Nanosecond Mobility of the Molecules in the Research of Supramolecular Assemblies of Dendrimers, DNA, or Fullerene-Containing Compounds

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Summary: The data concerning the structural organization of supramolecular assemblies containing the dendrimer molecules of various generations, DNA-polycation of various chemical structure were obtained by analysing the changes of nanosecond mobility of macromolecules or low-molecular weight organic cations. Effect of water-soluble fullerene-containing compounds on interaction of DNA molecules with low-molecular weight cations and polycations was studied as well. The data were obtained by luminescent methods.

Keywords: dendrimers; DNA; fullerene; nanosecond mobility; supramolecular assemblies

Introduction

The investigation of multicomponenet assemblies formed by noncovalent intermolecular bonds is an important problem since the functional properties of these assemblies are determined by their structure, stability, and their structural changes.

Special methods are necessary for investigating the changes of noncovalent bonds since they are very unstable. The most informative approach is the study of the effect of intermolecular noncovalent bonds on the mobility of noncovalently bonded components. The case in point is that the mobility occurring with relaxation times coinciding with the intermolecular contacts duration (H-bonds and others) is the nanosecond mobility. The study of nanosecond mobility by polarized luminescence is the most informative method for investigating multicomponent polymer systems consisting of labeled and unlabeled components. The labeled macromolecules were obtained by covalent attachment of luminescent markers of the anthracene structure (one marker per thousand units). The investigated objects are water-soluble assemblies including linear polymers, dendrimers, DNA, or fullerene molecules as well as the low-molecular weight organic ions of different conformations and metal ions.

Exchange or replacement reactions and competitive interactions are used for establishing the contribution of different intermolecular interactions. Nanosecond relaxation times were measured by polarized luminescence [1].

Results and Discussion

1. The data on nanosecond mobility studied by polarized luminescence were obtained for dendrimers (**D**) on the basis of L-lysine molecules in an aqueous solution. They show that the mobility of D molecules as a whole is the main nanosecond relaxation process in a solution. It was found for D on

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the basis of L-lysine that relaxation times τ increase with storage of dendrimers in an aqueous solution. This means that D molecules form associates. These data were obtained for D of the 3 - 5-th generation. However, the associates are not revealed for D molecules of the 6-th generation (Table).

In this table M_{theor}, $M_{S,D}$ and M_{PL} are the molecular weights calculated from chemical formula and determined by sedimentation and diffusion, and by polarized luminescence using the equation:

$$\tau = 1,2[\eta]M\eta/RT$$

The data show that higher increase in M and τ during the dendrimers storage in an aqueous solution is observed for dendrimers with lower generation number (D_3 and D_5). M_{PL} for D_6 does not differ from M_{theor}.

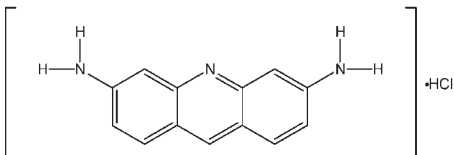
The data on the density of lysine side chains packing were also obtained by polarized luminescence from the values of the parameter $1/P_o$' characterizing the amplitude of high frequency motions of lysine side chains. The luminescent marker was at the end of these chains. The results (Table) show the dependence of $1/P_o$' on the number of generations in D molecules. The value of $1/P_o$' for D of the 3 - 5-th generation coincides with $1/P_o$' for a loose macromolecular coil. For D of the 6-th generation, $1/P_o$' coincides with $1/P_o$' for a compact macromolecular globule [1].

It was also found that the changes in lysine side chains packing correlate with association processes in the D aqueous solution. The comparison of data for homo- (on the lysine basis) and hetero (Lys-Glu, Lys-His) dendrimers show higher tendency for hetero dendrimers to form associates.

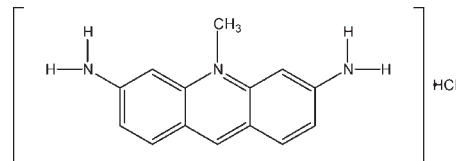
It was also revealed that the structure of intermolecular assemblies consisting of dendrimers and polyanions strongly depends on the number of generations, i.e. on the tendency of dendrimer molecules to form associates (Fig. 1, 2).

