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Molecular and Structural Characteristics of Lactodendrimers Based on Poly(amidoamine)

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Abstract—Six generations of lactodendrimers based on poly(amidoamine) were studied by the methods of hydrodynamics (velocity sedimentation) and optics (static and dynamic light scattering). The experimental evidence was analyzed and compared with the published data. The hydrodynamic invariant and average density of a substance in a dendritic molecule were estimated. A volume occupied by lacto groups was evaluated, and it was found that these groups experience no steric constraints in the molecules of lactodendrimers.

Dendritic molecules (molecular trees) have been extensively studied during the past decades [1–6]. The regular molecular structure of such macromolecules provides for their use in various fields of supramolecular chemistry, catalysis, surface phenomena, life science, and materials technology. Hyperbranched dendritic systems prepared via a one-step process [2, 3] are closely related to regular dendritic molecules. Amylopectin, glycogen, and lignin are the examples of naturally occurring hyperbranched dendrimers.

In recent years, the chemical modification of the parent dendritic molecules has attracted much attention [5–8]. This modification involves the attachment of functional groups to the core of a dendritic molecule which ensures the concentration of desired properties in a limited volume. The chemical nature of attached groups is, as a rule, different from the chemical composition of the core; as a result, hybrid molecules are synthesized; copolymers may be considered as the linear analogs of the resulting compounds. These molecules exhibit peculiar properties, which are the subject of intense investigations [5, 6]. Considerable attention has been given to glycodendrimers [7, 8], which provide an opportunity to model and assess glycoprotein interactions. These studies are motivated by the necessity to better understand the role of sugar-containing molecules in living systems. Glycodendrimers are also employed as hydrophilic molecular systems useful for the preparation and modification of medicines.

In addition, dendrimers offer an interesting object for molecular physics. However, dendrimers and their properties have been studied insufficiently.

In this work, we are concerned with new experimental data on the molecular parameters of poly(amidoamine)-based lactodendrimers whose structural formula was reported in [9]. These data are compared with the results obtained previously for lactodendrimers based on poly(amidoamine) [9, 10] and poly(propyleneimine) [11, 12].

EXPERIMENTAL

Dynamic light scattering from solutions was studied on a DynaPro-801TC photometer (Protein Solutions, Inc.) equipped with a laser operating at $\lambda = 780$ nm. A fixed scattering angle was 90°. This instrument allowed measurements of translational diffusion coefficients D for globular proteins and molecules with a shape close to spherical [13]. The scattered photons were counted using a system of cascade photodiodes. A membrane filter injected through a Whatman Anotop 10 had a pore diameter of 0.02 μm and a volume of 150 μl. The solution concentration was of the order of 10^{-2} g/cm^3.

The time of signal accumulation for one reading depends on the sizes of molecules and on the difference between the refractive indexes of a polymer and a solvent and varies within 2–25 min. For the solutions of lactodendrimers in 0.019 M (0.165%) NaCl, it was found that the measurements might be performed only for the sample of the highest generation. Obviously, this is due to the effect of incompletely shielded

charges and to the appearance of short-lived clusters of dendritic molecules. An increase in the concentration of the low-molecular-mass salt to 1.169% (~0.2 M) enabled us to solve this problem and to measure the diffusion coefficients of all generations (Fig. 1). In further experiments, 0.2 M NaCl with the following characteristics (at 25°C): $\rho_0 = 1.002$ g/cm^3 and $\eta_0 = 0.914$ cP was used as a solvent.

