Communications to the Editor

[Chem. Pharm. Bull. 32(5)2040—2043(1984)]

A FLUORESCENT METHOD FOR SELECTIVE ISOLATION OF TRYPTOPHAN-CONTAINING PEPTIDE: MODIFICATION OF TRYPTOPHAN RESIDUE IN A PROTEIN WITH A CHELATING AGENT, 2-CARBOXY-1-HYDROXY-4-NAPHTHYLMETHYLDIMETHYLSULFONIUM CHLORIDE

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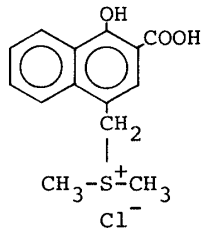
Protein was treated with 2-carboxy-1-hydroxy-4-naphthylmethyldimethylsulfonium chloride to modify the tryptophan residues in the chain. The product thus obtained was treated with trypsin and the tryptophan modified fragment(s) was selectively isolated by adsorption onto a Chelating Sepharose 6B column (Al^{3+} form).

This procedure may also be extended to molecular biology.

KEYWORDS——2-carboxy-1-hydroxy-4-naphthylmethyldimethylsulfonium chloride; tryptophan; tryptophan-containing peptide; Koshland reagent; fluorescence; metal chelate; glucagon; protein sequence analysis

In a previous paper, we described the synthesis and properties of 2-carboxy-1-hydroxy-4-naphthylmethyldimethylsulfonium chloride (**I**).[1] This molecule has three different functions: a) a phenolic hydroxy group ortho to carboxyl group to chelate metal ions; b) a naphthyl moiety to produce fluorescence; and c) a Koshland reagent type sulfonium chloride group to modify the tryptophan residue selectively in a peptide chain. The product of condensation with the tryptophan residue (in a solution at pH 3) fluoresces (λ_{ex}=255 nm; λ_{em}=416 nm). In the previous paper, we used this reagent to determine the number of tryptophan residues in a protein, while in the present report we have utilized this reagent for selective isolation of the tryptophan-containing fragments obtained by trypsin hydrolysis.



I

In this work, we selected glucagon as a model compound since the structure of this peptide is known and it contains one tryptophan residue.

TABLE I. Amino Acid Composition of Modified Glucagon

Amino acid	Glucagon	Modified glucagon
Ala	1	1.00*
Arg	2	2.15
Asp(Asn)	4	3.84
Glu(Gln)	3	3.21
Gly	1	1.02
His	1	0.73
Leu	2	1.91
Lys	1	0.91
Met	1	1.04
Phe	2	2.18
Ser	4	2.90
Thr	3	2.63
Tyr	2	2.25
Val	1	1.08
Trp	1	N.D.**

* Ala is arbitrarily taken as 1.00.
** not detected.

Glucagon[2] (1 mg; 2.87×10^{-7} mol) in 1 ml of 1 M acetic acid was mixed with 1 mg of reagent I (3.02×10^{-6} mol) and allowed to stand at room temperature for 30 min. The reaction mixture was loaded on a Sephadex G-15 column (1.6 × 40 cm) and gel filtration was carried out to remove the hydrolysate of the reagent. The modified glucagon fractions were collected and their amino acid composition was estimated.[3] As shown in TABLE I, the composition corresponding with that of glucagon was obtained except that tryptophan disappeared, indicating that selective modification of the tryptophan residue in glucagon was achieved by the reagent I.

The modified glucagon was then treated with an equivalent weight of trypsin in 0.1 M phosphate buffer (pH 8.0) at 37 °C overnight. The reaction mixture was loaded

