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STRUCTURAL TRANSFORMATIONS OF DNA UNDER THE INFLUENCE OF OLIGOMERS AND POLYMERS OF ETHYLENE GLYCOLS

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In this work, the effect of ethylene glycol oligomers and polymers of various molecular masses on the structural transformations of deoxyribonucleic acid (DNA) is studied exclusively by viscometry. The main goal of this study was to understand how polyethylene glycol (PEG) interacts, which binds to calf thymus DNA (ctDNA). This paper presents the results of these studies. It has been found that ethylene glycols with molecular masses of 600 and 1000, apparently due to their smallness, do not have a noticeable effect on the sizes of DNA macromolecules in the studied concentration ranges. For ethylene glycols with molecular masses of 6000 and 7000, a clearly expressed complex course of the dependence of the intrinsic viscosity of DNA solutions on the polymer content was established. A decrease in the viscosity of solutions was observed, which was interpreted as a result of a decrease in the size of DNA macromolecules. It was assumed that with a change in the concentration of ethylene glycols in the system, an increase in the density of DNA macromolecules occurs due to a change in the balance of hydrophobic-hydrophilic interactions. The results of the study in the presence of 20 000 molecular masses of PEG in a DNA solution practically exclude the presence of interaction between DNA and PEG molecules at such molecular masses.

Deoxyribonucleic acid (DNA) – ethylene glycol (EG) – polyethylene glycol (PEG) – calf thymus DNA (ct DNA) – viscometry

Այս աշխատանքում բացառապես մածուցիկության մեթոդով ուսումնասիրվել է էթիլենգլիկոլի օլիգոմերների և տարբեր մոլեկուլային զանգվածներով պոլիմերների ազդեցությունը դեզօքսիռիբոնուկլեինաթթվի (ԴՆԹ) կառուցվածքային փոխակերպումների վրա: Հետազոտության հիմնական նպատակն էր հասկանալ, թե ինչպես է փոխազդում պոլիէթիլենգլիկոլը (ՊԷԳ), որը կապվում է հորթի նշագեղձի ԴՆԹ-ին (հնԴՆԹ): Ներկա հոդվածը ներկայացնում է այդ ուսումնասիրությունների արդյունքները: Պարզվել է, որ 600 և 1000 մոլեկուլային զանգվածներով էթիլենգլիկոլները, ըստ երևույթին, իրենց փոքրության պատճառով, նկատելի ազդեցություն չունեն ԴՆԹ-ի մակրոմոլեկուլների չափերի վրա ուսումնասիրված կոնցենտրացիաների միջակայքում: 6000 և 7000 մոլեկուլային զանգվածներով էթիլենգլիկոլների համար հստակ արտահայտված բարդ ընթացք է հաստատվել ԴՆԹ-ի պոլիմերների պարունակությունից լուծույթների ներքին մածուցիկության կախվածության համար: Նկատվել է լուծույթների մածուցիկության նվազում, որը մեկնաբանվել է որպես ԴՆԹ մակրոմոլեկուլների չափերի նվազման արդյունք: Ենթադրվում է, որ համակարգում էթիլենգլիկոլների կոնցենտրացիայի փոփոխության ընթացքում ԴՆԹ-ի մակրոմոլեկուլների խտության աճ է տեղի ունենում հիդրոֆոբ-հիդրոֆիլ փոխազդեցությունների հավասարակշռության փոփոխության պատճառով: Հետազոտության արդյունքները ԴՆԹ լուծույթում ՊԷԳ-ի 20000 մոլեկուլային զանգվածով նմուշների առկայության պայմաններում գործնականում բացառվում է փոխազդեցության առկայությունը ԴՆԹ-ի և ՊԷԳ-ի մոլեկուլների միջև նման մոլեկուլային զանգվածների դեպքում:

Դեզօքսիռիբոնուկլեինաթթու (ԴՆԹ) – էթիլենգլիկոլ (ԷԳ) – պոլիէթիլենգլիկոլ (ՊԷԳ) – հորթի նշագեղձի ԴՆԹ (հն ԴՆԹ) – մածուցիկություն

