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Size and Solution Properties of Globular *tert*-Butyloxycarbonyl-poly(α,ϵ-L-lysine)

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ABSTRACT: Globular poly(α,ϵ-L-lysine) macromolecules protected on their surface by *tert*-butyloxycarbonyl residues were studied in dilute solution by means of viscosity determinations, photon correlation spectroscopy (PCS), and size exclusion chromatography (SEC). The three techniques yielded the following results: the homologues double their molecular weight with each incremented step, they are monodisperse, and the globular macromolecules are dense and behave as nondraining spheres. The sizes determined by viscosity and PCS are in agreement. In SEC they correlate well with the size of nondraining globular biopolymers. Globular poly(α,ϵ-L-lysine) macromolecules are thus suitable for use as molecular size "markers" in dilute solution studies of other polymers.

Introduction

Dense globular macromolecules of well-defined molecular weights, all with molecular weight distributions of $M_w/M_n = 1.0$, are usually biological in nature and obtained by extraction from living organisms. These biopolymers are usually soluble only in aqueous media. Furthermore, nature does not furnish us with globular macromolecules whose molecular weights and sizes change in uniform increments, making them suitable for studies of solution properties of dense globular macromolecules.

Denkewalter et al.[1] recently synthesized in our laboratories a series of *tert*-butyloxycarbonyl-blocked poly(α,ϵ-L-lysine) macromolecules. Models indicated them to be globular and dense. The molecular weight of each member of the series is twice that of the previous member. For each homologue the molecular weight distribution is extremely narrow, with $M_w/M_n \simeq 1.0$.

A detailed description of the synthetic procedure is presented in ref 1. The highlights of the procedure are summarized below. The nucleus of each globular macromolecule was *N*,*N*′-bis(*tert*-butyloxycarbonyl)-L-lysine benzhydrylamide. In reactions building on this nucleus, the nitrophenyl ester derivative of *N*,*N*′-bis(*tert*-butyloxycarbonyl)-L-lysine was found to be the most convenient reactive monomer. At each step, the Boc-protected product was deblocked in trifluoroacetic acid in dichloromethane, followed by the removal of excess acid and solvent under nitrogen. The resulting product was dissolved in dimethylformamide (DMF) and neutralized with triethylamine. A twofold molar excess (based on the calculated number of amino groups present) of Boc-L-NPE was added. The reaction mixture was then kept slightly basic by gradual addition of triethylamine. The reaction was allowed to proceed for 18 h in the case of the low molecular weight homologues and up to 3 days for the high molecular weight members of the series. The Boc-protected products were precipitated with ether, which allowed for the removal of excess reagents and byproducts. The Boc-protected products were then washed with acetonitrile and readied for the next synthetic step. For brevity, each member of the *tert*-butyloxycarbonyl-protected poly(α,ϵ-L-lysine) series was denoted by a symbol Boc-X. Thus the nucleus of each globular macromolecule was denoted by Boc-A, the second-stage product by Boc-B, the third-stage product by Boc-C, etc.

Because the Boc-X series was synthesized in a stepwise fashion with Boc-protected monomers, each member was expected to have a monodisperse molecular weight. Also, because of the amidation of both amino groups of each lysine residue, the products are highly branched and, thus, are supposed to have globular shape. Finally, because these globular molecules are protected on their surface by Boc residues, they were expected to be soluble in nonaqueous solvents capable of dissolving polyamides. Deblocking the Boc residues renders the globular molecules water soluble.

One purpose of this work was to measure the size of the members of the Boc-X series and attempt to demonstrate their usefulness as a series of molecular size "markers" of known and predetermined size and size increments, to be used in conjunction with other polymeric structures whose size is to be accurately determined.

Another purpose of this work was to determine the compactness of the Boc-X macromolecules. It is well-known[3] that the dilute solution viscosity and translational diffusion properties of random coil macromolecules are congruent with predictions based on the "free-draining" model. Accordingly, only a small fraction of the volume encompassed by the coiled macromolecule in solution is taken up by it; the rest is filled with solvent. The segments of the macromolecule are idealized as minute hard spheres connected by volumeless springs. The hard spheres perturb the flow of solvent around and through the macromolecular coil. However, the solvent is not retained within the coil and can freely "drain" out of it and be replaced by other solvent molecules.

