Binding of Poly(amido amine) Dendrimer on Sodium Poly-L-glutamate in Aqueous NaCl Solution†

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Binding of poly(amido amine) dendrimer on sodium poly-L-glutamate (NaPGA) in aqueous solutions of 0.01 and 0.1 M NaCl has been investigated by static light scattering. It was observed that the weight-average molecular weight and the radius of gyration increase sharply, when a certain molar ratio of dendrimer to NaPGA, N_{den}/N_{PGA}, was reached. The experimental results were simulated by using a model in which there was an equilibrium between dendrimers associated with NaPGA and those in solutions. The so-formed dendrimer−NaPGA complexes form aggregates in solutions through dendrimer junctions. The texture of such aggregates was observed by transmission electron microscopy. The aggregates bear a globular structure, the dimension of which was larger than that of a single NaPGA.

Introduction

The complex formation between large molecules such as protein−DNA and enzyme−substrate is a general phenomenon in living matter. Such complexation is important not only for molecular design of physiological phenomena but also for biological mimicry in basic science. Complexation of DNA with large molecules has special importance in gene delivery processes in mammalian organisms, and the complexation with polymers such as polylysine, poly(ethyleneimine), and poly(amido amine) (PAMAM) dendrimer has been investigated.[1] Since the dendrimer has a spherical architecture with explicitly regulated structure, it should be expected to behave like histone, which is known as a gene transport globular protein. Then, the in vitro investigation of DNA transfer in the living cells was carried out by using dendrimers.[2−9] The dendrimers interacting with DNA at an equinumber concentration of amino and phosphate groups form electroneutral interpolyelectrolyte complexes.[8] It has been reported that the concentration dependence of the interaction between polynucleotide and dendrimers depends on the dendrimer generation.[5] This behavior was compared with the Monte Carlo simulations, which were used to study the complexation between a polyelectrolyte and an oppositely charged spherical particle in the Debye−Hückel approximation.[10]

The binding of dendrimers to synthetic polyelectrolytes has also been investigated.[11−14] The complexation between polycations and carboxylated dendrimers occurred most readily for the 7.5th generation possessing high charge density[12] at a critical pH.[13] The interaction of poly(propyleneimine) dendrimer with linear polyanion has been compared with that of DNA.[14] By applying a theoretical model and computer simulation, the features of aggregates formed by charged dendrimers with oppositely charged polyelectrolytes and the conditions for their association were determined.[15] It was found that three different types of complexes were formed depending on the ionic strength of solution and the sizes of the dendrimer and polymer chain.

One of the authors of this paper (T.I.) has investigated with collaborators the complexation of sodium hyaluronate (NaHA) as a polyanion with a poly(amide amine) (PAMAM) dendrimer as a target molecule.[16] While NaHA held its wormlike character resembling that of NaHA in the absence of dendrimer upon the small addition of dendrimer in an aqueous NaHA solution, it behaved like a rigid rod at high dendrimer concentrations. It was also concluded that the hydrogen-bonding interaction along with the electrostatic interaction could be playing an important role for the NaHA−dendrimer complexation.

In the present work, the binding of PAMAM dendrimers on sodium poly-L-glutamates (NaPGA) was investigated by static light scattering that enabled us to evaluate the molecular weight, the radius of gyration of the formed complexes, the number of dendrimers bound to a NaHA, and the structure of complexes. Moreover, the dependence of the complexation on the mixing ratio was discussed and compared with the case of dendrimer−NaHA complexes.

Experimental Section

A methanol solution (10 wt %) of fourth-generation PAMAM dendrimer with NH_2 terminals (molecular weight 14 210, 64 terminal groups) was purchased from Aldrich. NaPGA (lot no. 350923, unit weight 151.1, unit contour length 0.362 nm) was a product obtained from Peptide Institute Inc. The weight-average molecular weight and radius of gyration obtained from static light scattering were 45 000 and 25.3 nm, respectively. Its degree of polymerization was 298. The chemical structures of PAMAM dendrimer and NaPGA are shown in Figure 1.

