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Note

Desalting of peptides by high-performance gel permeation chromatography

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The desalting of peptides, especially when dissolved in phosphate buffers, provides problems: for peptides with molecular weights less than 3500 daltons, dialysis is inadequate; when gel filtration or ultrafiltration is used the loss of peptides may be substantial. The use of chemically modified silica gels in high-performance gel permeation chromatography[1–3] suggests that high yields and short separation times might be obtained. However, for peptides dissolved in phosphate buffers, no detailed data are yet available for this technique.

MATERIALS AND METHODS

The experiments were performed with a Waters pump 6000A connected with an absorbance detector 440 (Waters, Koenigstein, F.R.G.). The columns were either TSK 2000 P SW (60 × 0.7 cm I.D.) purchased from LKB (Gräfelfing, F.R.G.), or Bio-Gel TSK 20 (30 × 0.7 cm I.D.) from Bio-Rad (Munich, F.R.G.). The separations were carried out at room temperature with a flow-rate of 1 ml/min, and UV detection at 254 nm. The eluents were 0.1 *M* formic acid for the TSK 2000 column and 0.01 *M* formic acid for the TSK 20 column. Formic acid was removed by lyophilization. The peptides (Table I) were dissolved in 100 μl of 0.1 or 1.0 *M* sodium phosphate. The injection volume was 100 μl. Phosphate was determined according to Fiske and Subbarow[4] (Sigma, Munich, F.R.G.). The columns were stored in methanol. A regular regeneration of the TSK 2000 P SW column with 0.1 *M* sodium dodecyl sulphate (SDS) was performed.

Water was of highly purified grade (Milli Q reagent water system). All chemicals were purchased from E. Merck (Darmstadt, F.R.G.).

Yields were estimated by plotting the peak areas obtained with different amounts of the peptides (10–50 μg).

RESULTS AND DISCUSSION

Peptides with molecular weights between 794 and 10,000 were effectively desalted from phosphate with the TSK 2000 P SW column. Six examples are shown in Fig. 1. The good yields of peptide material are shown in Table II. Peptides with molecular weights lower than 794, *e.g.*, thyrotropin-releasing hormone (TRH) and leu-enkephalin, had the same retention time as the salts, while 2,4-dinitrophenyl-

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TABLE I

MSH = Melanocyte-stimulating hormone; ACTH = adrenocorticotropin; LHRH = luteinizing-hormone releasing hormone. Sources: A, Serva (Heidelberg, F.R.G.); B, Bachem (Burgdorf, Switzerland); C, Sigma (Munich, F.R.G.); D, Paesel (Frankfurt, F.R.G.); E, gift from Hoechst (Frankfurt, F.R.G.); F, prepared as previously described[5].

Peptide	*Source*	*Molecular weight*
DNP-Alanine	A	255
Leu-phe	B	278
TRH	A	383
Leu-enkephalin	C	554
β-Lipotropin 39-45	D	794
Oxytocin	B	1007
Bradykinin	B	1060
LHRH	E	1182
Bacitracin	A	1450
Human gastrin I	B	2117
β-MSH porcine	B	2176
ACTH 18-39	B	2466
Glucagon	A	3483
Insulin B chain	A	3495
ACTH	A	4500
Aprotinin	A	6200
β-Lipotropin	F	9700

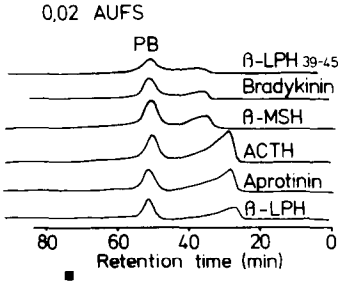

Fig. 1. Desalting of six peptides (each 10–20 μg) in the molecular weight range of 794–9700 from sodium phosphate (PB) using a TSK 2000 P SW column.

TABLE II

AREA UNITS (cm^2) OBTAINED WITH DIFFERENT CONCENTRATIONS OF PEPTIDES

β-LPH = β-lipotropin.

Peptide	*Concentration (μg)*				
	10	*20*	*30*	*40*	*50*
β-LPH 39-45	0.825	1.720	2.350	3.125	4.160
LHRH	1.865	3.890	5.776	7.516	9.216
Insulin B chain	0.630	1.260	1.852	2.660	3.380
Aprotinin	1.200	2.480	3.580	4.680	6.076
β-LPH	0.610	1.210	1.825	2.387	2.930

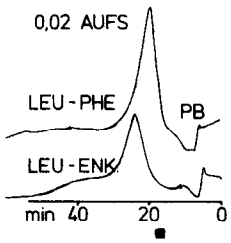

Fig. 2. Desalting of the dipeptide leu-phe (7·5 μg) and leu-enkephalin (50 μg) using TSK 20 column. PB = Sodium phosphate buffer.

alanine was eluted behind the salt peak. As an alternative, a TSK 20 column can be used for this molecular weight range (250–800 daltons) (Fuig. 2). In this case, the undesired non-specific interaction with the silica gel is of advantage. The effectiveness of this procedure was demonstrated by the fact that no phosphate could be detected in the desalted peptide sample with this sensitive method[4]. The samples dissolved in highly purified water had the same pH as water, demonstrating that no acidic equivalents of formic acid were present.

It should be stressed that the detailed conditions, *e.g.*, ionic strength, eluents and columns, for this desalting procedure are extremely important; *e.g.*, the change from 0.1 *M* to 0.01 *M* formic acid resulted in an insufficient separation from the salt peak of peptides having molecular weights lower than 3500 daltons. With the TSK 2000 P SW column we have performed more than 2500 desalting procedures without loss of the separational capacity. Regular regeneration of the column with 1% SDS (twice a week) is necessary.

In conclusion, this method allows the effective separation of peptides from salts with only a minor loss of peptide material and within a short time. So, it is suitable for small amounts of peptides. Furthermore, this method was applied successfully for other buffers, *e.g.*, Krebs–Ringer bicarbonate, ammonium acetate and sodium acetate.

Shively *et al.*[6] reported a partial formylation of N-terminal amino acids when formic acid was used for preparation of peptides. This effect depends on the concentration of formic acid, *e.g.*, 70%, used for cyanogen bromide degradation. We have been unable to observe such an effect with 0.1 *M* formic acid. Alternatively, we used highly purified water or acetic acid for desalting of these peptides, but no complete separation could be obtained in the range 800–2500 daltons. Problems can occur with strongly hydrophilic peptides where removal of the eluent may be difficult.

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