When the macromolecule is very dense and strongly deviates from a Gaussian distribution of its segments, it may prevent solvent molecules from easily passing through its encompassed volume and hinder the draining of solvent out of it. The model describing the extreme case of such a behavior is termed the "nondraining" model. In this case the whole macromolecule, and not its submolecular segments, behaves as a hard sphere. It is obvious that the dilute solution behavior of macromolecules conforming to the two extreme models will be different from one another. It is, naturally, expected that a gradual transition from the freely draining to the nondraining behavior will be associated with a gradual change in the viscosity, diffusion, and other functional properties of macromolecules in dilute solution. An excellent discussion of the subject is given by Yamakawa.[3] Accordingly, for the extreme case of free-draining behavior, the intrinsic viscosity $[\eta]$ is given by

$$[\eta] = N_A \zeta n \langle S^2 \rangle_0 / 6\eta_0 M \tag{1}$$

Here, N_A is Avogadro's number, ζ is the frictional coefficient of each of the n segments comprising the macromolecule having molecular weight M, $\langle S^2 \rangle_0$ is the mean-square unperturbed radius of gyration, and η_0 is the viscosity of the solvent. The translational friction coefficient f of the freely draining macromolecule is simply the sum of the friction coefficients of its segments

$$f = n\zeta \tag{2}$$

and the translational diffusion coefficient D of such a macromolecule is

$$D = k_B T / n\zeta \tag{3}$$

where k_B and T retain their usual meanings.

At the other extreme, that of nondraining behavior, the intrinsic viscosity is given[3] by

$$[\eta] = 10\pi N_A R^3 / 3M \tag{4}$$

where R is the radius of the nondraining hard sphere. The translational friction coefficient f of the nondraining macromolecule is

$$f = (9\pi^{3/2}/4)\eta_0 \langle S^2 \rangle_0^{1/2} \tag{5}$$

and its translational diffusion coefficient is

$$D = 0.192 k_B T / \eta_0 \langle R^2 \rangle_0^{1/2} \tag{6}$$

where $\langle R^2 \rangle_0$ is the unperturbed mean-square end-to-end distance of the polymeric chain. It should be recognized that in the case of a nondraining molecule, the root-mean-square end-to-end distance can be approximated by twice the radius R. Thus, eq 6 can be recast as

$$D = 0.096 k_B T / \eta_0 R \tag{7}$$

Obviously, a test of the compactness of the Boc-X macromolecules will be their adherence to the nondraining model expectations. Conversely, if the Boc-X molecules are not compact and dense, the volume encompassed by each will contain a large fraction of solvent, and the macromolecules will behave as if they are approaching freely draining behavior.

Experimental Section

Polymer samples, prepared as briefly described in the previous section, were supplied by Denkewalter and co-workers.[1] Anionically polymerized polystyrene of molecular weight $M = 2100, 4000, 20\,000$, and $51\,000$ and narrow molecular weight distribution, $M_w/M_n \leq 1.10$, was obtained from Pressure Chemical Co. A sample of polystyrene of $M \simeq 22\,000$ having a rather broad molecular weight distribution was obtained from Aldrich Chemical Co. The dense spherical biopolymers catalase, hemoglobin, and β-lactoglobulin were obtained from Sigma Chemical Co., catalog no. C-40, H-2500, and L-0130, respectively. Dimethylformamide (DMF), DMF containing 1% by weight LiCl, and water were the solvents used for visocosity, photon correlation spectroscopy, and size exclusion chromatography measurements. For the last two techniques the solvents were carefully filtered through 150- and 200-nm pore size Schleicher and Schuel membranes (designated RC-57 and RC-58, respectively) to remove dust particles.