The dendrimers of various generations were also studied by gel-permeation chromatography (GPC) by means of a three-detector registration [14] (Fig. 2). An Optilab 901 interference refractometer (Wyatt Technology, Santa Barbara, CA, USA) was used as a refractometric detector. A detector of static scattered radiation of a 5-mW He–Nr laser with λ = 632.28 nm (Wyatt Technology, Santa Barbara, CA, USA) was used in the scattered light measurements. Viscometric detection was performed with the aid of a viscometric detector of the hydrodynamic bridge type (analogous to the Winston electrical circuit) [15, 16]. The viscometric detector allows for differences in the viscosities of a solution and a solvent to be detected at the lowest concentrations and $(\eta - \eta_0)/\eta_0 c$ (or $\ln\eta_r/c$) may be taken as the intrinsic viscosity $[\eta]$. An injected volume (100 μl) passes during measurements through a series of three analytical columns TSK G6000PW, G5000PW, and G4000PW (Anachem, Luton, UK). The column resolution ensured separation of poly(ethylene oxide) macromolecules with M ranging from 1×10^3 to 2×10^6. An eluent was fed in a system at a rate of 0.8 ml/min at room temperature. The refractive index increments dn/dc were determined in independent experiments using an Optilab 901 interference refractometer.

The sedimentation coefficients were determined with a Beckman XLI analytical ultracentrifuge operating in an absorption mode (λ = 280 nm) (Fig. 3). The dendrimer solution concentrations were not above $c = 0.01 \times 10^{-2}$ g/cm^3; as a result, the obtained sedimentation coefficients can be taken as values extrapolated to the zero concentration ($c[\eta] < 0.0004$). The sedimentation coefficients s were calculated by a shift with time of the middle point of a tangent to the sedimentation curve enclosed by the extension of straight lines corresponding to $c = 0$ and $c = c_p$, where c_p is a concentration in the plateau region. For symmetric curves, the above point seems to be an inflection point and corresponds to a maximum on the curve plotted in the coordinates distance from a rotation axis–concentration gradient. The experimental data obtained at 25°C are summarized in Table 1.

RESULTS AND DISCUSSION

Hydrodynamic Invariant

The resultant system of molecular and hydrodynamic characteristics offers a means of estimating the hydrodynamic invariant A_0, which is commonly used in

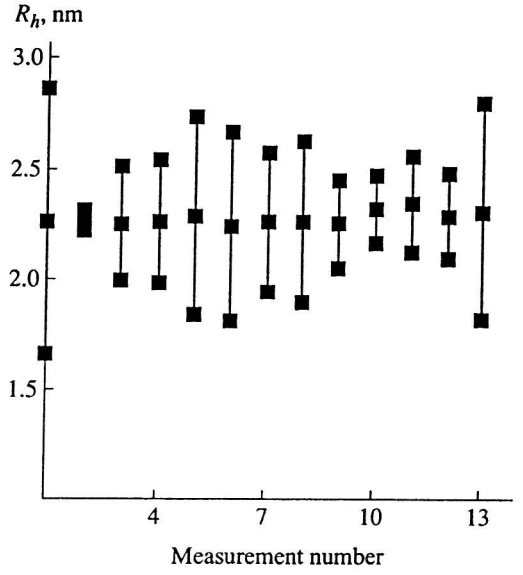

Fig. 1. Stokes radii (nm) of lactodendrimer (generation 2) determined by dynamic light scattering.

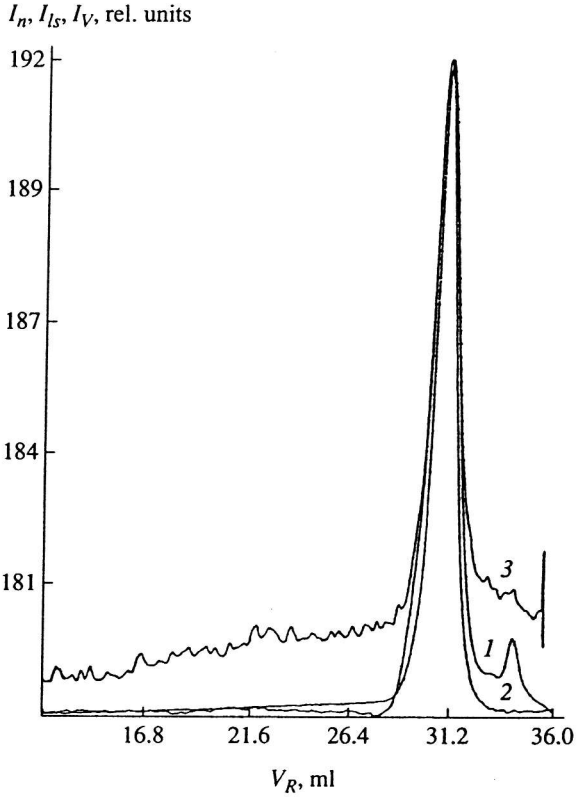

Fig. 2. Three-detector chromatogram of lactodendrimer (generation 4): signal of (*1*) refractometric detector, (*2*) scattered light detector, and (*3*) viscometric detector.

