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Note

Anomalous behavior of lectins in size-exclusion high-performance liquid chromatography and gel electrophoresis

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In characterizing the molecular structure of oligomeric proteins such as lectins, it is common practice to estimate the molecular weight of subunits by sodium dodecyl sulphate (SDS) gel electrophoresis and the native molecular weight by gel filtration. The oligomeric form of the protein can then be obtained by comparing these two molecular weights. Media such as Sephadex G-200 have sufficient resolving power that dimers can be readily distinguished from tetramers though in the fortunately rare case of trimers mistakes could be made. The advent of size-exclusion chromatography media such as the TSK SW series [1] has made it possible to rapidly obtain molecular weight estimates for proteins on high-performance liquid chromatography (HPLC) equipment. These media also have greater resolving power than the traditional gels. However in experiments with a group of lectins from leguminous plants reported here, we have found that results obtained by HPLC experiments were less reliable than those previously obtained by gel filtration.

MATERIALS AND METHODS

Published affinity chromatographic procedures were used to purify concanavalin A [2], pea (*Pisum sativum*) lectin [3], peanut (*Arachis hypogaea*) agglutinin [4], *Griffonia simplicifolia* GSI lectin [5] and *Phaseolus vulgaris* hemagglutinin [6]. The N-acetyl-D-galactosamine specific lectins from *Sophora japonica*, *Wisteria floribunda*, *Caragana arborescens* and *Glycine max* (soybean agglutinin) were purified on the affinity medium described for the *Cytisus scoparius* lectins [7]. *Ph. coccineus* and *Ph. lunatus* lectins were purified by chromatographic procedures [8, 9]. The *Dolichos biflorus* lectin was purchased

from Sigma (St. Louis, MO, U.S.A.), and the *Bauhinia purpurea*, *Vicia villosa* and *Cy. sessilifolius* lectins were purchased from E-Y Labs. (San Mateo, CA, U.S.A.).

SDS slab-gel electrophoresis was carried out by the method of Weber and Osborn [10] with a 10% gel and by the method of Laemmli [11] with a 12% gel. The standard proteins used were phosphorylase b (96,000 molecular weight, M_r) bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (30,000), chymotrypsinogen (25,600), β-lactoglobulin (18,400), myoglobin (17,200) and lysozyme (14,300). For size-exclusion HPLC, a TSK 3000-SW column (60 × 0.7 cm) and TSK-SW precolumn (10 × 0.7 cm) from Beckman Instruments were used. The column was eluted at 0.5 ml/min, using a Pharmacia P500 pump and the effluent was monitored at 280 nm with a Spectraphysics Model SP 8440 detector. Samples of approx. 100 μg of protein were injected in 20—50 μl vols., using a Pharmacia FPLC injector. Some preliminary experiments were carried out on a Beckman Model 330 HPLC system.

RESULTS

The subunit molecular weights (mol.wt.) previously reported for the lectins (Table I) were checked in experiments using two SDS gel electrophoresis methods. In both systems, the protein standards gave linear calibration plots and the lectins migrated in a narrow range of mobilities, close to the position of carbonic anhydrase (Fig. 1). The literature data of Table I were obtained mainly by the method of Weber and Osborn [10].

TABLE I

LECTIN MOLECULAR WEIGHTS FROM SIZE-EXCLUSION HPLC AND LITERATURE SUBUNIT MOLECULAR WEIGHTS

Lectin	Apparent mol.wt.	Subunit mol.wt.	Reference
Gr. simplicifolia	120,000	32,500	14
Ph. lunatus	120,000	31,000	15
B. purpurea	120,000	(195,000)*	16
W. floribunda	105,000	28,000	17
Ph. coccineus	100,000	34,000	8
Ph. vulgaris	96,000	34,000	18
S. japonica	96,000	32,500	19
V. villosa	96,000	30,000	20
Ca. arborescens	82,000	30,000	21
D. biflorus	78,000	27,500	22
Cy. scoparius	70,000	30,000	7
G. max	68,000	27,600**	13
Cy. sessilifolius	64,000	(110,000)*	23
A. hypogaea	48,000	27,500	24
Concanavalin A	29,000	25,600**	12
P. Sativum	37,000	7000 17,000	3

*Oligomer molecular weight.
**Calculated from amino acid sequence.