Dilute solution viscosity measurements of members of the Boc-X family and the polystyrene homologues were obtained with a Nametre direct-readout viscometer. From the instrumental readout and knowledge of the solutions' (or solvents') densities, the absolute viscosity in centipoise is obtained. In addition to measurements of absolute viscosity, relative viscosities of several Boc-X samples were obtained from comparison of efflux times of solution and solvent in Cannon-Ubbelohde dilution viscometers. In these viscometers solvent efflux time was of the order of 200 s, eliminating kinetic effects and making it possible to obtain a reasonable measure of precision. The reduced viscosity values calculated from data obtained by both methods were in reasonably good agreement with each other.

Viscosity measurements were performed in pure DMF and in DMF/1% LiCl. The measured solvent viscosities, η_0, were 0.784 and 1.051 cP, respectively. Solution densities were calculated from the density of DMF (0.944 g/cm^3), the density of LiCl (2.068 g/cm^3), and the density determined for several of the intermediate Boc-X solid samples (1.18 ± 0.01 g/cm^3) by means of Beckman gas comparison and pycnometry techniques. The volume additivity assumed in these calculations was not verified, however. Absolute viscosity values were converted to reduced viscosities, η_{sp}/c, by means of the common relationship

$$(\eta - \eta_0)/\eta_0 c = \eta_{sp}/c$$

in which η is the solution viscosity and c the polymer concentration.

Size exclusion chromatography (SEC) was performed in a Waters Associates ALC/GPC Model 201 chromatographic apparatus fitted with three DuPont porous silica columns designated SE1000, SE500, and SE100. All runs were conducted at ambient temperature. To limit variations of chromatogram broadening[4] to those arising from the polymeric samples themselves, all solutions were prepared at a concentration of 0.5% (w/v) and injection volumes of 60 μL were used for all samples.

Samples Boc-E, Boc-G, and Boc-K were selected for determination of their translational diffusion coefficient D by means

Table I
Number of Lysine Residues, Molecular Weight, and Radius of Globular Boc-Poly(α,ϵ-L-lysine)

code	stage	no. of Lys residues	calcd mol wt	R, nm
Boc-A	nucleus	1	511	0.59
Boc-B	2	3	967	0.73
Boc-C	3	7	1 900	0.91
Boc-D	4	15	3 700	1.14
Boc-E	5	31	7 300	1.43
Boc-F	6	63	14 600	1.80
Boc-G	7	127	29 200	2.26
Boc-H	8	255	58 400	2.85
Boc-I	9	511	116 800	3.59
Boc-K	10	1023	233 600	4.53

of photon correlation spectroscopy (PCS). Measurements were conducted for all three samples in DMF/1% LiCl and for Boc-K only in pure DMF. Stock solutions were prepared at least 24 h in advance, and other concentrations were obtained by dilution. Clarification of the solutions was accomplished with 150-nm Schleicher and Schuel filters. Scattering cells were 10-mm cylindrical cuvettes (Precision Cells) and were carefully cleaned with dust-free water and dried before use. The PCS apparatus is the same as described previously,[5] except that the autocorrelation functions were collected at a finite angle. Data for Boc-G and Boc-K were taken at a 90° scattering angle, and for Boc-E at 40°. No polarizers or analyzers were used in the collection optics. The solution temperature was maintained at 20 ● 0.1 °C.

The average and normalized variances, $\bar{\Gamma}$ and $\mu_2/\bar{\Gamma}^2$, respectively, of the autocorrelation function decay rate Γ were determined by using the cumulants method.[6,7] The PCS autocorrelation function $G(\tau)$ has the form

$$G(\tau) = A + B \exp(-2\Gamma\tau) \qquad (8)$$

where A is a measured base line, B is the amplitude of the signal, τ is the time per channel, and Γ is the decay rate of the exponential term. D is related to Γ by

$$\Gamma = DK^2 \qquad (9)$$

in which the magnitude of the scattering vector K is given by

$$K = (4\pi n/\lambda) \sin (\theta/2) \qquad (10)$$

where θ is the scattering angle, n is the refractive index of the scattering medium, and λ is the wavelength of the incident light in a vacuum.

