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Note

High-speed gel filtration of proteins in 6 *M* guanidine hydrochloride on TSK-GEL SW columns

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In the period since the introduction of high-performance liquid chromatography, progress in the field of gel filtration has been slow in comparison to other modes of liquid chromatography. Recently, however, several kinds of column packings have been developed for use in gel filtration. Among them, two column packings were particularly effective in high-speed gel filtration: TSK-GEL PW and TSK-GEL SW (Toyo Soda, Tokyo, Japan). The former is especially suitable for measurements of molecular-weight distributions of polymers[1] and oligomers[2], the latter for the separations of proteins[3]. Moreover, TSK-GEL SW, which is a microparticulate silica gel chemically bonded with hydrophilic compounds, could also be used in denaturing solvents such as aqueous SDS solution[4] and concentrated guanidine hydrochloride[5]. TSK-GEL SW comprises three grades of different pore sizes, G2000SW, G3000SW and G4000SW, whose separation ranges and separation efficiencies in a common buffer solution and in aqueous SDS solution have been described previously[6,7]. In this paper, the separation range and separation efficiency of TSK-GEL SW in 6 *M* guanidine hydrochloride are reported.

EXPERIMENTAL

Gel filtration was carried out at 25 °C on a Model HLC-803 liquid chromatograph (Toyo Soda) equipped with a UV detector at 280 nm. A column (60 cm × 7.5 mm I.D.) of G2000SW, G3000SW or G4000SW was used for each measurement. The eluent was 6 *M* guanidine hydrochloride containing 0.1 *M* sodium phosphate (pH 6). The flow-rate was 0.5 ml/min. The sample concentration was 0.2% and the injection volume was 0.1 ml. The sample solutions were prepared by incubating proteins overnight in the eluent containing 0.02 *M* dithiothreitol at 25 °C. Iodoacetamide was then added to a concentration of 0.04 *M* and the solutions were again incubated at 25 °C for more than 1 h.

The proteins used are listed in Table I.

TABLE I

PROTEINS USED IN EXPERIMENTS

Protein	*Mol.wt. of constituent polypeptide*	*Source**
Thyroglobulin	165,000	A
Bovine serum albulin	67,000	B
Ovalbumin	43,000	C
β-Lactoglobulin	17,500	D
Myoglobin	16,900	A
Ribonuclease	13,700	E
Cytochrome c	12,400	D
Insulin	2900 (average)	A
γ-Globulin (human serum)	50,000 (heavy chain) 23,000 (light chain)	F
Ovalbumin (crude)		B

* A, Sigma (St. Louis, MO, U.S.A.); B, Wako (Osaka, Japan); C, Seikagaku Kogyo (Tokyo, Japan); D, Miles Labs. (Elkhart, IN, U.S.A.); E, P-L-Biochemicals (Milwaukee, WI, U.S.A.); F, Nakarai (Kyoto, Japan).

RESULTS AND DISCUSSION

Calibration curves for polypeptide in 6 *M* guanidine hydrochloride on G2000SW, G3000SW and G4000SW columns are shown in Fig. 1. The separation ranges estimated from Fig. 1 are summarized in Table II. Both upper and lower limits in 6 *M* guanidine hydrochloride are lower than those in non-denaturing solvents. On the whole, polypeptides in the molecular-weight range from 1000 to 400,000 can be separated by gel filtration in 6 *M* guanidine hydrochloride on TSK-GEL SW. The exclusion limits of G2000SW, G3000SW and G4000SW were com-

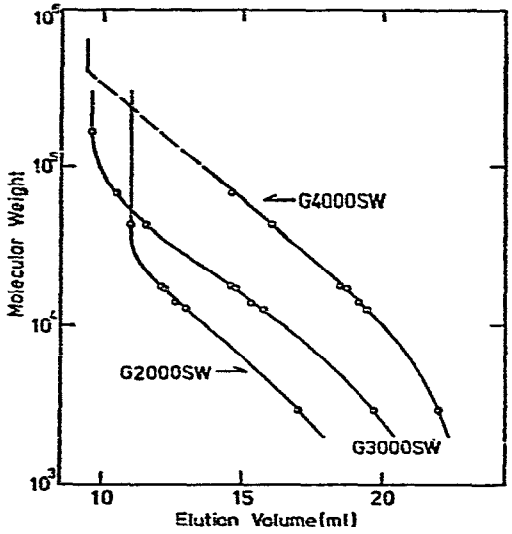

Fig. 1. Calibration curves of TSK-GEL SW columns for polypeptide in 6 *M* guanidine hydrochloride.