It can be seen that the plot of τ vs. the content of interacting components differs for D_3 – PMAA and D_6 – PMAA assemblies (**PMAA** –polymethacrylic acid). The data show that D_3 forms associates not only in an aqueous solution but also on the PMAA chains. For D_3 in a complex with PMAA the relaxation time τ increases to 240 ns. This means that a compact nucleus is formed. For D_6 in a complex with PMAA, the value of τ is 80 ns. This increase can be caused by the decrease of single dendrimer molecules content in a solution upon PMAA addition. The intramolecular mobility of PMAA molecules interacting with D_3 and D_6 dendrimers is also different (Fig. 1, 2).

2. In a research of supramolecular assemblies including DNA molecules, the nanosecond mobility of a low-molecular weight cations (proflavine, tripaflavine) interacting with DNA was studied. Its change upon polycation addition was also investigated. In DNA aqueous solution the relaxation times are 1000 ns and 1 ns in the absence and in the presence of polycation, respectively. Close coincidence was found in changes of the following two values: relaxation times of a luminescent low-molecular weight organic cation and its luminescence intensity upon the polycation addition. This permits us to study the change in luminescence intensity in DNA solution upon polycation addition (Fig. 3). DNA concentration in solution was 0,005 mg/ml. Polycations are polydimethylami-



Proflavine



Tripaflavine

Table .
Mobility of dendrimer molecule as a whole determined by polarized luminescence for dendrimers of various generations

Dendrimer	M_{theor}	$M_{S,D}$**	M_{PL} from τ_{whole}	Time of storage in solution	τ_{whole}	$1/P_o$
D_3*	2011	2700	2300	fresh	3,1	25
			2400	30 days	3,1	25
			9000	1 year	3,6	9
D_5*	8160	13000	17900		23	17
D^*_5 Lys-Glu	16500	24500	22500		29	10,2
D^*_5Lys-His	17000	15300	29700		38	8,1
D^{*5}_6	16300		20000		26	5,7
			18000		23	5,7
D^*_6	16300		16300		21	8,5

* Means a luminescent marker.
** $M_{S,D}$ were determined by G.M. Pavlov et. Col., (IMC RAS).

noethylmethacrylate, polyallylamine, polyvinylamine.

To estimate the lability of a polycation-DNA complex in an aqueous solution, the synthetic polyanion – polyvinylsulfonate, is added to a solution containing DNA, polycation and proflavine. When polycation passes from DNA complex on synthetic polyanion, the proflavine is bonded to DNA. The parameter χ, characterizing the lability of polycation-DNA bond in a complex is estimated from a change of proflavine portion connected with DNA when the polycation passes on synthetic polyanion.

The more labile is the polycation- DNA bond, the greater quantity of polycation molecules passes from DNA to polyanion and the greater quantity of proflavine molecules contacts with DNA. Polycations

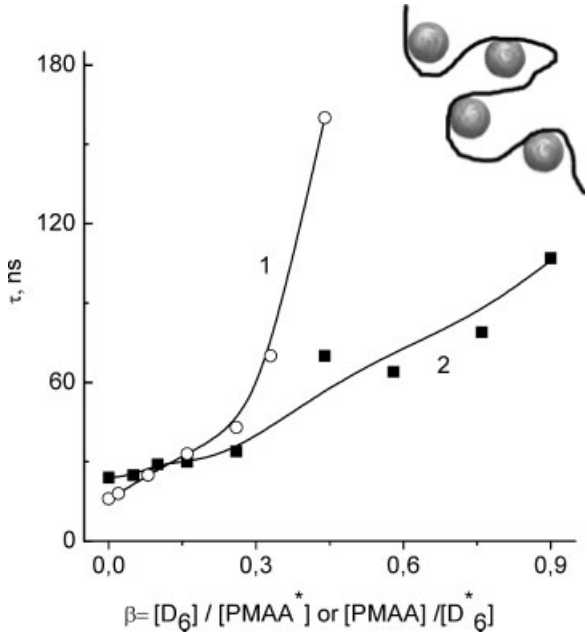


Figure 1.
Dependence of τ value on relation of interacting components β (in moles) in aqueous solution: PMAA* and D_6 (1); PMAA and D_6* (2). Index * means luminescently labeled component.