Ignoring higher terms, the cumulants expansion gives

$$\tfrac{1}{2} \ln [(G(\tau) - A)/(G(0) - A)] = -\bar{\Gamma}\tau + (\mu_2/2)\tau^2 \qquad (11)$$

where $G(0)$ is the value of $G(\tau)$ at $\tau = 0$ and μ_2 is the variance of Γ about $\bar{\Gamma}$. Since $G(0)$ is inaccessible for real correlation functions, the value of $G(\tau)$ at the second channel was used in approximating the amplitude $B = G(0) - A$. The values of Γ were converted to D by using eq 9, and the values of μ_2 were normalized by dividing by $\bar{\Gamma}^2$. The normalized variance $\mu_2/\bar{\Gamma}^2$ was less than 0.05 for Boc-E and Boc-G and 0.10 ± 0.05 for Boc-K. These small values of $\mu_2/\bar{\Gamma}^2$ are suggestive of essentially monodisperse material.

Results and Discussion

Samples of *tert*-butyloxycarbonyl-blocked poly(α,ϵ-L-lysine) were prepared by Denkewalter, Kolc, and Lukasavage[1] and are described in Table I. In each macromolecule the number of lysine residues is $2^n - 1$, where n is the stage of preparation. From these samples, all but Boc-F and Boc-I were obtained and studied by us.

Viscosity results for all available Boc-X samples were remarkably similar; typical ones are presented in Figure 1. Here, the upper curves represent the reduced viscosities as measured in pure DMF, and the lower curves represent the reduced viscosities measured in DMF/1% LiCl. In pure DMF, there is no apparent relationship between the sequence of Boc-X members and the relative position of their reduced viscosity curves. Thus, the reasons for the scatter within this group of reduced viscosities are not known at present. The strong upward curvature with dilution of the reduced visocity values in pure DMF, and its suppression in the presence of LiCl, suggests the presence of polyelectrolyte effects. Similar suppression of the increase of reduced viscosity values was obtained by the addition of up to 4% (saturation) urea to the DMF. The nature of the specific interactions responsible for the polyelectrolyte effect is not known at present.

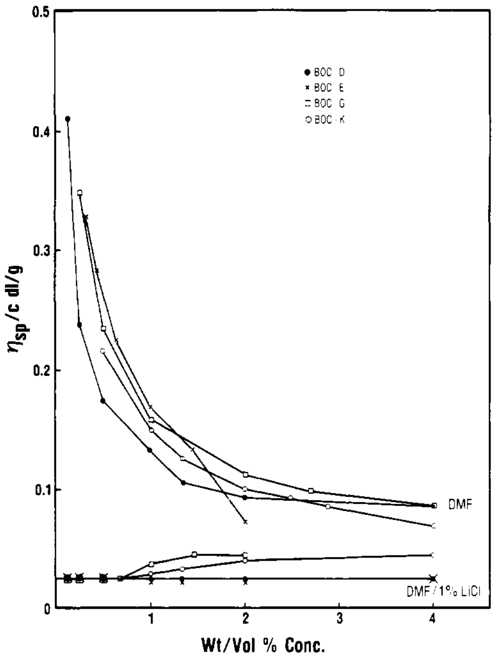

Figure 1. Typical reduced viscosity vs. concentration curves of Boc-X in DMF (top group) and in DMF/1% LiCl (bottom group).

Table II
Translational Diffusion Coefficients and Hydrodynamic Radius in DMF/1% LiCl

		R_H, nm	
sample	$D \times 10^7$, cm^2/s	non-draining	draining
Boc-E	7.619 ± 0.030	1.5	2.7
Boc-G	5.348 ± 0.028	2.1	3.8
Boc-K	2.581 ± 0.024	4.4	7.9

The reduced viscosity values in DMF/1% LiCl for all Boc-X homologues are remarkably low. The low molecular weight members Boc-A, Boc-B, Boc-C, Boc-D, and Boc-E maintain a value of $\eta_{sp}/c = 0.025$ dL/g over the full concentration range measured (0.125–4%). The higher molecular weight members Boc-G, Boc-H, and Boc-K maintain a value of $\eta_{sp}/c = 0.025$ in concentrations lower than 0.75%, with a slight divergence at concentrations of 1% and over. The small increase in η_{sp}/c of the higher Boc-X members at high concentrations in DMF/1% LiCl is not understood yet.

