Journal of Chromatography, 487 (1989) 450–455
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4531

Note

Separation of peptides from sodium dodecyl sulphate by high-performance liquid chromatography

BATIA KAPLAN* and MORDECHAI PRAS

Heller Institute of Medical Research, Sheba Medical Center, Sackler Medical School, Tel-Aviv University, 52621 Tel-Hashomer (Israel)

(First received July 1st, 1988; revised manuscript received October 4th, 1988)

Sodium dodecyl sulphate (SDS) is one of the most popular anionic detergents used in the separation of proteins and peptides. Such techniques as preparative SDS polyacrylamide gel electrophoresis and gel permeation chromatography in the presence of SDS are often needed for fractionation of complex mixtures of biological compounds containing proteins and peptides. However, the use of SDS in preparative separations is often limited by the difficulties in removing it from isolated proteins and peptides. As SDS binds with proteins [1], special procedures for the removal of SDS from proteins have been developed [2–9]. Most of these procedures involve dialysis or gel permeation techniques [2,3,6,7,9], which, however, may be not suitable for separation of SDS from small- and medium-size peptides.

In this paper we describe the application of reversed-phase high-performance liquid chromatography (HPLC) for the separation of peptides from SDS.

EXPERIMENTAL

Materials

Acetonitrile (Bio-Lab., Jerusalem, Israel) was of HPLC grade. Trifluoroacetic acid (TFA), guanidine hydrochloride and the peptides alanylserine (Ala–Ser), tri- and tetraglycine, adrenocorticotropic hormone 1–4 fragment, angiotensin II, bradykinin and neurotensin were obtained from Sigma (St. Louis, MO, U.S.A.). SDS and basic fuchsin were purchased from BDH (Poole, U.K.). The tetrapeptide tuftsin was kindly supplied by Abic (Natanya, Israel). Amyloid AA 6–12 fragment was synthesized in the Institute for Biological Research (Nes-Ziona, Israel).

0378-4347/89/$03.50 © 1989 Elsevier Science Publishers B.V.

HPLC equipment

The HPLC equipment consisted of a Spectra-Physics 8700 solvent delivery system, 8500 dynamic mixer and 8750 organizer, coupled to a Jasco Uvidec 100-IV spectrophotometer with an 8-μl cassette-type cell (10-mm pathway), Hewlett-Packard 3390 A integrator and LKB 2211 Superrack fraction collector. A LiChrosorb RP-18 (10 μm) (Knauer, Bad Homburg, F.R.G.) column (25 cm$\times$0.4 cm I.D.) was used.

Sample treatment

Reference solutions of peptides (0.1–1.0 mg/ml) were prepared in 0.01 *M* sodium phosphate buffer (pH 7.0). Solutions of SDS–peptide mixtures (10:1, w/w) were prepared in the same buffer and contained 0.1–1 mg/ml peptide and 1–10 mg/ml SDS. A 1-ml volume of each mixture was mixed with 0.1 ml of aqueous 6 *M* guanidine and allowed to precipitate for 0.5–1 h at room temperature. The precipitate was removed and the supernatant was collected by centrifugation at 13 700 *g* for 10 min with an Eppendorf 5415 centrifuge.

HPLC procedure

HPLC was carried out by isocratic elution with aqueous 0.1% TFA (7 min), followed by a linear gradient from 0 to 50% acetonitrile in 0.1% TFA (30 min) and subsequently by isocratic elution with acetonitrile–0.1% TFA (50:50) (35 min). A flow-rate of 1.0 ml/min was maintained and 0.5-ml fractions were collected. The elution of the peptides was monitored by measuring the UV absorbance at 220 nm. The elution of guanidine and SDS was checked in the collected fractions by the methods described below.

SDS determination

SDS was determined by measuring the light absorbance of the chloroform-soluble SDS–basic fuchsin complex at 553 nm [10].

Guanidine determination

Guanidine was detected by checking the precipitation of guanidinium dodecyl sulphate after the addition of SDS to the samples [11]: 0.1 ml of aqueous 10% SDS was mixed with 0.5 ml of sample and the mixture obtained was allowed to precipitate at room temperature for 1 h.

Recovery of peptides

The recovery of peptides purified from SDS was determined by using the ninhydrin reaction [12] and the method of Lowry et al. [13]. The ninhydrin reaction was applied to check the recovery of di-, tri- and tetrapeptides and the method of Lowry et al. to evaluate the amount of the larger peptides. The values obtained were referenced to those of a known initial amount of peptide in the sample.