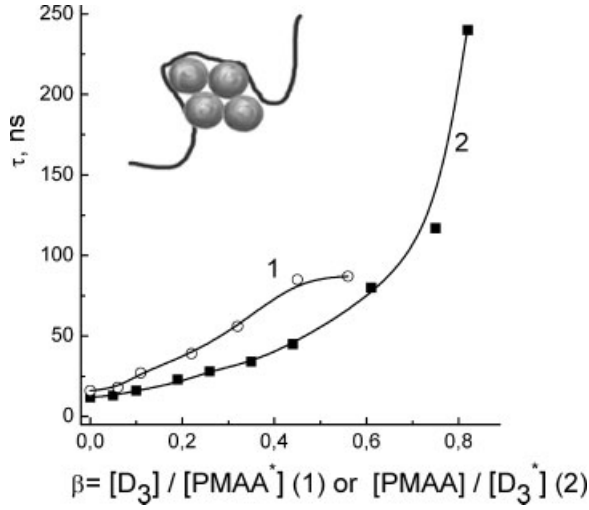
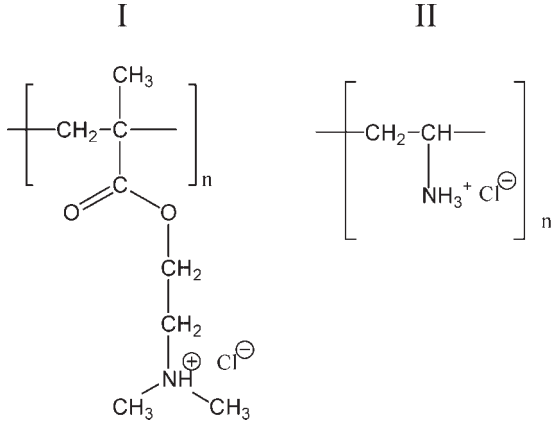


Figure 2.
Dependence of τ value on relation of interacting components β (in moles) in aqueous solution: PMAA* and D_3. (1); PMAA and D_3* (2). Index * means luminescently labeled component.

of the different chemical structures: poly-N-dimethylaminoethylmethacrylate (I), polyvinylamine (II), copolymers of N-dimethylaminoethylmethacrylate with N-vinylpyrrolidone or with N-methacryloylaminoglucose were used in experiments. They differ in the number of ionogenic groups and in their structure: $-NH_3^+$, $-NH(CH_3)_2^+$, and the presence of carbohydrate fragments.

The molecular weight M of DNA prepared from a cattle spleen is 16×10^6, that of polycations is $(1–8) \times 10^4$, and that of polyanion is 8×10^3.

Comparative research of the stability of polycation-DNA complexes depending on a polycation chemical structure showed (Fig. 4) that the stability increases (parameter of lability χ drops) on passing from the polycations with high intramolecular mobility (copolymers of N-vinylpyrrolidone with N-dimethylaminoethylmethacrylate for which the relaxation time describing intramolecular mobility τ is 17 ns) to polycations with lower mobility (poly-N-dimethylaminoethylmethacrylate, $\tau = 25$ ns, or polyvinylamine whose intramolecular



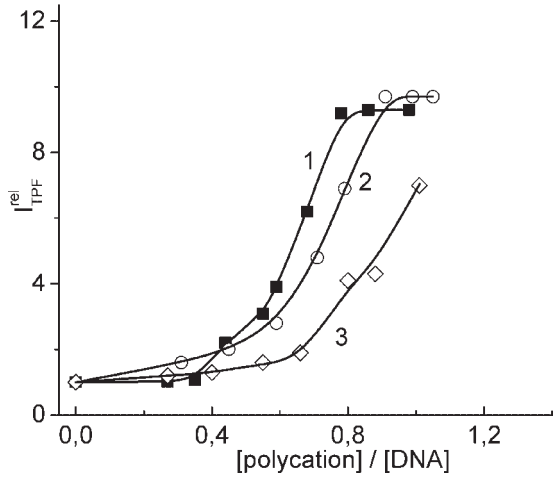


Figure 3.
Dependence of luminescence intensity of tripaflavine (arb. units) on content of polycation in aqueous solution of DNA β [polycation]/[DNA] (in moles).

hindrance grows because of proximity of the charged groups to the main polymer chain, $\tau = 28$ ns) [2].