As is obvious from Figure 1, the intrinsic viscosity of all Boc-X members in DMF/1% LiCl is 0.025 dL/g or 2.5 cm^3/g. This indicates that Boc-X homologues in DMF/1% LiCl behave as hard, impenetrable spheres. From the relationship in eq 4, the radius R of the nondraining hard spheres equivalent to the Boc-X globules can be calculated. The results are tabulated in Table I. Because all $[\eta]$ values in DMF/1% LiCl were 2.5 cm^3/g, the R values of Boc-F and Boc-I, which were not available for direct measurement, are added for completeness.

The results of the photon correlation spectroscopy are presented in Table II. The errors in the diffusion coef-

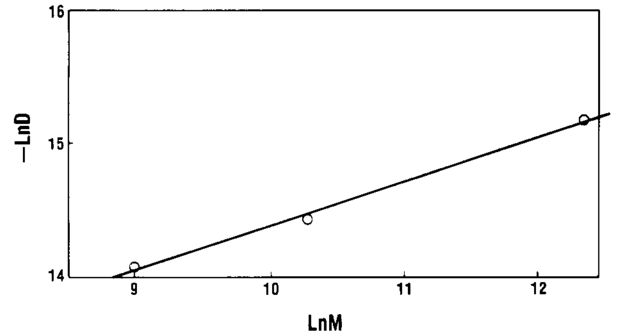

Figure 2. Logarithmic plot of D vs. M for three Boc-X samples in DMF/1% LiCl.

ficients are standard deviations of the mean value of 8–10 measurements per sample. For each sample two values of the hydrodynamic radius are reported. In the third column, the values calculated for nondraining macromolecules according to eq 7 are given and in the fourth column, the values calculated for the freely draining macromolecule according to

$$D = k_B T / 6\pi\eta_0 R_H \tag{12}$$

which is formally the same[3] as eq 3, are shown.

In Figure 2 a double-logarithmic plot of the translational diffusion coefficient as a function of the molecular weight M is presented. The slope, a, of the straight line connecting the data points of Boc-E, Boc-G, and Boc-K is 0.32. This indicates an empirical relation for Boc-X in DMF/1% LiCl of

$$D = kM^{-0.32} \tag{13}$$

in which k is a proportionality constant. It should be recognized that for the nondraining case the hydrodynamic radius R_H is equivalent to the radius R of the sphere. A comparison of the nondraining R_H values in Table I with the corresponding R values obtained from visocsity measurements in Table I shows an excellent agreement between the corresponding values. This indicates that the Boc-X homologues behave in DMF/1% LiCl as nondraining spheres and cannot be represented by freely draining or partly draining models.

According to Vollmert[8]

$$D = kM^{-1/3}\rho_{\text{coil}}^{1/3} \tag{14}$$

where ρ_{coil} is the average macromolecular coil density. It is obvious that in our measurements $\rho_{\text{coil}}^{1/3}$ must be unity or very close to it, rendering eq 13 and 14 essentially the same. A value of $\rho_{\text{coil}}^{1/3} \simeq 1 = \rho_{\text{coil}}$ reflects the strongly nondraining nature of the Boc-X homologues in DMF/1% LiCl and is in agreement with the viscosity measurements of $[\eta] = 2.5$ cm^3/g. It is of interest to note that for the freely draining hydrodynamic radii, shown in column four of Table II, the average macromolecular coil density would have been only 17% of that of the nondraining case. It is highly improbable that hydrodynamic spheres containing 83% of their volume as solvent will behave in the highly nondraining manner exhibited in both viscosity and PCS measurements in DMF/1% LiCl.