В данной работе исключительно методом вискозиметрии изучено влияние олигомеров и полимеров этиленгликоля различной молекулярной массы на структурные превращения дезоксирибонуклеиновой кислоты (ДНК). Основная цель этого исследования состояла в том, чтобы понять, как взаимодействует полиэтиленгликоль (ПЭГ), который связывается с ДНК тимуса теленка (цтДНК). В данной работе представлены результаты этих исследований. Установлено, что этиленгликоли с молекулярными массами 600 и 1000, по-видимому, в силу своей малости, не оказывают заметного влияния на размеры макромолекул ДНК в исследованных диапазонах концентраций. Для этиленгликолей с молекулярными массами 6000 и 7000 установлен четко выраженный сложный ход зависимости характеристической вязкости растворов ДНК от содержания полимера. Наблюдалось снижение вязкости растворов, что интерпретировалось как результат уменьшения размеров макромолекул ДНК. Предполагалось, что при изменении концентрации этиленгликолей в системе происходит увеличение плотности макромолекул ДНК за счет изменения баланса гидрофобно-гидрофильных взаимодействий. Результаты исследования в присутствии 20 000 молекулярных масс ПЭГ в растворе ДНК практически исключают наличие взаимодействия молекул ДНК и ПЭГ при таких молекулярных массах.

Дезоксирибонуклеиновая кислота (ДНК) – этиленгликоль (ЭГ) – полиэтиленгликоль ПЭГ) – ДНК тимуса теленка (кт ДНК) – вискозиметрия

As is known the viscosity measurement is regarded as the least ambiguous and the most critical test of a DNA-binding model in the solutions [15, 25, 29, 32]. Viscometry is widely used as one of the well-known methods of hydrodynamic studies of polymers to establish results of the interaction of small molecules with DNA, as it is one of the most sensitive methods to changes in the shape and size of polymer macromolecules. Along with all available methods for determining the type of binding of small molecules to DNA in solutions, the measurement of viscosity is considered the simplest and most illustrative [15, 21, 25, 29, 30, 32].

Viscometry gives primary information about the structure, size, shape, and molecular mass of compounds [26]. Currently, it is considered that the analysis of the shapes of viscometry titration curves is a convenient way to determine the type of binding of small molecules with DNA [32, 33]. Viscometry is widely used as one of the well-known methods of hydrodynamic studies of polymers, as it is one of the most sensitive methods to changes in the shape and size of polymer macromolecules.

In our studies to explore the interaction between the small molecules and DNA, viscosity measurements were carried out by keeping the DNA concentration constant and varying the con-centration of small molecules. The viscosity of DNA solutions was performed using an Ubbelohde capillary viscometer (the capillary's diameter is 0.56 mm, viscometer constant, mm^2 / s^2 – 0.01). All viscosity measurements were carried out in a thermostated bath at a temperature of 22 ± 0.01^0C.

Measurements were made in a thermostat, the temperature of which could be maintained within the $\pm0.01^0$C range from the required one. A phosphate buffer of 6.0 ml was transferred to the viscometer to obtain the reading of efflux time. The efflux time of the solvent (0.1 BPSE buffer) was 93.5 sec. For the determination of solution viscosity, 6.0 ml of 86 μM DNA in phosphate buffer was taken to the viscometer and a flow time reading was obtained. To avoid reducing the concentration of DNA when adding the appropriate amounts of solutions of PEG in the dynamics, the same quantity of solution of DNA was added simultaneously with redoubled concentration.

The efflux time of samples was measured after the thermal equilibrium of the viscometer was achieved (about 10 min). Time reading was obtained with a timer accurate to ±0.01s and viscosity measurements were taken until three consecutive readings differed by less than ±0.1s. In all measurements, the experimental errors were within allowed limits and did not exceed 1%.

Our previous studies on the interactions of small molecules with DNA also confirm the importance of using the viscometry method to obtain complete information with optical methods [(1-6)]. Therefore, in this work, we used exclusively the viscometry method.