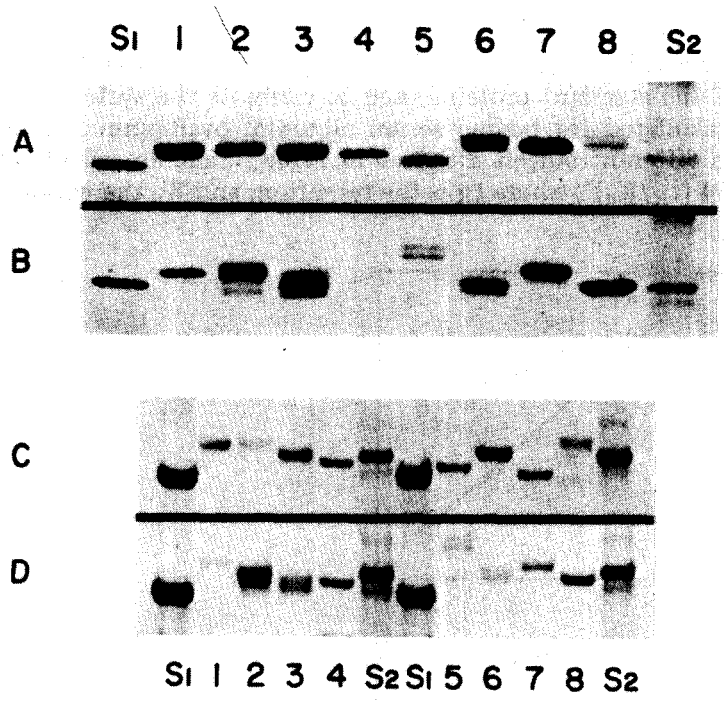

Fig. 1. SDS gel electrophoresis of lectins. The gel sections shown in A and B were obtained by the method of Laemmli [11] and those in C and D by the method of Weber and Osborn [10]. The standard proteins shown are carbonic anhydrase, mol.wt. 30,000 (AS_1, BS_1, CS_2, DS_2) and chymotrypsinogen, mol.wt. 25,600 (AS_2, BS_2, CS_1, DS_1). The lectins are *Ph. vulgaris* (A1, C1); *Ph. coccineus* (A2, C2); *Ph. lunatus* (A3, C3); concanavalin A (A4, C4, D4). *D. biflorus* (A5, C5); *G. max* (A6, C6); *A. hypogaea* (A7, C7); *Gr. simplicifolius* GSI (A8, C8); *S. japonica* (B1, D1); *Cy. scoparius* (B2, D2); *Cy. sessilifolius* CSIa (B3, D3); *B. purpurea* (B5, D5); *Ca. arborescens* (B6, D6); *V. villosa* (B7, D7) and *W. floribuda* (B8, D8).

Molecular weights were estimated for the same lectins by size-exclusion chromatography on a TSK 3000-SW column, run in 0.2 *M* potassium phosphate buffer, pH 7.0 (Table I). Eight of the lectins had mol.wt. between 95,000 and 120,000, consistent with tetrameric forms. Six others, including the well characterized soybean and peanut agglutinins had mol.wt. in the range 50,000—82,000. Concanavalin A should have been tetrameric under these buffer conditions [25] but eluted later than the position expected for the dimer species. The pea lectin also eluted a little later than expected for its $\alpha_2\beta_2$ structure. When the specific sugar inhibitors, D-galactose for peanut agglutinin, N-acetyl-α-galactosamine for soybean agglutinin and methyl-α-D-glucoside for concanavalin A, were added to the buffer, each lectin eluted slightly earlier.

The behavior of the soybean and peanut agglutinins when chromatographed with buffers of different pH, ionic strength and buffer ion is shown in Fig. 2. The apparent molecular weights were little changed by running in 0.1 *M* acetate buffer, pH 5.5. At lower ionic strengths, the column calibration changed considerably, but the apparent molecular weights of the lectins were still below those expected for tetrameric lectins. Similar results to those with the 20 m*M* sodium acetate and 20 m*M* potassium phosphate buffers shown in

Fig. 2, were obtained with 20 m*M* sodium acetate buffer, pH 5.5. One characteristic of the agglutinin peaks was that they were often significantly broader than those of the standard proteins used to calibrate the system. The column efficiencies calculated for bovine serum albumin, ovalbumin and the two agglutinins in the different buffers are given in Table II, and are calculated from the formula 5.54 $(t_R/W_0)^2$, where t_R is the retention, and W_0 the width at half-height.