The translational diffusion coefficient D of the sample Boc-K was measured by PCS also in pure DMF. As in the DMF/1% LiCl solvent, the D value showed a remarkable concentration independence of the measured range of concentration. The result of about 35 individual measurements at four different concentrations (0.5, 0.3, 0.2, and 0.1%) is $D = (2.940 \pm 0.059) \times 10^{-7}$ cm^2/s, yielding a nondraining radius of $R_H = R = 4.86$ nm. The concentration independence of the D values of Boc-K in DMF and the fact that its radius is only 10% larger than in DMF/1% LiCl indicate that even in pure DMF a substantial amount of the nondraining character of Boc-K is retained by the globular macromolecule. The insensitivity of D to concentration appears to contradict the increase in η_{sp}/c of the same sample upon dilution in DMF. The viscosity measurements were repeated twice and yielded in both cases similar sharp increases in η_{sp}/c, especially in the concentration rate $c \leq 0.3\%$, amply covered in the PCS measurements. We do not understand the difference of the results of viscosity and PCS of Boc-K in pure DMF.

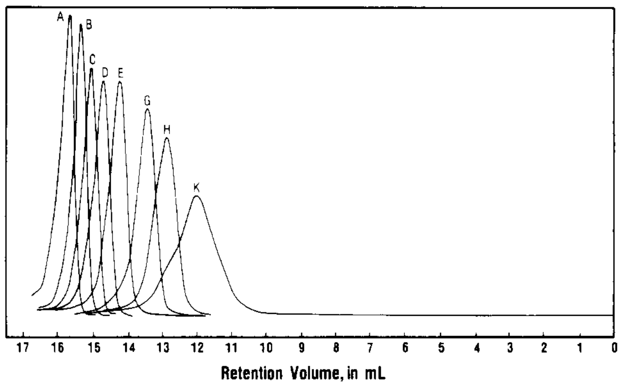

Figure 3. Composite of the size exclusion chromatograms of all available Boc-X samples in DMF/1% LiCl.

Table III
SEC Retention Volumes in DMF/1% LiCl

sample	V, mL	Δ, mL	M^a	$[\eta]$, cm^3/g
Boc-A	15.75		511	2.5
Boc-B	15.40	0.35	967	2.5
Boc-C	15.08	0.32	1 900	2.5
Boc-D	14.70	0.38	3 700	2.5
Boc-E	14.28	0.42	7 300	2.5
Boc-F	unavailable		14 600	
Boc-G	13.43	0.95	29 200	2.5
Boc-H	12.90	0.53	58 400	2.5
Boc-I	unavailable		116 800	
Boc-K	12.05	0.85 0.42 ± 0.07^b	233 600	2.5
polystyrene	15.15		2 100	4.2
polystyrene	14.78		4 000	5.0
polystyrene	13.30		20 000	7.0
polystyrene	12.25		51 000	11.7

[a] Molecular weights of Boc-X from Table I. [b] Average Δ per increment.

Size exclusion chromatography results were obtained from all available Boc-X homologues in DMF/1% LiCl. Figure 3 shows a composite of the GPC instrument traces. All were obtained under identical conditions. Retention volumes corresponding to the peak positions are given in Table III together with the difference, Δ, in milliliters between the retention volumes. Values obtained for several narrow-distribution polystyrene "markers" in DMF/1% LiCl are also included. The intrinsic viscosities of the polystyrene samples in DMF/1% LiCl are tabulated in Table III. The data in Table III are instructive. According to them, the doubling of the molecular weight of Boc-X homologues is associated with linear and about equal incremental decreases in the corresponding retention volumes, indicating a relationship of the form

$$\log M \sim V \tag{15}$$

with V being the retention volume. The values of V and M shown in Table III for Boc-X and polystyrene homo-

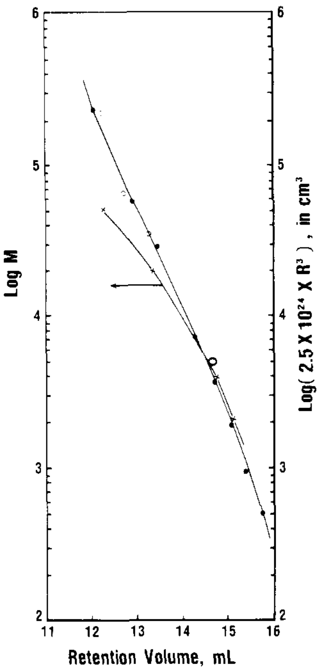

Figure 4. Semilogarithmic plot of SEC retention volumes vs. M and R^3. Boc-X (●) and three globular biopolymers (○) samples are plotted against both M and R^3. The polystyrene samples (×) are plotted against M only.