Amino acid analysis

The Hewlett-Packard HPLC 1090 system was adapted for amino acid analysis. A Pickering (Mountain View, CA, U.S.A.) column was used for the conventional (Stein and Moore) amino acid analysis procedure.

RESULTS

The retention of standard peptide samples analysed by HPLC (Fig. 1) is shown in Table I. The HPLC analysis of mixtures containing 0.2 mg/ml peptides and 2 mg/ml SDS demonstrated the same retention times of the corresponding peptides (listed in Table I). At higher concentrations of peptides (1 mg/ml) and SDS (10 mg/ml) in the mixture, almost all peptides demonstrated the standard retention behaviour, with the exception of tuftsin ($k'=17.36$) and amyloid AA 6–12 fragment ($k'=16.20$). The retention time of SDS was 48 min ($k'=18.20$) (Fig. 2).

The precipitation of guanidinium dodecyl sulphate was checked in samples containing 0.0025–10 mg/ml SDS and 0.55 *M* of added guanidine. Precipitation was observed with the samples containing more than 0.007–0.01 mg/ml SDS. After the precipitate had been removed, less than 0.01 mg/ml SDS was found in the supernatants obtained.

The supernatants of SDS–peptide mixtures (obtained after the removal of SDS precipitated with added guanidine) were subjected for HPLC analysis. All the peptides present in the SDS–peptide mixtures (tuftsin, amyloid AA 6–12 fragment, angiotensin II, bradykinin and neurotensin) were found in the corresponding supernatants at the same concentrations as their initial concentrations in the SDS–peptide mixtures (0.2–1.0 mg/ml). Further, the retention times of all the

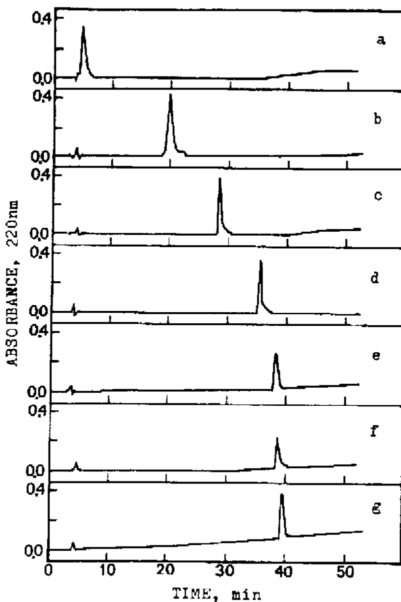

Fig. 1. HPLC of peptides on a LiChrosorb RP-18 (10 μm) column (25 cm × 0.4 cm I.D.). The samples of peptides (0.2–0.3 mg/ml) were analysed by isocratic elution with aqueous 0.1% TFA (7 min), followed by a linear gradient of 0 to 50% acetonitrile in 0.1% TFA (30 min) and subsequently by isocratic elution with acetonitrile–0.1% TFA (50:50) (35 min). Elution of peptides was monitored by UV absorbance at 220 nm: (a) triglycine; (b) tuftsin; (c) adrenocorticotropic hormone 1-4 fragment; (d) amyloid AA 6–12 fragment; (e) angiotensin; (f) bradykinin; (g) neurotensin.

TABLE I

RETENTION OF PEPTIDES CHROMATOGRAPHED ON A LICHROSORB RP-18 COLUMN

HPLC was performed by using isocratic elution with aqueous 0.1% TFA (7 min), followed by a linear gradient from 0 to 50% acetonitrile in 0.1% TFA (30 min) and subsequently with acetonitrile–0.1% TFA (50:50) (35 min). Elution was monitored by UV absorbance at 220 nm. The elution time of the unretained solute (ammonia solution), $t_0 = 2.5$ min, was determined and used for calculation of the k' values.

