

Dansylation and hydrolysis afforded dansyl-aspartic acid in the case of peptide $CNBrS_1$ and dansyl-alanine in the case of peptide $CNBrS_2$.

The degree of incorporation of tritium into the amino acids of phosvitin S_1S and peptide $CNBrS_1$ is shown in table 2.

4. Discussion

Peak S_0 in fig. 1 has the same elution volume as unreacted phosvitin S_1S and was confirmed by amino acid analysis to be unchanged starting material. The peaks preceding the position of S_0 were found to contain only small yields of intractable high molecular weight products similar to material encountered during fractionation of crude hen phosvitin [1].

The large peptide $CNBrS_1$ has an elution position which is very close to that of phosvitin S_3S, of molecular weight 2.8×10^4, found in whole hen phosvitin [1]. The absence of homoserine places this peptide on the *C*-terminal side of the cyanogen bromide cleavage point. Dansylation shows that aspartic acid follows methionine at the cleaved peptide bond.

The small peptide $CNBrS_2$ has the same *N*-terminal residue, alanine, as the whole protein. Homoserine content confirms its position as being on the *N*-terminal side of the cyanogen bromide cleavage point as well as showing by yield that there is approximately one residue of homoserine per molecular weight of 5,000.

Results in table 1 show that peptide $CNBrS_2$ is free of histidine, valine, leucine, isoleucine, tyrosine and glucosamine, but contains all the proline, phenylalanine and unphosphorylated threonine. The sum of the compositions of the peptides for the molecular weights shown gives a total which approaches that of the original phosvitin S_1S.

Tryptophan, detected only in the larger peptide $CNBrS_1$, is most likely to be present as one residue per molecule, since previous workers [9–11] report the tryptophan content of phosvitin as being 0.2 to 0.3 residues per 10^4 g and the S_1S fraction constitutes the major proportion of phosvitin [1].

Tyrosine is only found in small amounts in phosvitin S_1S [1], but it persists after cleavage in peptide $CNBrS_1$. The possibility of *O*-phosphorylation of this amino acid, or high destruction rates during hydrolysis prior to amino acid analysis could be responsible for low results in spite of the precaution of adding phenol. These factors could also explain the failure to recover tyrosine as a free amino acid on hydrazinolysis of phosvitin S_1S [1]. Assuming that one tyrosine residue is present per molecule of phosvitin S_1S, a 2.5 to 3-fold increase in its values in table 2 is necessary to correct for the low recovery. The high level of incorporation into aspartic acid has been found through experience to be an artefact with this tech-

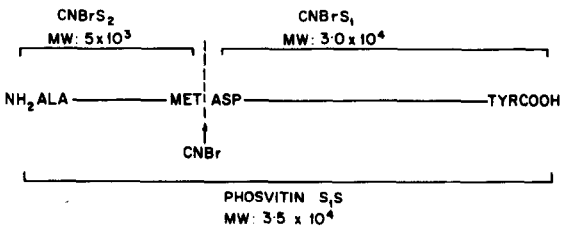

Fig. 2. Peptide locations in phosvitin S_1S.

nique [12], enhanced in the case of phosvitin S_1S by the abundance of aspartic acid. The result is therefore interpreted as being positive for tyrosine as a *C*-terminal residue to both the whole protein and peptide $CNBrS_1$.

Findings are summarised in fig. 2.

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