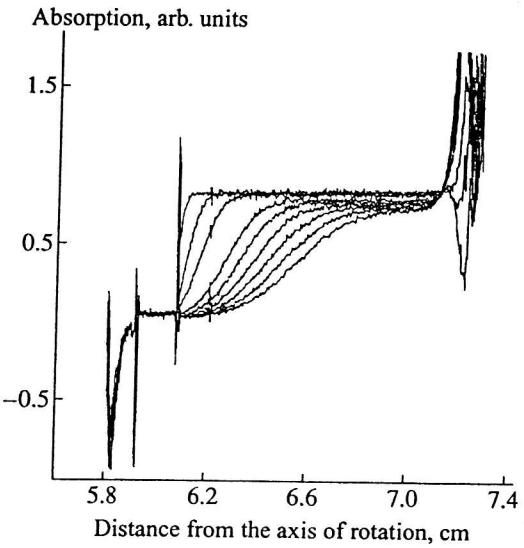

Fig. 3. Absorption sedimentogram for lactodendrimer (generation 3) at $c = 0.01 \times 10^{-2}$ g/cm^3. The scan interval was 20 min and $n = 50000$ rpm.

the molecular physics of polymers [17, 18]. The value of A_0 in this case may be calculated according to three equations:

$$A_{01} = [D](M_w[\eta])^{1/3},$$

$$A_{02} = R[s][\eta]M_w^{-2/3},$$

$$A_{03} = (R[s][D]^2[\eta])^{1/3},$$

where R is the universal gas constant, $[D] \equiv D_0\eta_0/T$, and $[s] \equiv s_0\eta_0/(1 - \upsilon\rho_0)$. The obtained hydrodynamic invariants are listed in Table 2 (it is clear that $A_{01}^2 A_{02} \equiv A_{03}^3$) together with the values of A_{03} calculated for the same samples in the solvent containing 0.019 M NaCl. The average value of A_0 obtained in 0.2 M NaCl is equal to $(2.47 \pm 0.11) \times 10^{-10}$ cm^2/(s^2 K mol$^{1/3}$); the corresponding average value in 0.019 M NaCl is $A_0 = (2.61 \pm 0.16) \times 10^{-10}$ [11, 12]. The common value averaged over the entire body of data is $A_0 = (2.51 \pm 0.08) \times 10^{-10}$. The data on A_0 are inconsistent with the value of A_0 reported for impermeable rigid spheres ($A_0 = 2.914 \times 10^{-10}$). This implies, in particular, that the values of $M_{D\eta} = A_0^3([D]^3[\eta])^{-1}$ derived from the theoretical value of A_0 may be overestimated by ~1.6 times, whereas the values of $M_{s\eta} = (R/A_0)^{3/2}([s]^3[\eta])^{1/2}$ will be underestimated by a factor of 1.3.

Table 1. Molecular and hydrodynamic characteristics of poly(amidoamine)-based lactodendrimers

N	M_{theor}	M_{sD}^* [9, 12]	M_w	M_w/M_n	dn/dc, cm^3/g	$D \times 10^7$, cm^2/s	s, Sv	$[\eta]$, cm^3/g
4	2420	2500	2900	1.05	0.177	15.0	0.77	4.0
8	5230	6600	7400	1.00	0.196	11.9	1.1	3.3
16	10840	13700	12400	1.02	–	10.4	1.7	3.8
32	22120	22800	24300	1.03	0.162	8.7	2.6	4.1
64	44640	47000	49700	1.07	–	7.5	4.2	3.7
128	89690	93000	92400	1.07	0.183	5.9	6.3	3.7

Table 2. Hydrodynamic invariant A_0 and density of molecules ρ of poly(amidoamine)-based lactodendrimers

