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Note

High-performance liquid chromatography of protein polypeptides on porous silica gel columns (TSK-GEL SW) in the presence of sodium dodecyl sulphate: comparison with SDS-polyacrylamide gel electrophoresis

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Although sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (SDS–PAGE)[1] has a high efficiency, it suffers from disadvantages such as difficulties in the precise determination of mobilities and in the recovery of samples. These shortcomings are inevitable in a separation technique that utilizes a continuous gel matrix. Gel chromatography is free from such shortcomings, but is inferior to gel electrophoresis with respect to resolution and sensitivity. Gel chromatography in the presence of SDS was first reported in advance of SDS–PAGE by Kretscher[2], but found only limited use for the above reasons.

The situation is now being changed by the introduction of porous silica gel suitable for packing in a high-performance liquid chromatographic (HPLC) column. A series of columns pre-packed with such gels has been developed by Toyo Soda, and are available under the name TSK-GEL SW. They have been shown to have high efficiency in protein fractionations[3,4]. Imamura *et al.*[5] have demonstrated that the columns can be used effectively to determine molecular weights of proteins in the presence of SDS. They have not, however, extended their work to a comparison of this promising technique and SDS–PAGE, with which the former must compete in performance. We have therefore studied HPLC (porous silica gel) in the presence of SDS (SDS–HPLC).

EXPERIMENTAL

Sodium phosphate buffer (0.10 *M*) of pH 7.0 was used throughout, and contained 0.1% of SDS (special grade for protein analysis from Nakarai Chemicals) and 0.02% of sodium azide. The buffer was supplied to two TSK-GEL G3000SW columns (each 60 cm × 7.5 mm I.D.) connected in series by a Milton-Roy Model SF Minipump, after filtration through a glass and a stainless-steel sintered-type filter. A Rheodyne injector with a 100-μl sample loop was used. Elution was monitored at 280 nm by the use of a flow cell with an internal volume of 8 μl. All of the above components comprised a Model HLC-803 liquid chromatograph (Toyo Soda). All operations were carried out at 20 ± 2°C.

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SDS–PAGE was carried out essentially according to the method of Weber and Osborn[1]. A frequently used sample was a lyophilized mixture of six kinds of purified proteins (Electrophoretic Calibration Kit; Pharmacia). The other proteins used were preparations of the best grade available.

RESULTS AND DISCUSSION

Fig. 1 shows a typical example of the elution patterns for the Pharmacia Kit obtained with the two G3000SW columns. About one third of a vial of the Kit, nominally containing 60–150 μg of each protein, was applied to the columns. Four of the six kinds of proteins could be separated but the first and second did not show a baseline region between their peaks. When only one of the columns was used all of the peaks were resolved fairly well, but there were no baseline regions between the neighbouring peaks. The anomaly in the elution curve at a retention time of about 130 min is an optical artifact due to the elution of the micelles of SDS added in excess in the sample solution. The elution of the micelles often gave a positive peak, contrary to the case shown in Fig. 1. The shape of the elution pattern of the micelles is dependent not only on the amount of SDS added to a sample but also on the extent of incorporation of materials absorbing at 280 nm into the micelles. Care should therefore be taken not to mistake it for a protein peak when it gives a positive deflection. Other materials of low molecular weight in the sample mixture were eluted far behind the micelle band (not shown).