Peptide	Retention time (min)	k'
Ala-Ser	3.6	0 44
Triglycine	3.4	0.36
Tetraglycine	3.7	0.48
Thr-Lys-Pro-Arg (tuftsin)	20.2	7.08
Ser-Tyr-Ser-Met (adrenocorticotropic hormone 1-4 fragment)	29.1	10.64
Phe-Leu-Gly-Glu-Ala-Phe (amyloid AA 6-12 fragment)	36.5	12 60
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe (angiotensin II)	37.9	14.16
Lys-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (bradykinin)	38.1	14.24
Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu (neurotensin)	41.0	15.40

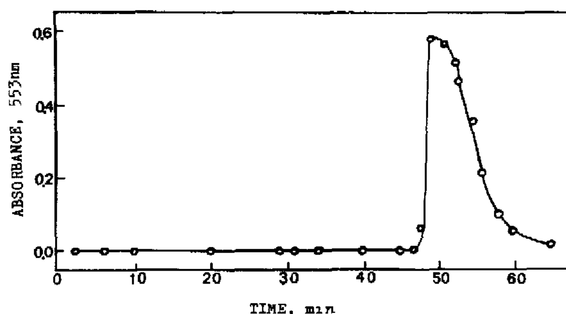

Fig. 2. Typical elution profile of SDS obtained by HPLC on a LiChrosorb RP-18 (10 μm) column (25 cm×0.4 cm I.D.). The samples of SDS (10 mg/ml) and peptide (1 mg/ml) mixtures (SDS-Ala-Ser, SDS-triglycine, SDS-tetraglycine, SDS-tuftsin, SDS-adrenocorticotropic hormone 1-4 fragment, SDS-amyloid AA 6-12 fragment, SDS-angiotensin, SDS-bradykinin, SDS-neurotensin) were analysed under the conditions described in Fig. 1. Fractions of 0.5 ml were collected and checked for SDS [10] by light absorbance of the SDS-basic fuchsin complex at 553 nm. The retention properties of the peptides are described in the text.

peptides found in the supernatants were the same as those of the corresponding standard peptides. The excess of guanidine used for SDS precipitation was also found in the supernatant; the retention time of guanidine eluted with aqueous 0.1% TFA was 3 min ($k' = 0.20$). The identities of the peptides recovered from SDS-peptide mixtures by SDS precipitation and subsequent HPLC were confirmed by amino acid analysis.

Table II demonstrates the efficiency of the removal of SDS from peptides. The peptides recovered from mixtures of 0.1–2.0 mg of SDS and 0.01–0.2 mg of peptide were lyophilized and redissolved in 0.1–0.4 ml of 0.01 M sodium phosphate buffer (pH 7.0). The amount of SDS determined in these peptide solutions was below

TABLE II

EFFICIENCY OF REMOVAL OF SDS FROM PEPTIDES OBTAINED USING REVERSED-PHASE HPLC

Sample	Initial amount in sample (mg)		Efficiency of SDS removal (%)	Recovery of peptide (%)
	SDS	Peptide		
One-step procedure: HPLC[a] of SDS-peptide mixture				
SDS-Ala-Ser	2.0	0.2	>99.8	98
SDS-triglycine	2.0	0.2	>99.8	99
SDS-tetraglycine	2.0	0.2	>99.8	
SDS-adrenocorticotropic hormone 1-4 fragment	2.0	0.2	>99.8	97
SDS-angiotensin II	0.3	0.03	>99	97
SDS-bradykinin	0.2	0.02	>98.5	96
Two-step procedure: SDS precipitation[b] and HPLC[a] of supernatant				
SDS-tuftsin	2 0	0.2	>99 8	80
SDS-amyloid AA 6-12 fragment	2 0	0.2	>99.8	78
SDS-angiotensin II	0.1	0.01	>97	79
SDS-bradykinin	0.2	0.02	>98.5	75
SDS-neurotensin	0.2	0.02	>98.5	82

[a]Chromatography was performed on a LiChrosorb RP-18 column The elution was carried out with aqueous 0 1% TFA (7 min), followed by a linear gradient of 0 to 50% acetonitrile in 0.1% TFA (30 min) and subsequently with acetonitrile-0.1% TFA (50:50) (35 min).
[b]Samples containing SDS (10 mg/ml) and peptide (1 mg/ml) mixture were mixed with aqueous 6 *M* guanidine (10:1, v/v) and allowed to precipitate (0.5-1 h). The supernatants were obtained using centrifugation with an Eppendorf centrifuge (13 700 *g*, 10 min)

the sensitivity limit of the method (0.3 μg of SDS). The yield of peptides purified from SDS by the one-step procedure (HPLC separation of SDS–peptide mixtures) varied from 96 to 99%. The peptides purified from SDS by the two-step procedure (precipitation with guanidine and subsequent HPLC) yielded 75–82% of the initial amount of peptides in the SDS–peptide mixtures. In this instance, the losses of the peptide material were mostly related to incomplete removal of supernatant which may be partially entrapped within the precipitate.