Generation	$A_0 \times 10^{10}$, g cm^2/(s^2 K mol$^{1/3}$)						
	A_1	A_2	A_3	A_3 [9, 12]	A_{av}	ρ_f^*	ρ_η
						g/cm^3	
0	2.24	2.91	2.45	2.68	2.57 ± 0.23	0.42 ± 0.13	0.63
1	2.28	2.09	2.21	2.50	2.27 ± 0.12	0.32 ± 0.03	0.76
2	2.48	2.40	2.45	2.85	2.55 ± 0.16	0.39 ± 0.02	0.66
3	2.67	2.40	2.57	2.76	2.60 ± 0.12	0.41 ± 0.05	0.61
4	2.82	2.32	2.64	2.46	2.56 ± 0.17	0.49 ± 0.10	0.68
5	2.73	2.31	2.59	2.39	2.51 ± 0.16	0.45 ± 0.08	0.68
Average values	2.54 ± 0.20	2.41 ± 0.17	2.49 ± 0.12	2.61 ± 0.16	2.51 ± 0.08	0.41 ± 0.04	0.67 ± 0.04

* The average value $\rho_f = (\rho_{sD} + \rho_D + \rho_s)/3$.

Comparison of Hydrodynamic Characteristics

Comparison between the hydrodynamic and molecular parameters presented in Table 1 and the results obtained previously for the same dendrimers using other apparatuses and techniques indicates that these data agree satisfactorily. The values of M_w (Table 1) and M_{sD} [11, 12] are virtually identical. Moreover, GPC measurements demonstrate that the polydispersity of the samples studied is insignificant ($M_w/M_n < 1.07$).

Below, the hydrodynamic parameters will be compared in log–log coordinates (Fig. 4). Figure 4a displays $\log[\eta]$ plotted versus $\log M$ (either M_w or M_{sD} were used) based on the data of Table 1 and the results taken from [11, 12]; this figure also displays the data obtained for poly(propyleneimine)-based lactodendrimers [9, 10] and globular proteins [19, 20]. A lower zone refers to the limiting theoretical value of $[\eta]$, which may be calculated for impermeable rigid spheres from the values of a specific partial volume determined experimentally. As evidenced by the comparison of the viscometry data, the intrinsic viscosity of lactodendrimers is virtually unaffected by their molecular mass and the nature of the core for the two structures under comparison; furthermore, the intrinsic viscosity of lactodendrimers is close to that of globular coils. At the same time, the values of $[\eta]$ are somewhat higher than the lower theoretical limit for impermeable rigid spheres. Figure 4a also shows the plot of $[\eta]$ versus M for the linear water-soluble poly(vinylpyrrolidone) [21]. The data derived from the velocity sedimentation data are compared in a similar manner (Fig. 4b).

As follows from Fig. 4 and as was noted more than once in [9, 11], the velocity sedimentation coefficient is the hydrodynamic characteristic, that is, the parameter controlled by the translational friction of molecules, which is sensitive to a change in the molecular mass of dendritic molecules. Therefore, the development of the theory of a translational friction coefficient of regularly branched molecules seems to be an extremely urgent problem.