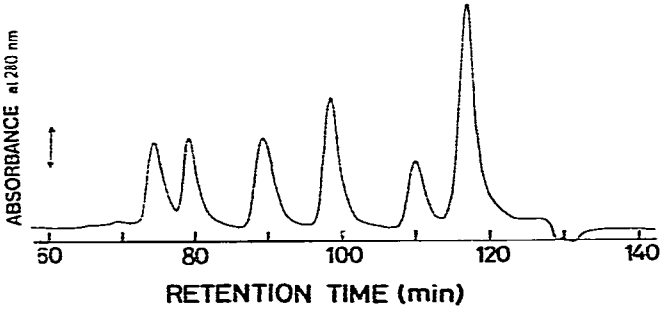

Fig. 1. Typical elution pattern from the two columns of TSK-GEL G3000SW connected in series. Flow-rate, 0.30 ml/min. The bar in the left indicates 0.01 absorbance unit. The contents of a sealed vial from the Pharmacia Kit were dissolved by injection of 200 μl of 0.10 *M* sodium phosphate buffer (pH 7.0) containing 30 mg of SDS and 10 mg of dithiothreitol. The mixture was heated in a water-bath at 100°C for 5 min. One third of the mixture was applied to the SDS–HPLC system. The vial nominally contains 64, 83, 147, 83, 80 and 112 μg of rabbit phosphorylase *b* (mol.wt. 94,000), bovine serum albumin (mol.wt. 67,000), ovalbumin (mol.wt. 43,000), bovine carbonic anhydrase (mol.wt. 30,000), soybean trypsin inhibitor (mol.wt. 20,100) and bovine α-lactalbumin (mol.wt. 14,400).

Fig. 2 shows the elution patterns obtained when the weights of protein sample applied were successively halved. Retention times were also measured with more than ten other proteins. The retention time was reproducible within 1 % provided that the run was continued without resetting the pumping rate.

Fig. 3 shows a plot of the molecular weights of the proteins *versus* retention times relative to that of bovine serum albumin. The retention time of bovine serum

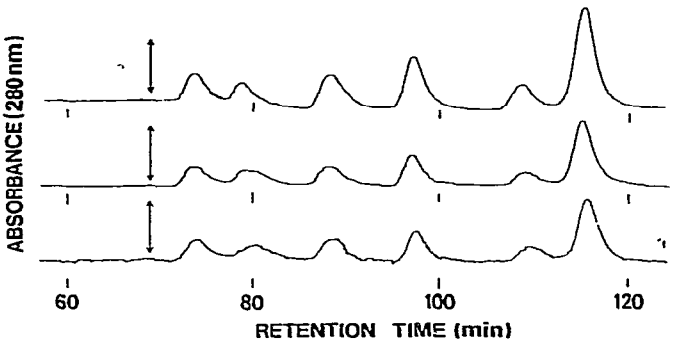

Fig. 2. Elution patterns obtained with the Pharmacia Kit in experiments in which the weights of the sample proteins were successively halved in comparison with Fig. 1 from the top to the bottom. The bars on the left indicate 0.01, 0.01 and 0.005 absorbance unit from top to bottom.

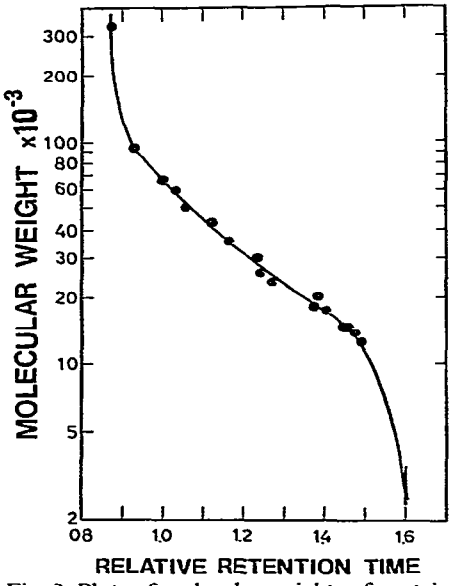

Fig. 3. Plots of molecular weights of proteins *versus* their retention times relative to that of bovine serum albumin in SDS–HPLC. Proteins from left to right: thyroglobulin, phosphorylase *b*, bovine serum albumin, catalase, immunoglobulin G heavy chain, ovalbumin, lactic dehydrogenase, carbonic anhydrase, chymotrypsinogen A, immunoglobulin G light chain, β-lactoglobulin, trypsin inhibitor, myoglobin, lysozyme, α-lactalbumin, ribonuclease, cytochrome *c* and insulin A and B chains. The insulin chains failed to separate, and their elution position is denoted by the bar on the right. Relative retention times of blue dextran (frontal edge) and DNP-alanine were 0.885 and 2.41, respectively.

albumin was measured for every series of experiments in order to obviate the effect of fluctuation of the pumping rate. The correlation between the two parameters is good enough for the SDS–HPLC technique to be used to determine molecular weights of proteins between 10,000 and 100,000.