The effect of intramolecular hindrance of polycation side chains bearing charged units on the stability of a polycation-DNA complex shows the importance of the analysis of polycation chains relaxation characteristics for choice of objects for the formation of such complexes.

A new supramolecular assemble is formed. It includes besides three components – DNA, a polycation, and a low-molecular weight luminescent cation also the fourth one: the water-soluble fullerene-containing polymer – poly-N-vinylpyrrolidone, $M = 24 \times 10^3$ (**PVP-C_{60}**). In this fullerene-containing polymer, four chains are without C_{60} and only one chain contains the C_{60} molecule. These data are obtained both from the calculation of bonded portion of

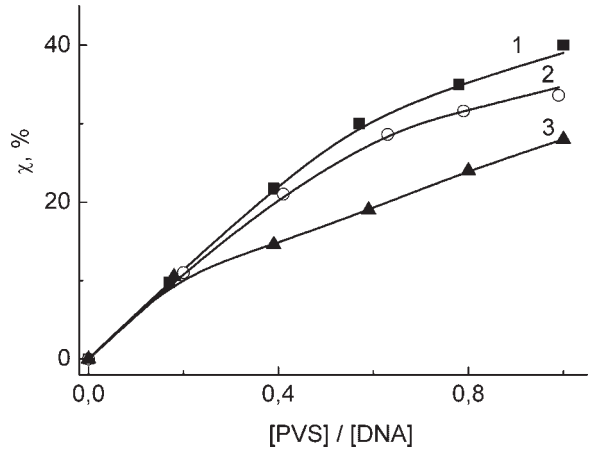


Figure 4.
Dependence of χ parameter of DNA-polycation complexes lability on the content of PVS polyanion [PVS]/[DNA] (in moles) in aqueous solution of complex. Polycations are polydimethylaminoethylmethacrylate (1), polyallylamine (2), polyvinylamine (3).

C_{60} molecules (1%) and from the analysis of inclusion of PVP chains in supramolecular formations at the interaction with PMAA. It is shown that PVP without C_{60} does not interact with DNA. This means that only one PVP chain bearing the C_{60} molecule interacts with the DNA molecule containing a low-molecular weight cation (proflavine or tripaflavine).

This interaction is only observed on the addition of a polycation that replaces a low-molecular weight cation from DNA. It was found that PVP-C_{60} retains a low-molecular weight cation in DNA. This is impossible to find out without addition of the polycation to DNA aqueous solution since a low- molecular weight cation in DNA is considerably hindered. This follows from high values of τ describing the mobility of the low-molecular weight cation in DNA. When PVP-C_{60} is present, greater portion of proflavine (tripaflavine) is bonded by DNA upon the addition of polycation. It is quite reasonable to suppose that the proflavine-PVP-C_{60} complex is formed in DNA. This is quite possible because the interaction of low-molecular weight organic cations with PVP-C_{60} is observed in an aqueous solution in the absence of DNA but in DNA this process occurs more effectively. The other question is what intermolecular contacts are formed between PVP-C_{60} and DNA: C_{60}-DNA contacts or PVP-C_{60}–DNA contacts. The latters are more probable since the PVP molecules bonded to C_{60} change their conformation and functional activity.

Hence, DNA in supramolecular assemblies can be used for separating fullerene-containing polymer chains from those without fullerene in fullerene-containing polymer systems of donor-acceptor type and for the study of functional properties of such complexes.

Conclusion

It follows from all data obtained that the study of nanosecond mobility, namely, of intramolecular mobility of polymer chains or of molecules as a whole (for low-molecular weight compounds, $M < 10000$) allows us to solve problems of a structure of supramolecular assemblies and of structurizations in solutions of molecules of different structures and architectures.

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