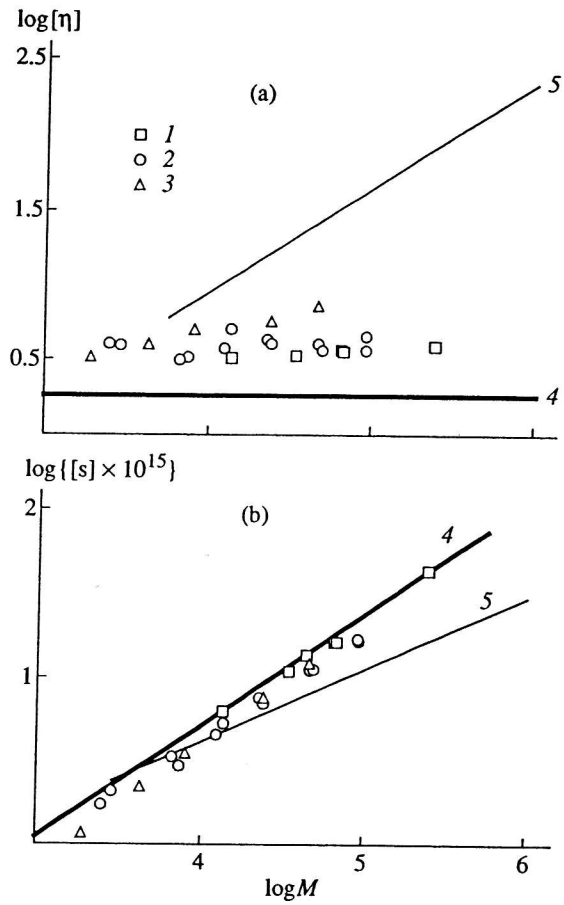

Fig. 4. Double logarithmic plot of (a) $[\eta]$ and (b) $[s]$ vs. M for: (*1*) globular proteins [19, 20], (*2*) poly(amidoamine)-based lactodendrimers according to the data of Table 1 and [11, 12], and (*3*) poly(propyleneimine)-based lactodendrimers according to [9, 10]; (*4*) the limiting value of $[\eta]$ calculated in terms of undraining rigid sphere model and (*5*) the curve for linear polymer (poly(vinylpyrrolidone) according to [21]).

Average Density of Dendrimer Substance in a Volume Confined by a Dendritic Molecule

Based on hydrodynamic and molecular characteristics, the average density of a dendrimer substance in a volume confined by a dendritic molecule may be determined. It is easily seen that in terms of the sphere model density may be calculated by three equations:

$$\rho_{sD} = 3^4 2\pi^2 \eta_0^3 (1 - \upsilon\rho_0)^{-1} (kT)^{-2} (sD^2),$$

$$\rho_D = 3^4 2\pi^2 N_A^{-1} (\eta_0/kT)^3 M_w D^3,$$

$$\rho_s = 3^4 2\pi^2 N_A^2 (\eta_0/(1 - \upsilon\rho_0))^3 s^3/M_w^2,$$

based on the translational friction data. Proceeding from the viscometry data, the density may be calculated by the relationship

$$\rho_\eta = 2.5/[\eta].$$

The density ρ_η and the average density $\rho_f = (\rho_{sD} + \rho_D + \rho_s)/3$ are summarized in Table 3. These data give no way to reveal any trend demonstrating how the values of ρ vary with the number of generation (or molecular mass) determined by both translational friction and viscometry. The values of density derived from the viscometry data are ~1.5 times higher than the corresponding values based on the translational friction data. This apparently reflects that the rigid sphere model is incompletely consistent with the actual shape of molecules.

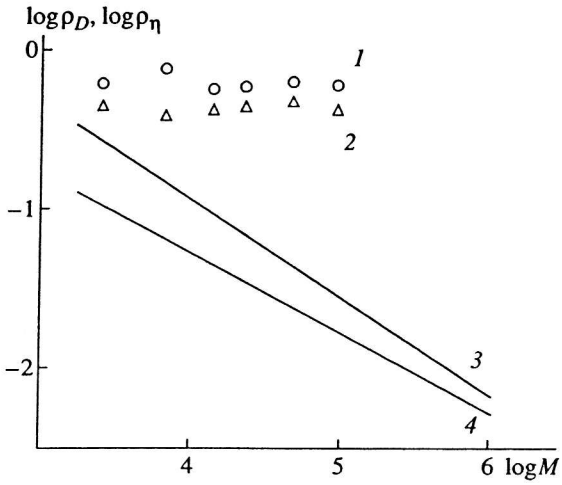

Fig. 5. Double logarithmic dependence of the density of the dendrimer substance ρ vs. M: (*1*) density derived from the rotational friction data, (*2*) density calculated by the translational friction data, and (*3*, *4*) the density of linear molecules of polysaccharide (pullulan) determined from the results of rotational and translational friction, respectively [22].