Fig. 4 shows a typical electrophoretic pattern obtained with the Pharmacia Kit using SDS–PAGE with the standard procedure[1]. Comparison of Fig. 4 with Figs. 1

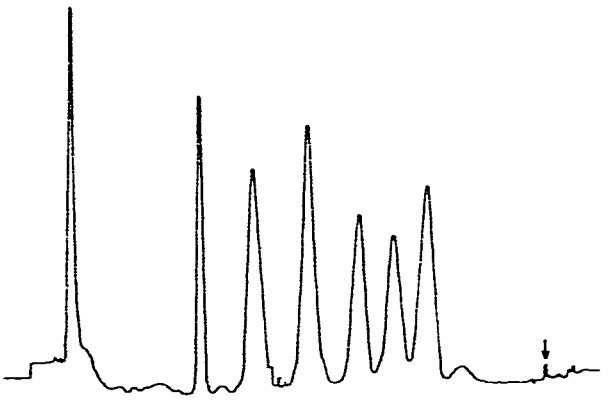

Fig. 4. Typical SDS–PAGE profile obtained with the Pharmacia Kit. The contents of a vial from the Kit were dissolved by injection of 1 ml of 0.1 *M* sodium phosphate buffer (pH 7.0) containing 25 mg of SDS, 50 mg of mercaptoethanol and a small amount of malachite green. The mixture was heated in a water-bath at 100 C for 5 min, then 10 μl of the mixture were applied to the top of the gel (5%). Electrophoresis (left to right): 6 mA per tube, 140 min, 25°C. The peak on the left is an artifact due to the gel top. Other major peaks correspond to those in Fig. 1 in the same sequence. The arrow on the right indicates the position of the marker dye.

and 2 indicates that the SDS–PAGE and the SDS–HPLC techniques give similar resolutions except for proteins of higher molecular weight, for which the former technique performed better. The use of TSK-GEL SW of other grades (G4000 and G2000) in addition to G3000 can expand the range of molecular weights covered by the SDS–HPLC technique. Clearly the SDS–HPLC technique can act as an alternative to SDS–PAGE. The former exceeds the latter in ease of operation, sample recovery, reproducibility, time required and variability of solvent composition. The latter technique, on the other hand, exceeds the former in the amount of sample required and the simultaneous operation of many runs at low cost.

The bottom SDS–HPLC elution curve in Fig. 2 was obtained by application of a protein sample only five times larger than that used to obtain the SDS–PAGE electrophoretic pattern in Fig. 4. The detector used to monitor the eluate in the present study was of a conventional type. If a more sensitive detector were to be used, there might be no significant difference in the amounts of sample required in the two techniques.

The SDS–HPLC technique separates complexes between SDS and various polypeptides derived from proteins predominantly on the basis of molecular sieving. The SDS–PAGE technique, on the other hand, separates them on the basis of both molecular sieving and electrophoresis. The determination of protein molecular weights by either of these techniques is therefore empirical in nature. Such empirical methods do not always give a correct estimate of molecular weight, but the development of the SDS–HPLC technique has made available two techniques with comparable performances.

A particular protein is expected to behave differently in the two techniques. Thus, the SDS–HPLC technique is promising not only as a substitute for the SDS–PAGE technique but also as a means for examining any abnormal behaviour of a protein in the latter. The SDS–HPLC technique is free from the restrictions imposed

by the electrophoresis conditions in the SDS–PAGE technique. In principle, various aqueous solutions can be used as solvents in SDS–HPLC, thus making modifications of the technique possible, unless the solvents attack the chromatographic system or have a high viscosity.

The resolution in SDS–HPLC is reported to be affected markedly by the concentration of salt added to the buffers[5–7]. A study on the parameters that affect the elution behaviour of proteins in SDS–HPLC is needed, as a rapid expansion of the use of this technique is expected in the near future.

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