As already known, polyethylene glycol (PEG) is a high-quality chemicals polyether compound derived from petroleum with many applications. Oligomers and polymers of ethylene glycol are organic compounds with the structural formula HO-CH_2-(CH_2-O-CH_2)$_n$-CH_2-OH, with different molecular masses (Other names are also used - polyoxyethylene (POE), polyethylene oxide (PEO) [20]. Oligomers weighing up to 2000 g/mol ape wax flakes or length of the compound's molecular chain. Oligomers weighing up to 400 g/mol are colorless powders [21]. Oligomers and polymers of ethylene glycols for various purposes are widely used in everyday life, in the fields of the food industry, medicine, agriculture, and industry. PEG is widely used to improve the properties of drugs [32] for cell immobilization [17], and in technical applications [22, 24]. PEG is incorporated into DNA complexes of a number of cationic polymers, including polyethyleneimine (PEI) [17, 28] and poly(amidoamine) [5].

As we have already noted, PEG is a very common food supplement. As is known, PEG is widely used in the process of DNA condensation [14]. Considering this fact, we devoted the present work to studying the effect of PEG on the DNA structure. Our main goal was to investigate the influence of the molecular mass of PEG on the nature of its interaction with DNA exclusively by the viscometry method.

Thus, our main goal was to study conformational changes in DNA under the influence of PEGs of different molecular masses. For our studies, we used only the viscometry method, since it is rightly considered the method most sensitive to the shape and mass of macromolecules [14, 19, 23].

Viscometry gives primary information about the structure, size and mass of compounds [23]. Currently, it is considered that the analysis of the shapes of viscometry titrations curves is a convenient way to determine the type of binding of small molecules with DNA [20, 23].

Materials and methods. It is well known, that viscometry is a key indicator of changes in the conformational properties of macromolecules in different processes [29, 30]. Optical, measurements and studies provide necessary but not sufficient clues to explain binding between DNA and the complex, while hydrodynamic measurements are regarded as the least ambiguous tests of a binding model in solution [10, 36].

Thus, viscosity measurements are carried out as an effective tool to clarify the binding mode of PEG to ctDNA. Intercalates are known to cause a significant increase in the viscosity of DNA solution due to lengthening the DNA helix as base pairs are separated to accommodate the binding ligand. In contrast, partial, non-classical ligand intercalation in grooves causes a bend in the DNA helix reducing its effective length and thereby its viscosity [19, 27, 28].To explore the interaction between the PEG to DNA, viscosity measurements were carried out by keeping the DNA concentration constant and varying the concentration of PEG.

Viscometry is widely used as one of the well-known methods of hydrodynamic studies of polymers to establish results of the interaction of small molecules with DNA. Indeed, according to

the Flory-Fox famous equation, the dependence of the intrinsic viscosity on the size of macromolecules is expressed by the following expression

$$[\eta]_\theta = \Phi(\theta)\frac{(\overline{h_\theta^2})^{3/2}}{M} = \Phi(\theta)\frac{(LA)^{3/2}}{M}\alpha^3, \quad (1)$$

where – is Flory constant, is the average molecular mass of the polymer, is the mean square distance between the ends of the macromolecules, A is the Kuhn segment, L is the contour length and α is the swelling degree of macromolecules [12]. Experimental data show, that from a thermodynamic point of view, the value is greater in good solvents than in bad ones. These data are in good agreement with the theoretical conclusions since it is proven that macromolecules acquire a minimum size in θ solvents and swell as the quality of the solvent improves.

In this work, the viscosity of DNA solutions was performed using an Ubbelohde capillary viscometer of the Diadem company, which, along with the main office in Moscow, also includes a branch in Armenia. As known, it used to determine the kinematic viscosity of transparent Newtonian fluids. The capillary's diameter is 0.56 mm, and the temperature of all studies is 22^0C.

Flow times were measured with a digital stopwatch. Each sample was measured three times and an average flow time was calculated.

The efflux time of samples was measured after the thermal equilibrium of the viscometer was achieved (about 10 min). Time reading was obtained with a timer accurate to ±0.01s and viscosity measurements were taken until three consecutive readings differed by less than ±0.1s. In all measurements, the experimental errors were within allowed limits and did not exceed 1%.