TABLE II

SEPARATION RANGE OF TSK-GEL SW FOR POLYPEPTIDE IN 6 *M* GUANIDINE HYDROCHLORIDE CONTAINING 0.1 *M* SODIUM PHOSPHATE (pH 6)

Column	*Separation range*
G2000SW	1000– 25,000
G3000SW	2000– 70,000
G4000SW	3000–400,000

pared with those of other column packings described previously. Sephadex G-100 (20,000[8]) and Sephacryl S-200 (30,000[9]) had similar exclusion limits to G2000SW. Bio-Glass 500 (70,000[10]), Bio-Gel A-5m (100,000[8] or 80,000[11]) and Sepharose CL-6B (80,000[12]) had similar exclusion limits to G3000SW. Sepharose 4B (250,000[11]) had a slightly lower exclusion limit than G4000SW.

Specific resolution (R_s) was calculated for some pairs of proteins (bovine serum albumin and ovalbumin, ovalbumin and myoglobin, myoglobin and cytochrome *c*, cytochrome *c* and insulin) to compare separation efficiencies of G2000SW, G3000SW and G4000SW, using the expression

$$R_s = 2(V_2 - V_1)/(W_2 + W_1)(\log M_1 - \log M_2) \quad (1)$$

where V, W and M represent the elution volumes, peak widths at the base and the molecular weights, respectively, of two components. Specific resolutions are plotted against average molecular weights of the two components in Fig. 2, which indicates that the highest separation efficiencies of G2000SW, G3000SW and G4000SW are found in the molecular-weight ranges below 10,000, 10,000–70,000 and above 70,000 respectively. This is also illustrated by Fig. 3 which shows elution curves for a mixture of proteins obtained on G2000SW, G3000SW and G4000SW. The A chain (molecular weight = 2380) and B chain (molecular weight = 3420) of insulin are partially separated on G2000SW, but only very slightly on G3000SW and not at all on G4000SW. Bovine serum albumin, ovalbumin, myoglobin and cytochrome *c* are best separated on G3000SW. The non-homogeneity of polypeptide chains derived from commercial thyroglobulin can be demonstrated only on G4000SW.

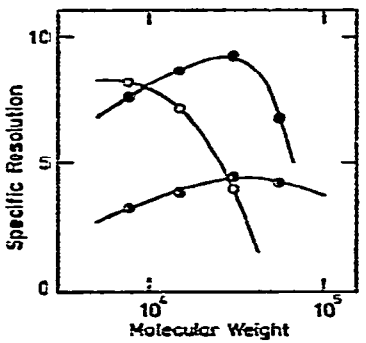

Fig. 2. Comparison of specific resolution for some pairs of proteins on G2000SW (○), G3000SW (●) and G4000SW (◑).

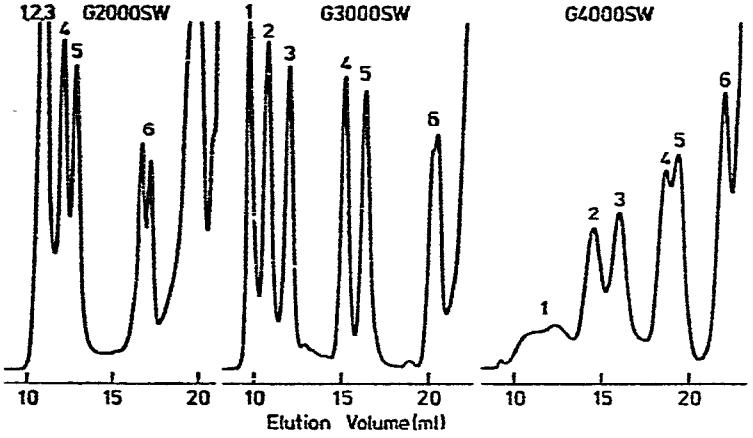

Fig. 3. Elution curves of a mixture of thryoglobulin (0.03%) (1), bovine serum albumin (0.06%) (2), ovalbumin (0.04%) (3), myoglobin (0.03%) (4), cytochrome *c* (0.02%) (5) and insulin (0.04%) (6) obtained by gel filtration in 6 *M* guanidine hydrochloride on G2000SW, G3000SW and G4000SW.

