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Note

High-efficiency cation-exchange chromatography of polypeptides and polyamines in the nanomole range

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High-efficiency ion-exchange columns, in which the ionic functional group is bonded to a spherical porous polystyrene bead, have been available for resolving amino acids and small peptides (below 30 residues) for a number of years[1]. Larger peptides bind too strongly to this resin and cannot be resolved. We wish to report the use of another resin, having the functional group bonded to a spherical porous silica bead, for the chromatography of polypeptides ranging from 1000 to over 20,000 daltons, as well as for polyamines.

EXPERIMENTAL

Pyridine was distilled twice over ninhydrin and reagent-grade acetic acid (Fisher, Springfield, N.J., U.S.A.) was used without further purification. Water having a resistance greater than 10^6 ohms was produced by a Hydro Service and Supplies (Durham, N.C., U.S.A.) purification system. Caprylic acid and a 25% solution of thiodiglycol were from Pierce (Rockford, Ill., U.S.A.) and oxytocin and vasopressin (Arg_8) were from Chemical Dynamics (South Plainfield, N.J., U.S.A.). Angiotension II, parathyroid hormone$^{1-34}$, and substance P were from Beckman (Palo Alto, Calif., U.S.A.

A prepacked 25 × 0.46 cm I.D. column of Partisil SCX (cation exchange) was obtained from Whatman (Clifton, N.J., U.S.A.). An LKB (Hicksville, N.Y., U.S.A.) gradient mixer and a Milton Roy (Riviera Beach, Fla., U.S.A.) minipump were used in conjunction with an automated fluorescamine-column monitoring system[2]. The column was pumped at 16 ml/h at room temperature, giving a back pressure of 200–300 p.s.i. Volatile pyridine–acetate buffers were used for elution. A 50-min linear gradient from 5×10^{-3} *M* pyridine, pH 3.0 to 5×10^{-2} *M* pyridine, pH 4.0, was followed by a 60-min linear gradient to 5×10^{-1} *M* pyridine, pH 5.0. The initial gradient buffer was used for pre-equilibration of the column. All column buffers contained thiodiglycol (0.01%) as an antioxidant and caprylic acid (0.01%) to prevent growth of microorganisms. Samples were applied to the column in 200 µl of 0.01 *N* HCl and column eluate was collected in glass ampules (Bellco, Vineland, N.J., U.S.A.) which had been pretreated with siliclad (Clay Adams, Parsippany, N.J., U.S.A.). Samples were dried *in vacuo*.

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Recovery of peptide in the column eluate was measured by several procedures. A fluorescent assay in which fluorescamine derivates were prepared was utilized for vasopressin[3]. Parathyroid hormone[1–34] was quantitated on an amino acid analyzer with fluorometric detection[4]. Immunoglobulin light chain[5], synthesized from ^{14}C-labeled amino acids in a cell-free system, was determined by counting radioactivity. NSILA (non-suppressible insulin-like activity) was followed by a semiquantitative binding assay[6].

RESULTS AND DISCUSSION

It was found that all amino acids, including arginine as well as the dipeptide lysylglycine, were eluted within one to two column volumes (10–20 min) with the gradient mentioned above. Retention time increased with both size and basicity of the