PEG fractions of different average sizes (600, 1 000, 6 000, 7 000, and 20 000 from Sigma) were used in the concentration-dependent viscosity measurements. Samples were prepared by dissolving a weighed amount of PEG in twice-distilled water, followed by stirring at room temperature to achieve complete dissolution. Measurements were carried out in a thermostat, the temperature of which could maintain ± 0.01^0C of the required one. 10.0 ml of phosphate buffer was transferred to the viscometer to obtain the reading of efflux time. The efflux time of the solvent was 93.5sec. The viscosity of the solution was measured until three consecutive readings differed by less than ±0.1s. For the determination of solution viscosity, 10.0 ml of 86 μM DNA in phosphate buffer was taken to the viscometer and a flow time reading was obtained. Each point measured was the average of at least five readings. The data obtained were presented as relative viscosity, versus r, where is the reduced specific viscosity of DNA in the presence of PEG and is the reduced specific viscosity of DNA alone.

An appropriate amount of PEG in a buffered solution was added to the viscometer to give the needful relative concentration (r) while keeping the DNA concentration constant, and the flow time read. To avoid reducing the concentration of DNA when adding the appropriate amounts of solutions of DNA in the dynamics, simultaneously the same quantity of solution of DNA with redoubled concentration was added. Time readings were obtained with a timer accurate to ±0.01s and viscosity measurements were taken until three consecutive readings differed by less than ±0.1s. The experimental errors did not exceed 1 %. In our studies, the samples were prepared by dissolving a weighed portion of PEG in bi-distilled water, followed by stirring at room temperature until complete dissolution. All samples were used without further purification. During investiga-tions, increasing amounts of ethylene glycol oligomers or polymers were added to DNA.

Results and Discussion. We characterize the viscosity behavior of the PEG-DNA solution through the relative viscosity, defined as the ratio $\eta/\eta_{0,}$ where the reduced specific viscosity of DNA in the presence of DNA is and the reduced specific viscosity of DNA alone is η_0. Obtained data were presented as (η/η_0) versus r (r = [PEG/DNA]).

The effect of ethylene glycol oligomers of various molecular masses (600 and 1000) on the conformation of DNA macromolecules in buffer solutions was studied.

Relevant studies have shown that ethylene glycol oligomers in the studied molecular size ranges do not affect the size of DNA macromolecules at all (fig.1).

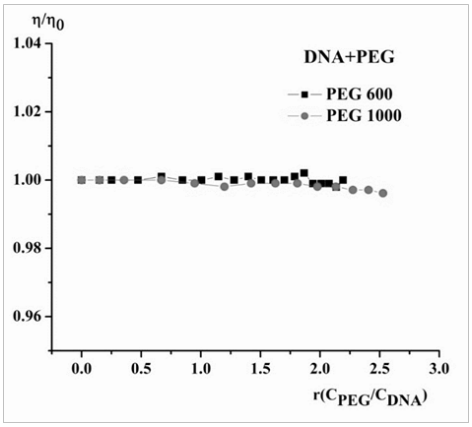

Fig. 1. Dependence of the relative viscosity of DNA on the concentration of ethylene glycol oligomers with molecular masses of 600 and 1000.

Quite a different result for PEG with molecular masses of 6000 and 7000 (fig. 2). The condensing properties of PEGs on DNA macromolecules have clearly demonstrated by studying the effect of PEGs with such molecular masses.

Fig. 2 shows the effect of 6000 and 7000 molecular mass polyethylene glycols (PEG 6000, PEG 7000) on the relative viscosity of the DNA solution and hence on the size of the macromolecules. The data show that under the influence of PEG there are clear structural changes in DNA macromolecules.