DISCUSSION

Nine peptides of different molecular mass, ranging from 176 to 1673, were purified from SDS using reversed-phase HPLC. The possibility of employing this method for the separation of peptides from SDS was revealed by a careful HPLC examination of the retention behaviour of peptides, SDS and SDS–peptide mixtures. SDS was found to be strongly retained by a LiChrosorb RP-18 column. The tested peptides varied in their retention behaviour, some being only weakly retained and the retention of other peptides being stronger and close to that of SDS. Direct application of HPLC to the separation of SDS–peptide mixtures was found

to be effective for the purification of peptides that have a retention behaviour essentially different from that of SDS. A two-step procedure is recommended for the purification of peptides with retention times similar to that of SDS, viz., precipitation of SDS by the addition of guanidine followed by HPLC of the supernatant obtained. The peptide and guanidine in the supernatant are easily separated by HPLC: guanidine is eluted from the column with aqueous 0.1% TFA and the retained peptides are recovered by increasing the acetonitrile content in eluent. The very small amounts of SDS unprecipitated by guanidine can also be separated from the peptides by HPLC.

The two-step SDS removal procedure is also recommended for the purification of those peptides whose retention times increase significantly in the presence of SDS. These changes in the retention behaviour of peptides are probably related to their interaction with SDS, and may depend on the nature of the peptide and on the concentrations of SDS and peptide in the sample. It was suggested previously that SDS–protein complexes dissociate in acidic aqueous acetonitrile solutions [9]. Hence, the use of gradient elution in HPLC by increasing the acetonitrile content in the eluent may also cause the dissociation of SDS–peptide complexes. This may explain the high efficiency of the separation of SDS from peptides by the proposed procedures even when binding of SDS to peptides occurred.

Finally, the retention behaviour of the peptides used in these experiments is typical of that of most of the known peptides [14–16]. We therefore consider that the procedures applied successfully here for the separation of SDS from nine peptides may also be useful for the purification of many other small- and medium-sized peptides. Only those peptides with the retention times similar to that of SDS may be purified somewhat less effectively. However, even in this instance, most of the SDS can be removed owing to the high efficiency of the precipitation of SDS by guanidine (99% in samples containing 1 mg/ml SDS).

The procedures for the separation of peptides from SDS employed here are simple and rapid and may be applied to the purification of micro amounts of peptides.

REFERENCES

1 K. Weber and M. Osborn, J Biol. Chem., 244 (1969) 44.
2 K. Weber and J. Kutter, J. Biol. Chem., 246 (1971) 4504.
3 G.P. Tuszynski and L Waren, Anal. Biochem., 67 (1975) 55.
4 L.E. Henderson, S. Oroszlan and W. Konigsberg, Anal. Biochem , 93 (1979) 153.
5 D A. Hager and R.R. Burges, Anal. Biochem., 109 (1980) 76.
6 R. Amons and P.J. Schrier, Anal. Biochem., 116 (1981) 439
7 M.W. Hunkapiller, E. Lujan, F. Ostrander and L.E. Hood, Methods Enzymol., 91 (1983) 227.
8 B. Kaplan and M. Pras, Clin Chim Acta, 163 (1987) 199.
9 B. Kaplan and M Pras, J. Chromatogr., 423 (1987) 376.
10 J.H. Waite and C.Y. Wang, Anal. Biochem., 70 (1976) 279
11 K. Weber, J R. Pringle and M. Osborn, Methods Enzymol., 26 (1972) 3.
12 S. Moore and W.H. Stein J. Biol. Chem., 211 (1954) 907.
13 D H. Lowry, N.J. Rosenbrough, L. Farr and R.J. Randall, J Biol. Chem., 193 (1951) 265.
14 P. Bohleh, F. Castilo, N. Ling and R. Guillemin, Int. J. Pept. Protein Res., 16 (1980) 306.
15 J.L. Di Cesare, Chromatogr. Newslett., 9 (1981) 16.
16 C A. Bishop and M.T.W. Hearn, J. Liq. Chromatogr., 4 (1981) 66.