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Letter to the Editor

Removal of sodium dodecyl sulphate from proteins by gel permeation chromatography

Sir,

The use of sodium dodecyl sulphate (SDS) in protein chemistry is often limited by the difficulties of removing it from SDS–protein complexes. In some removal procedures a poor recovery of proteins was obtained [1]. In other cases special electrophoretic equipment was needed [2], or the procedure was performed under strongly acidic conditions [3]. SDS extraction by the organic solvents was found to be effective [4–6]. However, the proteins were recovered as precipitates and their further solubilization was complicated.

We present a procedure for SDS removal from proteins by applying gel permeation chromatography in moderately acidic aqueous acetonitrile solution. This procedure requires no special equipment and helps to overcome the problems of protein recovery and solubility.

EXPERIMENTAL

SDS (BDH, Poole, U.K.) complexes with insuline, lysozyme, β-lactoglobulin, trypsinogen, ovalbumin, bovine serum albumin (Sigma, St. Louis, MO, U.S.A.) and amyloid proteins (AA and AL proteins, obtained as reported earlier [7]) were used in the experiments.

The aqueous solutions of SDS–protein complexes were obtained according to Weber et al. [8] and contained 0.5–2.0 mg/ml protein and at least a three-fold concentration of SDS in 0.01 *M* phosphate buffer (pH 7.0). Aliquots of 1 ml of these solutions were lyophilized, redissolved in 50 μl of distilled water and pipetted (with an intensive mixing) into 1.5–2.0 ml of aqueous 50% acetonitrile (Bio-Lab., Jerusalem, Israel), containing 0.1% trifluoroacetic acid (Sigma). The soluble samples obtained were applied to a Fractogel TSK HW-40 (F) (Merck, Darmstadt, F.R.G.) column (160×14 mm I.D.). The elution was performed using aqueous 50% acetonitrile solution containing 0.1% trifluoroacetic acid, and 0.8-ml fractions were collected at a flow-rate of 0.2 ml/min. Protein was determined by the method of Lowry et al. [9], and SDS using basic fuchsin [10].

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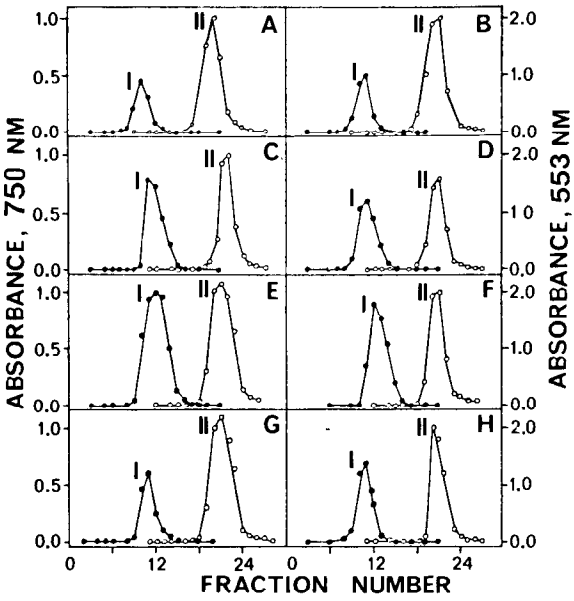

Fig. 1. Separation of proteins (peak I) from SDS (peak II) by gel permeation on a Fractogel TSK HW-40 column (160×14 mm I.D.) in aqueous 50% acetonitrile solution containing 0.1% trifluoroacetic acid. Fractions of 0.8 ml were collected and checked for SDS (○, absorbance at $\lambda = 553$ nm) according to Waite and Wang [10] and for protein (●, absorbance at $\lambda = 750$ nm) by the method of Lowry et al. [9]. (A) Protein AA, 0.7 mg; SDS, 10 mg; (B) protein AL, 0.8 mg; SDS, 10 mg; (C) bovine serum albumin, 0.9 mg; SDS, 8 mg; (D) β-lactoglobulin, 0.8 mg; SDS, 6 mg; (E) insulin, 1.0 mg; SDS, 10 mg; (F) trypsinogen, 1.0 mg; SDS, 8 mg; (G) ovalbumin, 0.5 mg; SDS, 10 mg; (H) lysozyme, 0.6 mg, SDS, 8 mg.

RESULTS

The samples containing SDS and protein in acidic aqueous 50% acetonitrile were stable without any visible precipitate formation up to the following protein concentrations: ovalbumin, 0.3 mg/ml; amyloid proteins, 0.4 mg/ml; lysozyme, 0.5 mg/ml; β-lactoglobulin, 0.9 mg/ml; trypsinogen, 1.2 mg/ml; bovine serum albumin, 1.6 mg/ml; insulin, > 2.0 mg/ml. Gel permeation profiles of these samples (Fig. 1) showed a good separation of proteins (peak I) from SDS (peak II). The eluted proteins yielded 65–85% of the protein applied to the column (Table I). The amount of SDS determined in eluted proteins was below the sensitivity limit of the method (0.3 μg of SDS). Thus, the recovered proteins contained less than 0.5% of the initial SDS in the samples.

DISCUSSION

The removal of SDS from proteins by the procedure described is obviously based on dissociation of SDS–protein complexes in acidic aqueous acetonitrile solution. The procedure is simple, rapid and allows the proteins to be recovered in a soluble state.

Previously, we found that addition of acetonitrile to neutal aqueous solutions

TABLE I

EFFICIENCY OF SDS SEPARATION FROM PROTEINS OBTAINED BY GEL PERMEATION CHROMATOGRAPHY

Fractogel TSK HW-40 column, acidic aqueous 50% acetonitrile.

Sample*	Amount applied to column (mg)		Recovery of protein eluted from column** (%)	Efficiency of SDS removal from protein** (%)
	Protein	SDS		
SDS-protein AA	0.7	10	65	>99.7
SDS-bovine serum albumin	0.9	8	85	>99.6
SDS-β-lactoglobulin	0.8	6	70	>99.5
SDS-insulin	1.0	10	80	>99.7
SDS-trypsinogen	1.0	8	75	>99.6
SDS-ovalbumin	0.5	10	65	>99.7

*Sample represents SDS-protein complex dissolved in aqueous 50% acetonitrile containing 0.1% trifluoroacetic acid.

**The fractions of eluted protein (peak I, Fig. 1) were pooled, lyophilized, redissolved in 1 ml of eluent and checked for protein [9] and SDS [10].

of SDS–protein complexes caused precipitation of proteins free from SDS [6]. However, a number of proteins are soluble in acidic aqueous acetonitrile, an organic solvent system that is widely used in reversed-phase high-performance liquid chromatography of proteins and peptides [6, 11–13]. In the present study we showed that SDS–protein complexes may be also solubilized in this organic solvent system. It allowed us to perform the gel permeation chromatographic separation of samples, containing SDS and protein, in acidic aqueous acetonitrile. Fractogel TSK was chosen as a chromatographic medium because of its high chemical stability towards organic solvents.

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