The solutions with different mixing ratios of dendrimer to NaPGA were prepared by mixing an aqueous 0.25 M NaCl solution of dendrimer (10 or 30 wt %), a 0.25 M NaCl solution of NaPGA (0.2 wt %), and a 0.25 M NaCl solution. Now, in the prepared mixture, the concentration of NaPGA was 0.01 or 0.1 wt %, and the concentration of NaCl was a constant equal to 0.25 M. The molar ratios of dendrimer to NaPGA, N_{den}/N_{PGA}, were varied up to 512, where the number ratio of the NH_2

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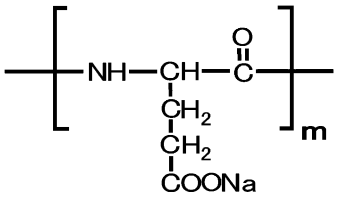
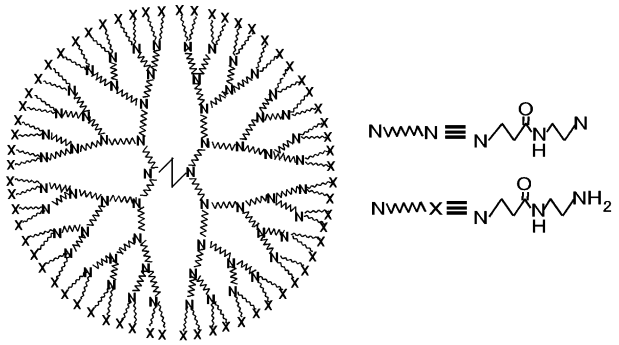


sodium poly-L-glutamate (NaPGA)



G4 poly(amido amine) dendrimer with amino terminals (den)

Figure 1. Chemical structures of a repeating unit of sodium poly-L-glutamate (NaPGA) (top) and a fourth-generation amino-terminated poly(amido amine) (G4 PAMAM) dendrimer (bottom).

terminal groups in dendrimer to the carboxylate groups in NaPGA, $[NH_2]/[COO^-]$, was 110.

Static light scattering was measured at 25 °C at 30° ∼ 150° scattering angles (through 10° interval) on an Otsuka Electronics DLS-700 spectrophotometer with an Ar ion laser at a wavelength of 488 nm. The weight-average molecular weight (M_w) and radius of gyration (R_G) were calculated by the equations

$$Kc/\Delta R_\theta = (1/M_w)(1 + R_G^2Q^2/3) + 2B_2c \quad \text{at low scattering angle} \quad (1)$$

$$Q = 4\pi n_0 \sin(\theta/2)/\lambda \quad (2)$$

where K is the optical constant, c is the total solute concentration, ΔR_θ is the reduced scattering intensity at scattering angle θ, Q is the scattering vector amplitude, B_2 is the second virial coefficient, n_0 is the refractive index of solvent, and λ is the wavelength of light in vacuo. Under the conditions of dilute solute concentration and high salt concentration in the present case, the second term in eq 1 is smaller than the first term, which allows not taking the second term into account. More detailed procedures of the measurements and analyses are described in the literature.[17]

Transmission electron microscopic (TEM) observation was carried out on a Hitachi H-800 electron microscope. An aqueous NaCl solution of dendrimer−NaPGA mixture was dropped on a carbon-coated copper grid and excess solution was drained. The dried grid was used for the TEM observation.

Results and Discussion

The weight-average molecular weight (M_w) and the radius of gyration (R_G) of mixed solutions of PAMAM dendrimer with NaPGA in 0.25 M NaCl evaluated by static light scattering are plotted as a function of N_{den}/N_{PGA} (or $[NH_2]/[COO^-]$) in Figure 2. The values of M_w and R_G increase slightly from those for a

