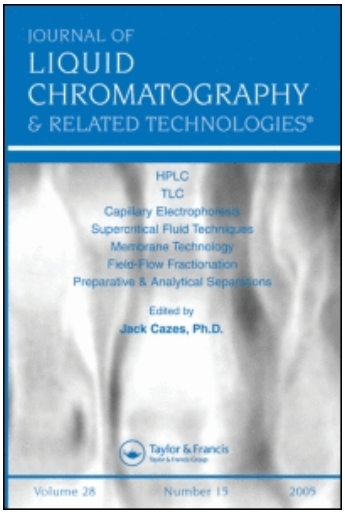

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HPLC Separation of Cholecystokinin Peptides-Two Systems

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HPLC SEPARATION OF CHOLECYSTOKININ PEPTIDES--TWO SYSTEMS

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ABSTRACT

Two HPLC systems are described which separate cholecystokinin (CCK) peptides from each other and from gastrin. One system utilizes a Supelcosil LC-18 reverse phase column with an acetonitrile-triethylamine phosphate buffer. The other system utilizes a Varian 2000 SW micropack molecular sieve column run in 0.1 M phosphate buffer. These HPLC systems offer improved resolution over conventional gel filtration of CCK and should be useful, when coupled with radioimmunoassay, for determining the molecular forms of CCK present in biological samples.

INTRODUCTION

Several cholecystokin-like peptides occur in varying proportions in different tissues (1). Some of these are undoubtedly the biologically active, secreted forms of the hormone; others are probably precursors or inactive metabolites. Still others may be post-mortem artifacts. There has been considerable effort made to develop methods for separating and quantitating the various CCK related peptides. Sephadex gel chromatography combined with sequence-

specific radioimmunoassay has been used for this purpose, but this combination is not ideal. The HPLC systems described here are an improvement in speed and resolution over conventional gel chromatography systems.

MATERIALS

Sulfated and desulfated CCK8 were the gift of Dr. Miguel Ondetti of Squibb. Desulfated CCK8 was also purchased from Peninsula Labs. CCK4 (Trp-Met-Asp-Phe-NH_2) was purchased from Sigma. CCK4, CCK5 (Gly-CCK4), CCK6 (Met-Gly-CCK4), CCK7 (Tyr-Met-Gly-CCK4), Gastrin 17 sulfated were the gift of Dr. Robert Jensen. Gastrin 17 desulfated was purchased from Calbiochem. CCK33 and CCK39 were the gift of Viktor Mutt. The freshly distilled triethylamine was the gift of Tom O'Donohue. Acetonitrile was from Fisher (HPLC grade). A Varian 5020 liquid chromatograph equipped with a Verichrom detector was used for these studies. The absorbance of the samples was followed at 210 nM.

METHODS

A. C-18 Reverse Phase System

A Supelcosil (Supelco) column was eluted with a 22% acetonitrile-78% phosphoric acid-triethylamine buffer, pH 6.5 (17 ml of 85% phosphoric acid was added to distilled deionized-Millipore filtered water and freshly distilled triethylamine is added until the pH reaches 6.5. The volume of the solution is then adjusted to 1 l). The buffer is passed through a Waters C-18 sep pack prior to use.

B. Molecular Sieve System

A Varian 2000 SW micro pack molecular sieve column is run with 0.1 M Na phosphate buffer pH 7.0. The buffer is passed through a sep pack ^{R} prior to use.

C. Sample Preparation

The standards are dissolved in a compatible solvent (one that does not absorb heavily at 210 nM). Biological samples were pre-purified on a sep pack ^{R} or extensively extracted to prevent column clogging. A method which yields 55-60% recovery of CCK-like material from biological is to prewash sep pack with 15 ml absolute methanol followed by 20 ml distilled water. The sample is applied in any convenient volume, washed with 20 ml distilled water and eluted with 3 ml absolute methanol. The methanol is evaporated and the sample is redissolved in water and injected. In separating biological samples care should be taken to wash the columns and injection systems thoroughly between injections of standards and biological materials so that the biological materials are not contaminated by the standards. It is advisable in order to test the thoroughness of working procedures, to run a water blank between standards and biological samples and assay the fractions in the radioimmunoassay.

Substantial amounts of partially and completely oxidized CCK are present in some tissue extracts. Therefore, it is important to know the retention times of partially and completely oxidized peptides on the C-18 system. To determine the retention times of partially and completely oxidized CCK, 1 µl of 30% hydrogen peroxide was added to 50 µl of peptide (1 µg/µl) and aliquots of the resulting solution were chromatographed at several times afterwards.

RESULTS

Table 1 lists the retention times of some CCK peptides on the C-18 reverse phase column. The peak width of the peptide peaks are sufficiently narrow that there is baseline separation between the peaks. It is also possible to separate the sulfated and desulfated forms of CCK8 and sulfated and desulfated gastrin 17. Retention times are reported for reduced, partially and completely oxidized

TABLE 1

Retention Times of CCK Peptides Run in 22% AN-TEAP on Supelcosil LC-18 Column

Peptide	Retention Time
CCK4	8.9
CCK4 oxidized	3.0
CCK5	8.9
Gastrin 17 sulfated	11.7
Gastrin 17 desulfated	16.5
Gastrin 17 desulfated oxidized	5.5
CCK8 sulfated	17.4
CCK8 sulfated partially oxidized	5.1, 7.9
CCK8 sulfated completely oxidized	3.3
CCK8 desulfated	19.8
CCK8 desulfated partially oxidized	6.6, 9.8
CCK8 desulfated completely oxidized	3.7
CCK6	21.2

forms of CCK. CCK8 has two methionine residues so that two partially oxidized forms are generated with two unique retention times. After several hours at 4°C both methionine residues are oxidized and a single unique retention time is observed. CCK4 and gastrin 17 only have one methionine residue so no partially oxidized forms are generated.

TABLE 2

Retention Times of CCK Peptides Run in 0.1 M Phosphate Buffer pH 7.0 on Varian 2000 SW Micropack Molecular Sieve Column

Peptide	Retention Time (minutes)
Gastrin 17 sulfated	15.0
Caerulein	15.1
CCK33	15.2
Gastrin 17 desulfated	16.3
CCK8 sulfated	19.8
CCK8 desulfated	23.4
CCK4	25.6
CCK5	25.9
CCK6	27.8
CCK7 desulfated	31.3

Table 2 lists the retention times of the CCK peptides on the molecular sieve column. The column was designed to separate molecules on the basis of molecular weight but some peptides run anomalously. The column easily separates some of the major forms of CCK.

DISCUSSION

Two HPLC systems are described here which permit rapid and complete separation of CCK peptides. Utilizing these systems in conjunction with CCK radioimmunoassay has facilitated the task of determining what molecular forms of CCK are found in biological samples and how they are interconverted.

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