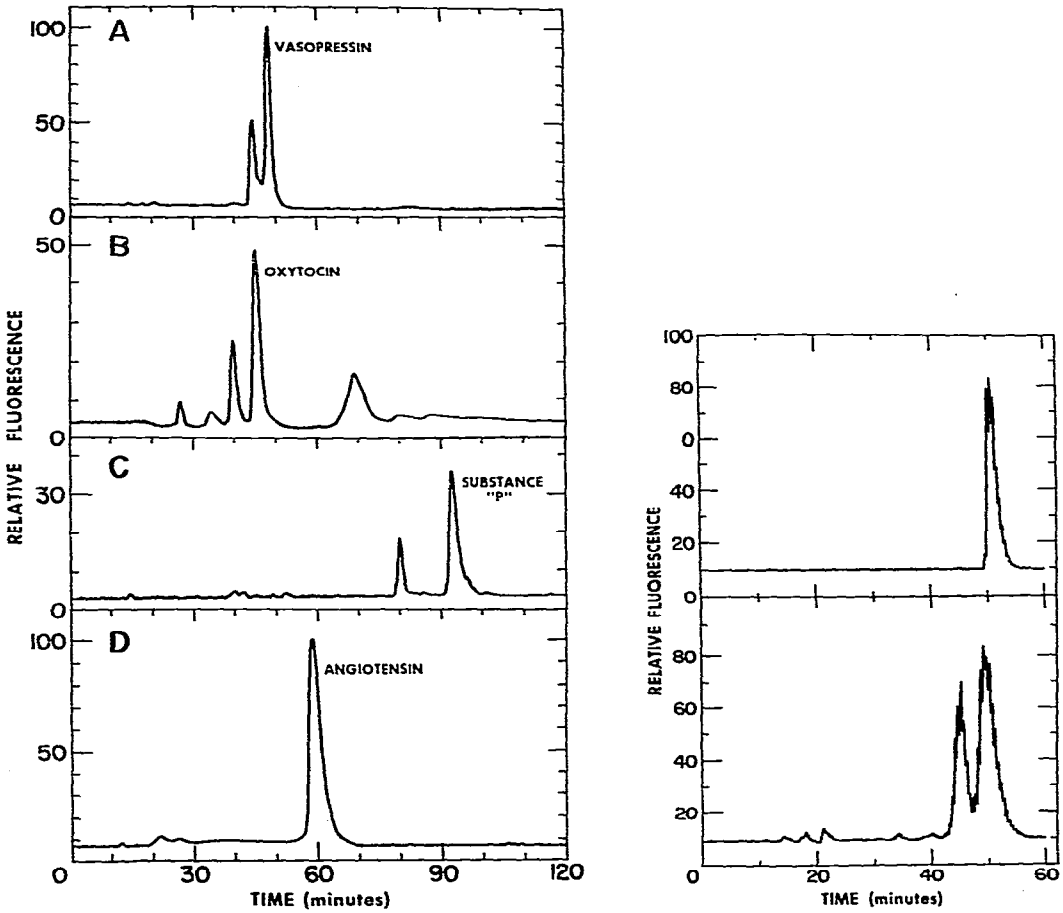

Fig. 1. Chromatography of synthetic peptides. Approximately 10 nmoles of each sample were applied to the column and 8% (800 pmoles) was utilized for detection.

Fig. 2. Purification of vasopressin. The major peak (bottom panel) was collected and rechromatographed (top papel)

peptide. The 0.5 *M* pyridine–acetate buffer was found to be sufficient to elute the tetradecapeptide substance P (Fig. 1).

NSILA (6,000 daltons), parathyroid hormone$^{1-34}$ (4,000 daltons and an immunoglobulin light chain (24,000 daltons) were retained on the column with 0.5 *M* pyridine–acetate as the limiting buffer. However, 2.0 *M* pyridine–acetate, pH 5.5, as the limiting buffer, was sufficient to elute each of the above as a sharp peak.

Chromatography of several chemically synthesized peptides, purportedly of high purity, indicated that they were not homogeneous (Fig. 1). The presence of multiple peaks was shown not to be an artifact by the following experiments with vasopressin. Two major peaks were observed. The peak eluting between 47 and 52 min was collected (Fig. 2) and rechromatography of an aliquot of this eluate resulted in the appearance of a single peak with the same retention time (Fig. 2). The identity of this peptide was confirmed by preparing a fluorescent derivative, which co-chromatographed on a reversed-phase column with pituitary-derived vasopressin-fluorophor[3].

A recovery of 70% was obtained for the chromatography of 1 nmole of vasopressin. Similar recoveries were obtained with 1 nmole of parathyroid hormone$^{1-34}$ and with nanogram quantities of the ^{14}C-labeled immunoglobulin light chain. Recovery may be affected by various parameters including flow-rate, buffer composition, column temperature, and sample size. The capacity of the column was not determined, but as much as 250 nmoles of vasopressin have been purified in a single run.

Polyamine resolution on Partisil SCX is presented in Fig. 3. It may be possible to perform quantitative polyamine analyses at the picomole level in a fraction of an hour with this methodology provided recovery and linearity are checked. Possible interference from peptides could be eliminated by acid hydrolysis of each biological

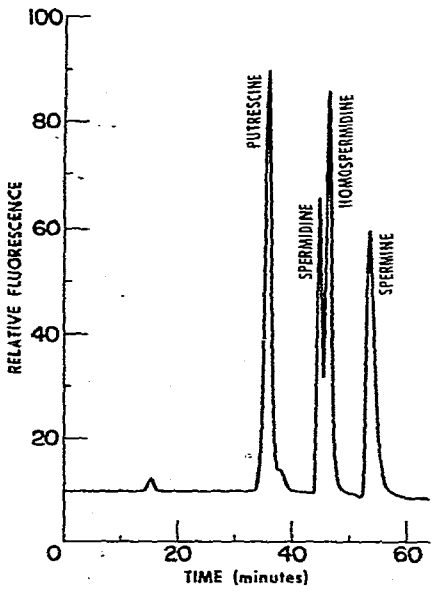

Fig. 3. Chromatography of polyamines. A mixture containing 10 nmoles of each polyamine was applied to the column and 8% was utilized for detection.

sample prior to chromatography. The amino acids produced by this treatment should not significantly affect the polyamines since they would elute well before putrescine.

The final point to be considered is the proper care of the column. Since the silica beads are degraded by alakli, samples and all column buffers must be below pH 7.6, thereby precluding the use of sodium hydroxide for column washing. No elevated or irregular baselines nor pressure build-ups have been encountered while working with these partially purified peptide samples. Sulfuric acid of moderate strength has been recommended by the manufacturer for column washing, if necessary. Other standard chromatography procedures, such as removal of particulate matter from samples and buffers, should be observed.

A silica-based anion-exchange column, Partisil SAX, is also available from Whatman, but has not yet been evaluated. A recent report has shown the utility of a similar silica-based cation-exchange column for the chromatography of biogenic amines[7]. These columns should also find wide application in peptide and polyamine chemistry.

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