logues are plotted semilogarithmically in Figure 4. The points of the Boc-X homologues follow a relatively straight line, similar in shape to universal GPC plots of log $M[\eta]$ vs. retention volume.[9] This is understandable since the intrinsic viscosity of all Boc-X samples is 2.5 cm^3/g and multiplication of M by a constant will merely displace the curve parallel to the logarithmic axis and not change its shape. The curve for the polystyrene samples increasingly deviates with M from the Boc-X curve, reflecting the fact that polystyrene retains its draining nature in DMF/1% LiCl. When multiplied by the intrinsic viscosities, the points of the polystyrene samples define a curve similar in shape and slope to the Boc-X curve but significantly displaced upward along the log axis.

When plotted against M on a log-log scale, the $[\eta]$ points of the three lower polystyrene homologues fall on a straight line of defining slope $a = 0.50$. The intrinsic viscosity of a polystyrene sample of $M = 20\,000$ having a broad molecular weight distribution ($[\eta] = 7.2$ cm^3/g) falls on the same straight line

$$[\eta] = KM^{0.50} \tag{16}$$

From this relationship it is apparent that at least the lower molecular weight samples are at or very close to their unperturbed size. Calculations based on unperturbed dimensions in the literature[10] yield, for example, a hydrodynamic radius for the polystyrene sample of $M = 51\,000$ about twice the size of Boc-H with $M = 58\,400$. Thus, the smaller retention volumes of the higher polystyrene homologues are explained by their hydrodynamic volumes being substantially larger than the corresponding volumes of Boc-X homologues.

After the SEC determinations in DMF/1% LiCl were completed, the columns of the GPC instrument were carefully washed with water, and three globular nondraining[11] biopolymers were chromatographed in the form of a 0.5% solution in water/1% urea. From DMF impurity markers in the water/urea solutions, it was found that the retention volumes of solutions in water/urea were 3.25 times larger than in DMF/1% LiCl. When the retention volumes in water/1% urea of globular catalase, hemoglobin, and β-lactoglobulin were divided by 3.25 and the resulting values plotted in Figure 4, the data points fell on the Boc-X curve. The radius of the catalase sphere ($M = 225\,000$) is 5.1 nm, and that of hemoglobin ($M = 68\,000$) is 2.6 nm.[11] Comparison with the data in Table II indicates that the molecular weight-size relationship of the biopolymers is essentially the same as that of the Boc-X family. The good fit of the (corrected for solvent exchange) SEC data of the biopolymers to the curve of the Boc-X homologues merely emphasizes the similar hydrodynamic behavior of both groups.

The fact, clearly observable from Tables I and II, that for the nondraining dense sphere of Boc-X there exists a linear relationship between M and R^3 allowed us to add a size scale on the right-hand side of Figure 4. Thus, for the nondraining case where $[\eta] = KM^0$, with K being 2.5 cm^3/g or slightly larger, a direct relationship is realized between the SEC retention volume and the macromolecular size.

Thus, it is clear that the well-defined size of the Boc-X homologues in Tables I and II in conjunction with their nondraining nature makes them suitable for use as size markers for studies such as SEC and PCS.

Finally, let us return to Figure 3. There we observe that the chromatogram width at its base line is very small for Boc-A and Boc-B and gradually increases toward Boc-K. We would first like to emphasize that the base line widths of all Boc-X samples, except Boc-K, are smaller than those shown by the four polystyrene samples obtained under identical conditions. This difference in broadening is thought to be due to the monodispersity of Boc-A through Boc-H, as compared with the small, yet present, polydispersity of the polystyrene samples, all with $M_w/M_n \leq 1.10$. The increased width of the Boc-K peak is associated, we believe, with the onset of an incomplete polymerization reaction on the surface of the sterically very hindered sphere of the precursor Boc-I.