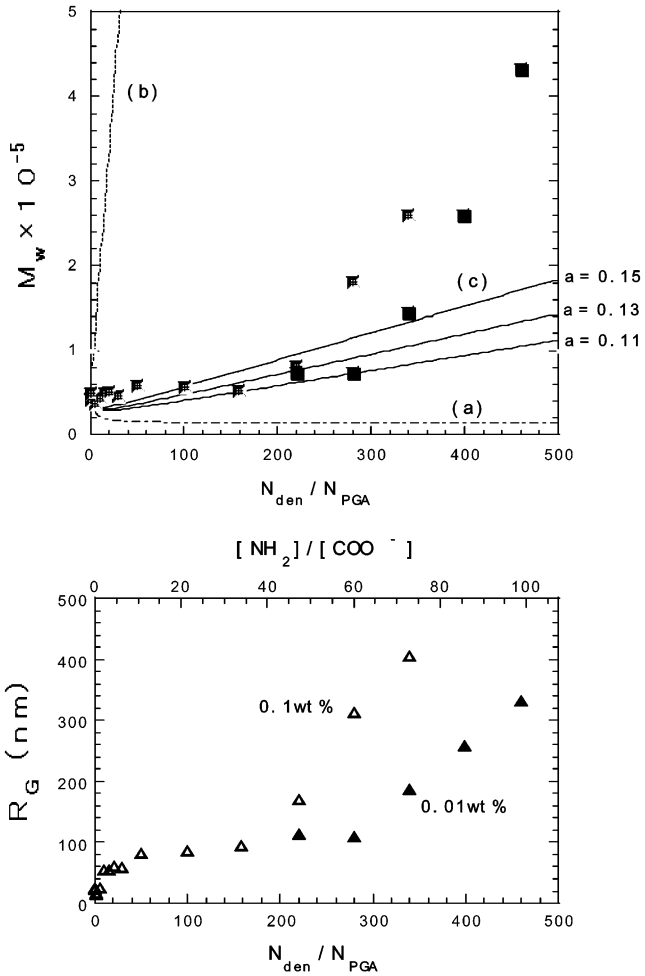


Figure 2. M_w and R_G for mixed solutions of PAMAM dendrimer and NaPGA in 0.25 M NaCl as a function of N_{den}/N_{PGA}. (Top) Observed M_w: ■, 0.01 wt % NaPGA; □, 0.1 wt % NaPGA. Calculated M_w: (a) a nonbinding model; (b) an average binding model; (c) a distribution-binding model at $a = 0.11$, 0.13, and 0.15. (Bottom) Observed R_G: ▲, 0.01 wt % NaPGA; △, 0.1 wt % NaPGA.

solution of NaPGA without added dendrimer up to certain critical ratios of N_{den}/N_{PGA} and then rise steeply, indicating complexation. The critical ratios for 0.01 and 0.1 wt % NaPGA are 300 and 220, respectively, thus implying the dependence of the aggregation on the mixing ratio and NaPGA concentration.

If dendrimers are mixed with NaPGA without any interaction between them, as illustrated in Figure 3a, the weight-average molecular weight of the mixture ($M_{w,nb}$) is described by

$$M_{w,nb} = \frac{N_{PGA}M_{PGA}^2 + N_{den}M_{den}^2}{N_{PGA}M_{PGA} + N_{den}M_{den}} \quad (3)$$

where N_i and M_i are the number and weight, respectively, of molecule i. The subscripts i = PGA and den denote NaPGA and PAMAM dendrimer, respectively. This "nonbinding model" is not the case of the mixture of PAMAM dendrimer and NaPGA in the present work, as seen in Figure 2.

It is reasonable to consider a model in which cationic PAMAM dendrimers are adsorbed on an anionic linear polymer chain through electrostatic interaction. It was shown previously that sodium hyaluronate could form a complex with the PAMAM dendrimer binding a certain average number of dendrimers.[16] The same "average binding model" (Figure 3b)

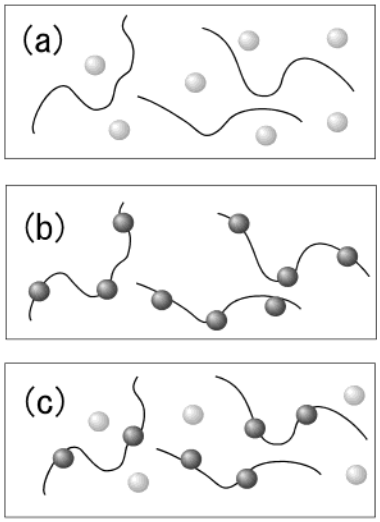


Figure 3. Schematic presentation of binding models of dendrimers on NaPGA chains: (a) a nonbinding model, (b) an average binding model, (c) a distribution-binding model.

is applicable to the mixture of PAMAM dendrimer and NaPGA. The weight-average molecular weight ($M_{w,ab}$) in the framework of this model can be written as

$$M_{w,ab} = \frac{N_{PGA}\{M_{PGA} + (N_{den}/N_{PGA})M_{den}\}^2}{N_{PGA}\{M_{PGA} + (N_{den}/N_{PGA})M_{den}\}} = M_{PGA} + (N_{den}/N_{PGA})M_{den} \quad (4)$$

The M_w values based on eq 4 are far larger than observed ones, as seen in Figure 2.

The third possibility can be presented by a "distribution-binding model", in which a part of dendrimers are associated with NaPGA and others are in solution. This is schematically presented in Figure 3c. If we denote "*a*" as the distribution-binding coefficient (=the number fraction of dendrimer bound on NaPGA in the total number of dendrimers), the weight-average molecular weight can be given as

$$M_{w,db} = \frac{N_{PGA}\{M_{PGA} + a(N_{den}/N_{PGA})M_{den}\}^2 + (1-a)N_{den}M_{den}^2}{N_{PGA}\{M_{PGA} + a(N_{den}/N_{PGA})M_{den}\} + (1-a)N_{den}M_{den}} \quad (5)$$

The M_w values calculated for $a = 0.11$, 0.13, and 0.15 were fitted well to the experimentally observed values up to the critical mixing ratios N_{den}/N_{PGA} of 300 and 219 for 0.01 and 0.1 wt % NaPGA solutions, respectively. However, a deviation of calculated values from experimental ones was found beyond the critical mixing ratio. This is obvious from Figure 2.