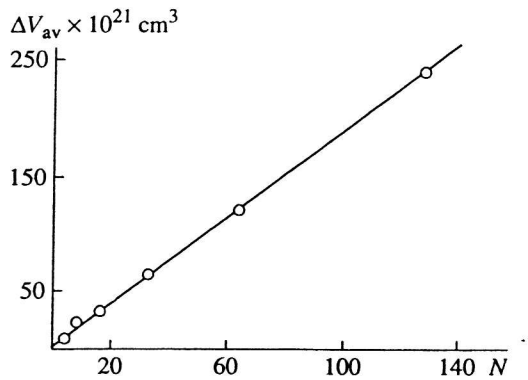

Fig. 6. Variation in the excess volume of lactosylated dendritic molecules based on poly(amidoamine) with their number of lactose groups.

Figure 5 compares the densities of the dendrimer and polymer substances in a volume occupied by a dendritic molecule and a molecule of a linear polymer. The flexible-chain linear polysaccharide poly(maltotriose) (pullulan) studied in water [22] was used as the polymer under comparison. The density of a polymer coil was calculated under a Gaussian approximation by the formulas

$$\rho_{sD} = P^3\eta_0^3(0.36)^{-1}(1-\upsilon\rho_0)^{-1}(kT)^{-2}(sD^2),$$

$$\rho_\eta = \Phi/N_A 0.36[\eta],$$

where P and Φ are the Flory hydrodynamic parameters [18].

For the dendritic molecules of higher generations, the density is greater by an order of magnitude and above than that of a polymer substance in a coil formed by a linear molecule. The density of a polymer substance in a volume confined by a rigid-chain macromolecule will be smaller by another order of magnitude. In the case of lactodendrimers, the density is almost independent of molecular mass, whereas for linear molecules, the density drops by an order of magnitude on passing to higher molecular masses. These are averaged estimates, and they do not allow one to estimate the substance distribution on passing from the center of a dendritic molecule to its periphery.

Volume Occupied by Lactose Groups in Lactodendrimers

In the lactodendrimers examined, up to 80% of the weight is concentrated in lactose units. Let us calculate what additional volume is occupied by lactosylated dendritic molecules compared to the parent dendrimers and compare this volume with the amount of lactose radicals in each generation. This volume will be estimated as the volume of a spherical layer $\Delta V = V_1 - V_2 = 4\pi/3(R_1^3 - R_2^3)$, where V_1 is the volume of a lactosylated dendritic molecule and V_2 is the volume of the parent poly(amidoamine) dendrimer. The values of V_2 were calculated from the radii of the parent poly(amidoamine) dendrimers [23]. Figure 6 plots ΔV (a mean value from the data on translation and rotational friction) as a function of the number of lactose groups N in the dendrimers of corresponding generations. As can be seen from Fig. 6, the values of ΔV are in direct proportion to the number of lactose end groups in a dendritic molecule. This implies that the volume occupied by a lactose group (within $1100 < (\Delta V/N) \times 10^{24}$ cm^3 < 2900) remains almost invariable on passing from low to higher generations. Let us compare this volume with the van der Waals volume of a lactose group $V_L = 275 \times 10^{-24}$ cm^3 calculated taking into account the molecular structure of this group [24]. The volume occupied by a lactose group in lactosylated dendritic molecules is several times greater than its intrinsic volume. It is easy to verify [19, 25, 26] that the volume occupied by a lactose group in dendritic molecules is comparable to a volume which a free lactose group may exclude for its unbound neighbors V_{ex} occurring in solution ($V_{ex} \approx 8V_L \approx 2200 \times 10^{-24}$ cm^3). It appears that this estimate is evidence that no particular steric restrictions exist for the end groups in lactosylated dendritic molecules.

The data obtained in this work indicate that the end lactose groups in the lactodendrimers under study are located in periphery and do not penetrate into the bulk of the parent dendrimers. The experimental molecular masses are in good agreement with the calculated data, thereby confirming that the amine end groups of the parent poly(amidoamine) dendrimers are completely

replaced by the lactose moieties. This also implies that the end amine groups in the parent dendrimers should also be located in the periphery of molecules rather than distributed within the bulk of the parent dendrimers.

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