The areas under the elution peaks were measured and were found to be about the same, with a ±10% scatter. The decrease in peak height is in accord with the peak broadening, maintaining a uniform peak area. The maintenance of a constant peak area is expected, since the detector is sensitive to the concentration of solute in solution and all injected samples contained the same amount of polymer. However, the reason for the gradual change in peak shape is not understood by us at present.

Conclusions

Size exclusion chromatography indicates that the molecular weight of members of the Boc-X family of homologues doubles with each successive step, in agreement with expectation. For each homologue the molecular weight is essentially monodisperse.

Viscosity and photon correlation spectroscopy measurements in DMF/1% LiCl reveal the Boc-X homologues to behave as nondraining spheres. The diameters of the spheres were obtained from both techniques and were in excellent agreement. The nondraining character was ascertained from the viscosity behavior and from the molecular weight-translational diffusion coefficient relationship.

The behavior of Boc-X homologue in SEC determinations was similar to that of three nondraining globular biopolymers used as markers.

Thus, the Boc-X homologues (and their deblocked analogues) may be useful as molecular size markers in chromatography and/or spectroscopy of unknown low to moderate molecular weight polymers in certain organic solvents. Their density and nondraining nature minimize size variability with changes in solvent quality.

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Lignin. 19. Kraft Lignin Component Conformation and Associated Complex Configuration in Aqueous Alkaline Solution[†]

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ABSTRACT: Discrete kraft lignin components behave ideally when eluted with 0.10 M aqueous NaOH from a cross-linked dextran gel: fractions with apparent polydispersity indices of 1.04 ± 0.01 are described by elution profiles that are close to a Gaussian shape. Of those selected for the present study, the weight-average molecular weights ($\bar{M}_w$) in aqueous solution at pH 9.5, ionic strength 0.10 M, were found by ultracentrifuge sedimentation equilibrium to vary between 1680 and ~140 000. On the basis of a logarithmic-normal component distribution, the polydispersities of the fractions during chromatographic elution have been compared with those observed under the conditions imposed for the absolute molecular weight determinations. Below the excluded limit for the gel, the plot of log $\bar{M}_w$ vs. column retention volume is linear and parallel to that for poly(styrenesulfonates). The kraft lignin components adopt an appreciably expanded random coil conformation in aqueous alkaline solution; there is no indication of a hydrodynamic effect arising from long-chain branching. The hydrodynamic behavior, on the other hand, of associated kraft lignin complexes is in accord with a flexible lamellar configuration, which is distinct from the conformation of the discrete components.

Introduction

It has been established that one of the most prominent physicochemical properties of lignin components is a pronounced tendency to form high molecular weight associated complexes.[1–4] Such effects dominate the colligative behavior of lignin samples under a variety of conditions ranging from nonaqueous[3] to aqueous alkaline solutions.[4] Furthermore, association, a reversible phenomenon between lignin components, can be complicated by nonreversible aggregation between the resulting complexes.[5–7]

Among lignin derivatives, the most abundant are the industrial byproducts formed under the relatively severe degradative conditions encountered during the kraft pulping process (typically at 170 °C for 5 h in aqueous solution containing 45 g L^{-1} NaOH and 12 g L^{-1} Na_2S). These kraft lignins are thought to have undergone significant structural modifications compared with the native polymer, a view based largely upon appropriate model compound studies[8] that have been partly confirmed by the structures of monomeric and dimeric components identified as being present in the spent liquor.[9]

Size-exclusion chromatography of kraft lignin samples using cross-linked dextran (Sephadex G series) gels with 0.10 M aqueous NaOH generates profiles that represent molecular weight distributions generally within an order of magnitude of those for the discrete components.[3,4] Appreciable variations may be expected in the effective volumes occupied by associated complexes and individual components of the same molecular weight. These may affect the accuracy with which a single calibration curve can be used to characterize the elution profiles of lignin samples differing only in their degree of association. The present work was directed toward investigating the hy-

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[†] All experimental work was conducted at the University of Washington. The analysis of the results was developed by S.S. after transferring to his present position at the University of Minnesota, where the manuscript was also mainly written.

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