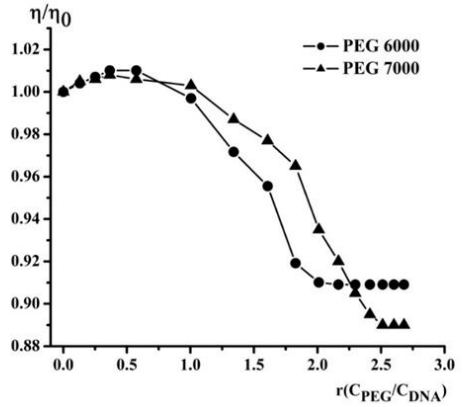

Fig. 2. Dependence of the relative viscosity of DNA on the concentration of ethylene glycol oligomers with molecular masses of 6000 and 7000.

Unexpected were the results in a low range of relative concentrations of PEG – the growth of viscosity of the solution and, as a consequence, an increase in the size of DNA macromolecules. Therefore, we performed additional measure ments in the specified range with smaller steps. The results presented in fig. 3 demonstrate the reliability of the data obtained. In our opinion, it is possible that, at a concentration of PEG, its condensing effect on DNA first manifests itself with a slight increase in the size of macromolecules.

Then, due to the increase in PEG concentrations, the condensation properties of DNA macromolecules change, which is expressed by a sharp decrease in the size of its macromolecules to a constant value [33].

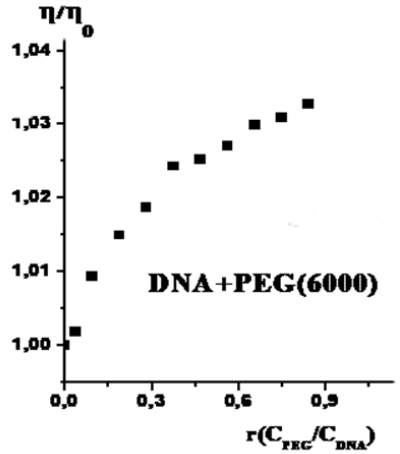

Fig. 3. Change in DNA viscosity in the presence of PEG 6000 in the low concentration range.

The dependence of the relative viscosity on the concentration of PEG/DNA can be divided into three parts. The relative viscosity increases in the range $r < 0.9$ decreases in the range $0.9 < r < 2.0$, and remains constant in the range $r > 2.0$. At high PEG concentrations, when 1 base pair contains more than 1 repeating unit, a decrease in solution viscosity is observed, which is interpreted as a result of a decrease in the size of DNA macromolecules.

Then, due to the increase in PEG concentrations, the condensation properties of DNA macromolecules change, which is expressed by sharp decrease in macro m olecules to a consatant value size of its [33].

This was interpreted because of a change in the balance of hydrophilic-hydrophobic interactions or otherwise as a result of screening of negative charges on phosphoric acid resin. Thus, the data show that under the influence of PEG there are clear structural changes in DNA macromolecules.

On fig. 4 shows the effect of 20, 000 molecular mass polyethylene glycol on the relative viscosity of the DNA solution. The results obtained practically exclude the presence of interaction between DNA and PEG molecules. The linear dependence of the viscosity of DNA and PEG on the composition of the solution corresponds to the principle of additivity.

On fig. 4 shows the effect of 20, 000 molecular mass polyethylene glycol on the relative viscosity of the DNA solution. The results obtained practically exclude the presence of interaction between DNA and PEG molecules. The linear dependence of the viscosity of DNA and PEG on the composition of the solution corresponds to the principle of additivity.

It should be noted that we have already conducted similar studies in the past - the influence of oligomers and polymers of ethylene glycols with a different molecular mass on the structural transformations of aqueous solutions of a surfactant - sodium pentadecylsulfonate (SPDS) depending on their content in the system. The studies were carried out by the methods of viscometry and light scattering [7]. It established that ethylene glycol with a molecular mass of 2,000 and 40,000 does not affect the structure

of the system. For ethylene glycol with a molecular mass of 4,000, 6,000, and 20,000, a clearly expressed complex course of the dependence of the intrinsic viscosity of the micelle system on the polymer content was established. It is assumed that with a change in the concentration of ethylene glycols in the system, micelles are compacted due to a change in the balance of hydrophobic-hydrophilic interactions. In parallel with the change in apparent micelle masses and asymmetry coefficients determined by the light scattering method, the intrinsic viscosity also changes depending on the composition of the system. The results of the study in the presence of 20 000 and 40 000 molecular masses of DNA in a DNA solution substantially exclude the interaction between SPDS and PEG molecules. The linear dependence of the viscosity of SPDS and PEG on the composition of the solution corresponds to the principle of additivity.