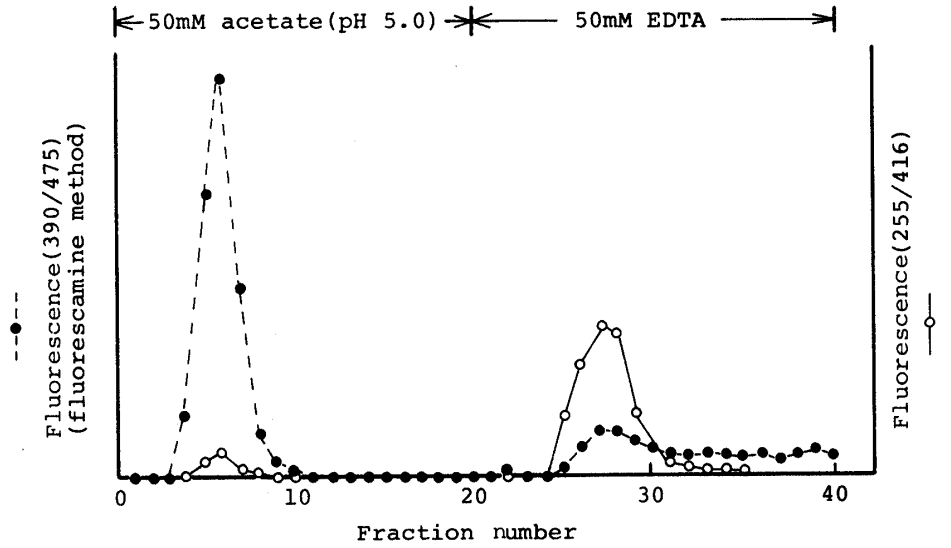


Fig. 1. Elution Profile of the Digests of the Modified Glucagon with Trypsin on a Chelating Sepharose 6B Column

TABLE II. Amino Acid Composition of Tryptophan-Containing Fragment

Amino acid	Expected*	Analysed
Ala	1	1.00**
Asp(Asn)	2	1.52
Glu(Gln)	2	1.65
Leu	1	0.67
Met	1	0.58
Phe	1	0.65
Thr	1	0.74
Val	1	0.71
Trp	1	N.D.***

* Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr.
** Ala is arbitrarily taken as 1.00.
*** not detected.