Commercial crude ovalbumin was chromatographed on G3000SW as an example of the applications of gel filtration in 6 *M* guanidine hydrochloride on TSK-GEL SW. The elution curve obtained is shown in Fig. 4. Four peaks 1, 2, 3 and 4 can be distinguished besides peaks eluting near the void volume. The molecular weights corresponding to these four peaks were estimated from the calibration curve in Fig. 1 and are summarized in Table III. Since the main components of egg white, from which the ovalbumin must have been obtained, are ovalbumin (60%, molecular

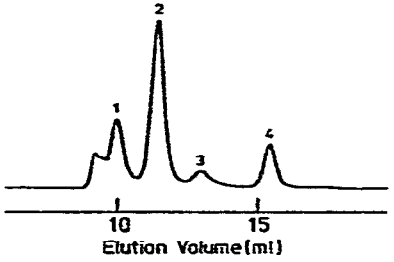

Fig. 4. Elution curve of crude ovalbumin obtained by gel filtration in 6 *M* guanidine hydrochloride on G3000SW.

TABLE III

MOLECULAR WEIGHTS OF POLYPEPTIDE CHAINS OF COMPONENTS OF CRUDE OVALBUMIN DETERMINED BY GEL FILTRATION ON G3000SW IN 6 *M* GUANIDINE HYDROCHLORIDE CONTAINING 0.1 *M* SODIUM PHOSPHATE (pH 6)

Component	*Mol.wt.*
1	77,000
2	43,000
3	26,000
4	13,000

weight = 43,000), conalbumin (14%, molecular weight = 70,000), ovomucoid (14%, molecular weight = 27,000–29,000) and ovoglobulin (12%, molecular weight = 14,000–17,000), peaks 1, 2, 3 and 4 are presumed to correspond to conalbumin, ovalbumin, ovomucoid and ovoglobulin, respectively. If this identification is correct, the molecular weights estimated agree with values generally admitted within 10%.

Human γ-globulin was also examined on G3000SW and on G4000SW. The elution curves obtained are shown in Fig. 5. Two main peaks are observed in both the elution curves, as is the case for rabbit γ-giobulin[13]. The larger and the smaller

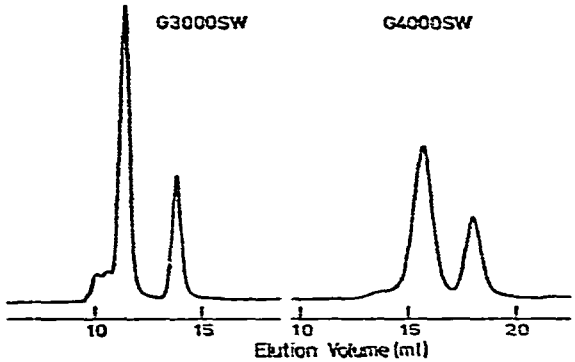

Fig. 5. Elution curves of γ-globulin (human serum) obtained by gel filtration in 6 *M* guanidine hydrochloride on G3000SW and G4000SW.

peaks are probably due to heavy and light chains, respectively. The molecular weights corresponding to these two peaks were estimated from the calibration curves in Fig. 1 and are summarized in Table IV. The molecular weights are in good agreement with the values obtained by other methods (see Table I) except that the molecular weight of heavy chain obtained on G3000SW is approximately 8% smaller than the value in Table I. Ratios of the molecular weights and peak areas of heavy and light chains are also listed in Table IV. If the extinction coefficients of heavy and light chains at 280 nm are identical, these two ratios should be equal. The two ratios obtained on G4000SW are in good agreement, while those obtained on G3000SW differ by about 20%.

TABLE IV

GEL FILTRATION OF REDUCED γ-GLOBULIN (HUMAN SERUM) ON TSK-GEL SW IN 6 *M* GUANIDINE HYDROCHLORIDE CONTAINING 0.1 *M* SODIUM PHOSPHATE (pH 6)

	G3000SW	*G4000SW*
Mol.wt. of heavy chain	46,000	49,000
Mol.wt. of light chain	22,000	22,000
Mol.wt. ratio*	2.09	2.23
Peak area ratio*	2.52	2.21

* Of heavy chain to light chain.

A flow-rate of 0.5 ml/min was employed here owing to the high viscosity of 6 *M* guanidine hydrochloride, although the flow-rates were 1 ml/min in previous work on TSK-GEL SW columns in phosphate buffer[6] and in aqueous SDS solution[7]. However, since 60-cm columns were employed instead of the 120-cm columns used in the previous experiments, the times required for the measurement of one sample were almost identical (30–50 min). Specific resolutions in 6 *M* guanidine hydrochloride were slightly higher than those in aqueous SDS solution and similar to those in phosphate buffer, although the separation ranges were different.

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