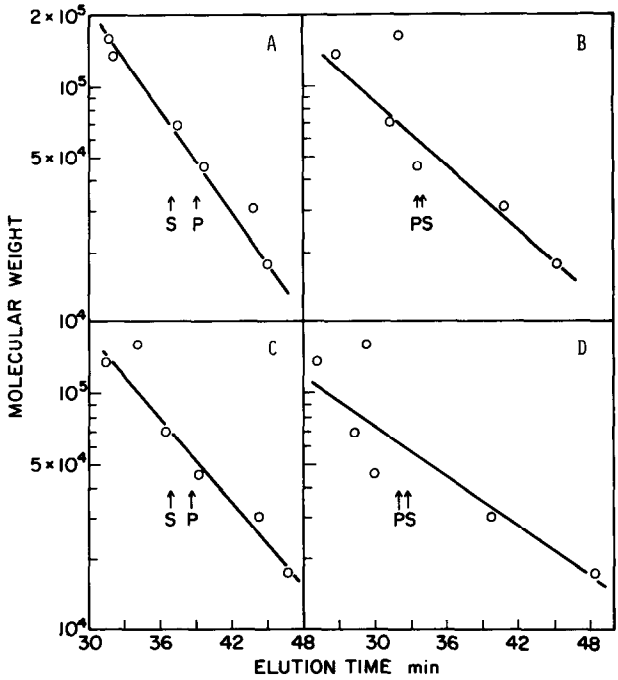

Fig. 2. Size-exclusion chromatography on TSK 3000-SW in various buffers. The standard proteins (○) from top to bottom in each calibration are IgG (160,000), bovine serum albumin dimer (136,000), bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (30,000) and myoglobin (17,000). The elution positions of soybean (S) and peanut (P) agglutinins are indicated by arrows. The buffers were (A) 0.2 *M* potassium phosphate, pH 7.0; (B) 0.02 *M* potassium phosphate, pH 7.0; (C) 0.1 *M* sodium acetate, pH 5.6; (D) 0.02 *M* sodium acetate, pH 6.5.

TABLE II

EFFICIENCIES OF THE TSK 3000-SW COLUMN MEASURED WITH VARIOUS PROTEINS AND BUFFERS

Protein	Column efficiency (theoretical plates)				
	0.2 *M* phosphate, pH 7.0	0.02 *M* phosphate, pH 7.0	0.02 *M* acetate, pH 6.5	0.1 *M* acetate, pH 5	0.02 *M* acetate, pH 5
Bovin serum albumin	7900	4700	4600	9200	5500
Ovalbumin	6100	4300	5800	7800	3700
Peanut agglutinin	4800	1900	600	4200	800
Soybean agglutinin	6200	2800	1200	6200	2300

DISCUSSION

The gel electrophoresis experiments showed that (a) the range of molecular weights for lectin subunits is much narrower than current literature values (Table I) suggest, and (b) the subunit weights can be significantly overestimated by these methods, when standard proteins such as carbonic anhydrase are used rather than lectins such as concanavalin A. With the exception of the *B. purpurea* lectin, all the lectins ran close to the positions of soybean agglutinin and concanavalin A, whose amino acid sequences [12, 13] give mol.wt. of 27,600 and 25,600, respectively. Thus estimates of 30,000 or above for some of these lectins (Table I) are probably 10—20% too high. Values for the subunit molecular weights of the *B. purpurea* and *Cy. sessilifolius* lectins have not been previously reported; they are 32,000 and 28,000, respectively. The oligomeric form of *B. purpurea* lectin may therefore be different from that of other lectins since the mol.wt. of 195,000 obtained by ultracentrifugation [16] would be consistent with a hexameric structure, though the HPLC value is consistent with a tetramer.

The average mol.wt. for the various lectins is approx. 27,000, giving a tetramer weight of 108,000. Several lectins, including the well characterized soybean and peanut agglutinins, had substantially lower apparent molecular weights. Therefore if TSK 3000-SW is used to determine lectin molecular form, an apparent mol.wt. of 100,000 or more may reliably indicate a tetrameric structure, but an apparent mol.wt. of 50,000—60,000 cannot be taken as evidence for a dimeric structure. The peak width may also be an indicator of aberrant behavior, as the data of Table II show.

Experiments conducted with various buffers for peanut and soybean agglutinins did not suggest a mechanism for their late elution. These two proteins have been well characterized by ultracentrifuge experiments and they are clearly tetramers [24,26,27]. Also, their behavior on Sephadex G-200 was consistent with their molecular weight [24, 26]. Their behavior on TSK 3000-SW was not improved by changing to a pH further from their isoelectric points, nor by lowering the ionic strength to reduce hydrophobic interactions. Adding the specific sugar inhibitors had little effect, hence interactions of their combining-sites with hydroxyl groups on the packing is not significant. Dissociation of the lectin tetramers into dimers might be occurring but the elution position of soybean agglutinin was not changed over a 40-fold range of sample load, including amounts equivalent to those used in the Sephadex G-200 experiments of Lotan et al. [26].

Since even members of one protein family behaved differently in these experiments, it is evident that molecular weight estimates obtained by size-exclusion HPLC must be viewed with caution, particularly when dealing with oligomeric proteins, and preferably be confirmed by gel chromatography on Sephadex or similar media.

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