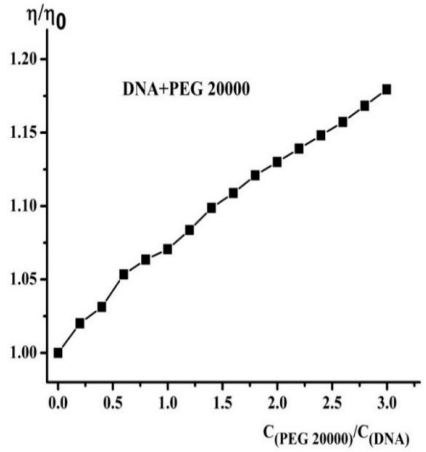

Fig. 4. Dependenceoftherelative viscosity of DNA of the concentration of ethylene glycol oligomers with molecular mass 120 000.

On fig. 4 shows the effect of 20, 000 molecular mass polyethylene glycol on the relative viscosity of the DNA solution. The results obtained practically exclude the presence of interaction between DNA and PEG molecules. The linear dependence of the viscosity of DNA and PEG on the composition of the solution corresponds to the principle of additivity.

It should be noted that we have already conducted similar studies in the past - the influence of oligomers and polymers of ethylene glycols with a different molecular mass on the structural transformations of aqueous solutions of a surfactant – sodium pentadecylsulfonate (SPDS) depending on their content in the system. The studies were carried out by the methods of viscometry and light scattering [7]. It established that ethylene glycol with a molecular mass of 2,000 and 40,000 does not affect the structure of the system. For ethylene glycol with a molecular mass of 4,000, 6,000, and 20,000, a clearly expressed complex course of the dependence of the intrinsic viscosity of the micelle system on the polymer content was established. It is assumed that with a change in the concentration of ethylene glycols in the system, micelles are compacted due to a change in the balance of hydrophobic-hydrophilic interactions. In parallel with the change in apparent micelle masses and asymmetry coefficients determined by the light scattering method, the intrinsic viscosity also changes depending on the composition of

the system. The results of the study in the presence of 20 000 and 40 000 molecular masses of DNA in a DNA solution substantially exclude the interaction between SPDS and PEG molecules. The linear dependence of the viscosity of SPDS and PEG on the composition of the solution corresponds to the principle of additivity.

The described circumstance allows us to be skeptical of the opinion spread in the literature, according to which the interactions observed in the DNA-PEG system are tried to be interpreted by the structural features of DNA.

Conclusion. The work is devoted to the elucidation of the pathways of PEG-DNA interaction. The results of studying the effect of oligomers and polymers of ethylene glycols of various molecular masses on the structural transformations of DNA, studied only by the viscometry method, are presented. It has been established that ethylene glycol oligomers do not affect the structure of DNA at all. In polymers of ethylene glycols (with molecular masses of 6000 and 7000), a well-pronounced complex character of the dependence of the intrinsic viscosity of DNA solutions on the content of the polymer has been established. It was assumed that with a change in the concentration of ethylene glycols in the system, a change in the structure of DNA macromolecules occurs due to a change in the balance of hydrophobic-hydrophilic interactions. The presence of 20,000 molecular mass of PEG in a DNA solution excludes the interaction of DNA and PEG molecules.

We hope that with further research we will be able to more clear:

- to find out the cause of a slight increase in the size of DNA macromolecules at initial doses of adding PEG of a certain molecular mass to its solution and to clarify the mechanisms of change in the size of macromolecules;
- clarify the range of PEG molecular mass values, in which changes in the size of DNA macromolecules are clearly observed.

Thus, these studies have demonstrated that viscometry is apparently a very cogent method for demonstrating the influence of small molecules for monitoring the structural changes in the solution of DNA using other methods if necessary to confirm the results obtained.

ACKNOWLEDGEMENTS

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