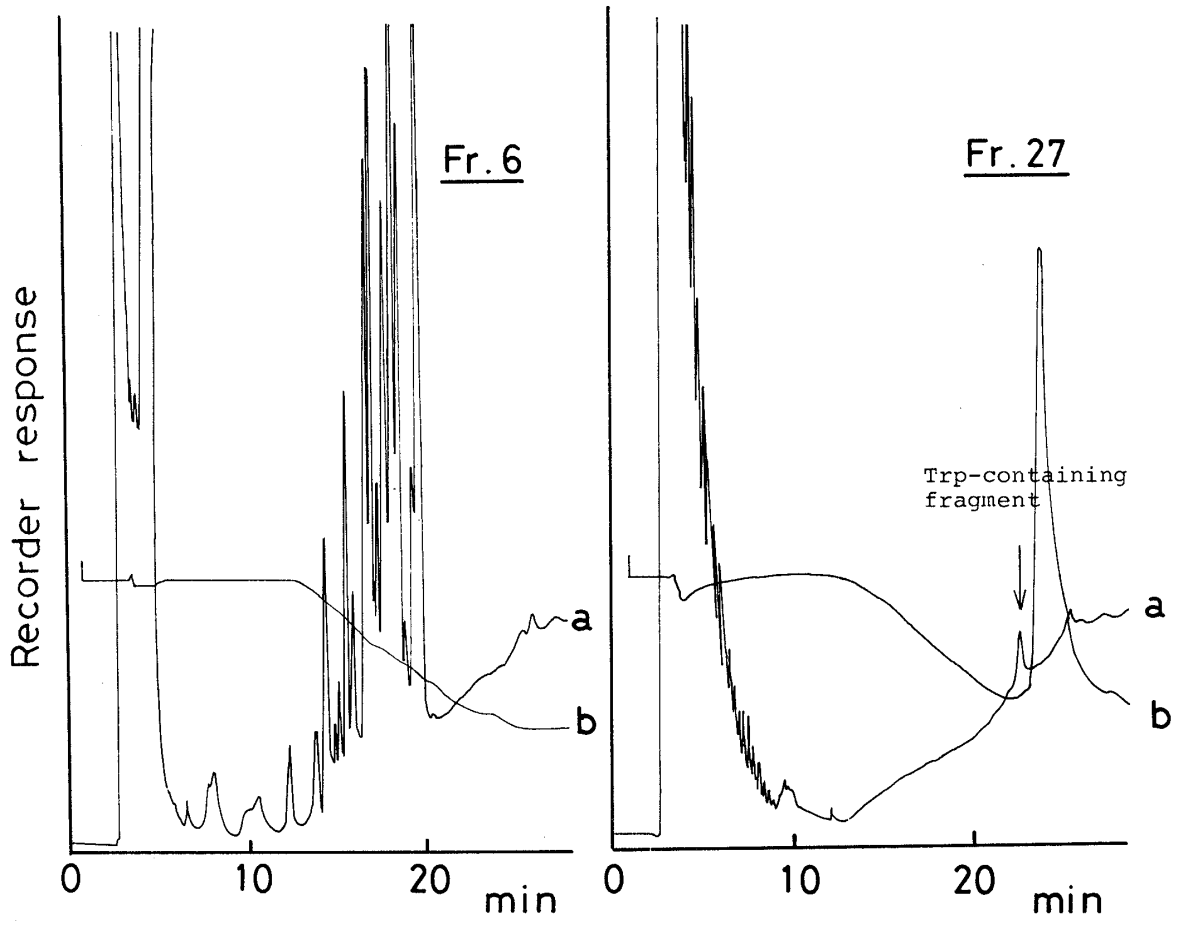


Fig. 2. Chromatograms Obtained from the Fractions 6 and 27
a: absorption at 210 nm.
b: fluorescence (Ex: 255 nm, Em: above 402 nm).

on a Chelating Sepharose 6B column (Al^{3+} form, 1.6 x 5 cm). The unbound materials, including trypsin, its autolysates or the peptide fragment not containing tryptophan, were washed from the column with 50 mM acetate buffer (pH 5.0), and then the elution with 50 mM EDTA solution was carried out (Fig.1). Fraction 27 in Fig.1, in which the amount of peptide determined by the fluorescamine method[4] and the fluorescence (excitation:255 nm, emission:416 nm) corresponded, had an amino acid composition identical to the expected tryptophan-containing peptide fragment of glucagon (TABLE II). Figure 2 shows the chromatograms obtained from fractions 6 and 27 in Fig.1.[5] Although they were many peaks in fraction 6 derived from the peptide fragment in glucagon containing no tryptophan or trypsin and its autolysates, in fraction 27 a single peak of tryptophan-containing peptide was observed. This suggests that the selective isolation of the tryptophan-containing fragment in glucagon was achieved using the Chelating Sepharose 6B column.

As expected, only one tryptophan-containing fragment from glucagon was isolated from the Chelating Sepharose 6B column. This selective isolation of tryptophan-containing fragments can be extended not only to other protein but also to the cloning of DNA sequences.

ACKNOWLEDGEMENT The authors are grateful to Dr. Mahesh C. Khosla for his valuable discussion.

REFERENCES AND NOTES

1) T. Hojo, H. Nakamura, Z. Tamura and T. Nakajima, Chem. Pharm. Bull., 31, 3350, (1983).
2) Glucagon used was purchased from Sigma Chemical Co. This was extracted from a mixture of bovine and porcine pancreases.
3) The hydrolysis of peptide was carried out in 5.7 N HCl containing 4% thioglycollic acid at 110°C for 20 h.
4) To a mixture of 50 µl of an aliquot of each fraction and 1 ml of 0.2 M borate buffer (pH 9) was added 0.5 ml of fluorescamine solution (20 mg/ 100 ml acetone) with vigorous shaking. The fluorescence measurement was performed with a Hitachi 650-10S fluorescence spectrophotometer (excitation:390 nm, emission:475 nm).
5) Chromatographic conditions: column, TSK GEL LS-120T (4.6 × 250 mm); eluent, 0.02% HCl-CH_3CN(95:5,v/v)(solvent A) and 0.02% HCl-CH_3CN(40:60,v/v)(solvent B). Linear gradient elution of 100% A to 100% B during 16 min was carried out; flow rate, 1.0 ml/min. UV detection at 210 nm and fluorescence detection (excitation:255 nm, emission:above 402 nm) were performed with a Jasco UV-100-V UV spectrophotometer and a FP-540 spectrofluorometer, respectively.

(Received March 1, 1984)