The sharp increase of observed molecular weight beyond the critical mixing ratio could be explained by the aggregation of dendrimer−NaPGA complexes. This will be called an "aggregation model". The aggregation starts at a critical mixing ratio X to form *i*-mers such as dimer, trimer, and tetramer, as illustrated in Figure 4. The fraction of *i*-merized complexes in aggregates of $(2 \sim n)$-mer is denoted as f_i (=the number fraction of $(i-1)$-mers that formed *i*-mers of the total $(i-1)$-mers), and it is in proportion to the binding ratio $(N_{den}/N_{PGA} - X)$ with proportional coefficient A_i. Then, the weight-average molecular weight can be calculated by using the following equation

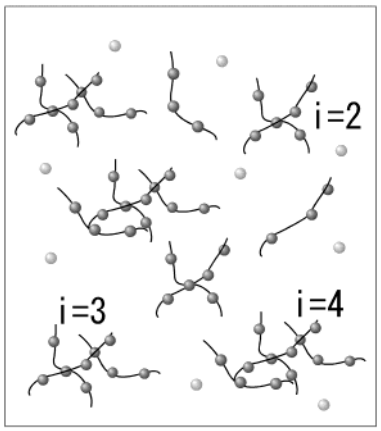


Figure 4. Schematic presentation of an aggregation model.

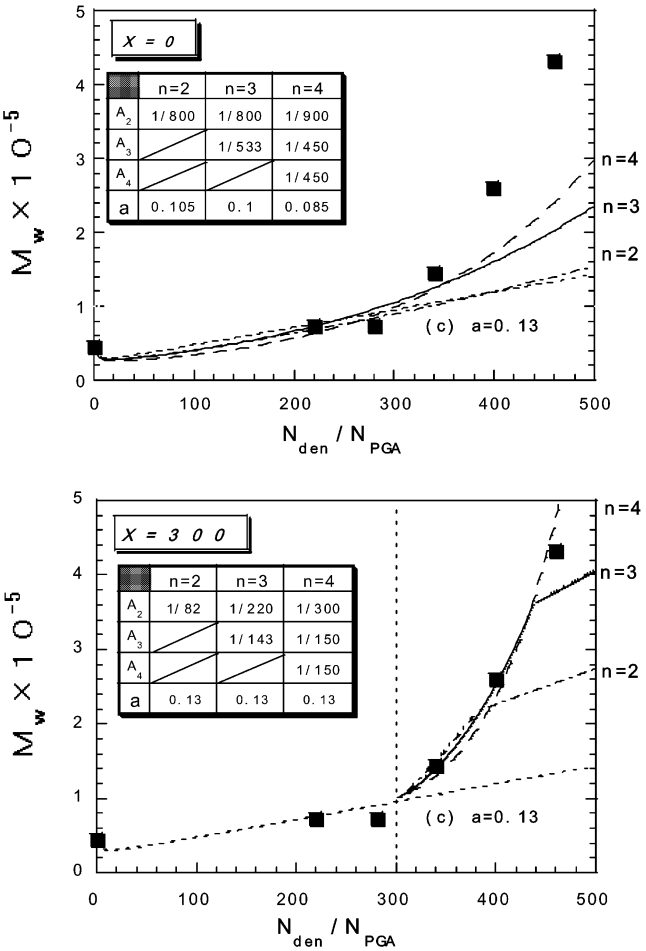


Figure 5. Calculation of M_w for mixed solutions of PAMAM dendrimer and NaPGA in 0.25 M NaCl as a function of N_{den}/N_{PGA} on the basis of the aggregation model. NaPGA concentration, 0.01 wt %. ■, observed. The optimum parameters for the best fitting are included in the figures.

$$M_w = \frac{\{1 + \sum_{i=2}^{n}(i-1)\prod_{i=2}^{n}f_i\}N_{PGA}\{M_{PGA} + a(N_{den}/N_{PGA})M_{den}\}^2 + (1-a)N_{den}M_{den}^2}{N_{PGA}\{M_{PGA} + a(N_{den}/N_{PGA})M_{den}\} + (1-a)N_{den}M_{den}} \quad (6)$$

where

$$f_i = A_i\{(N_{den}/N_{PGA}) - X\} \quad (7)$$

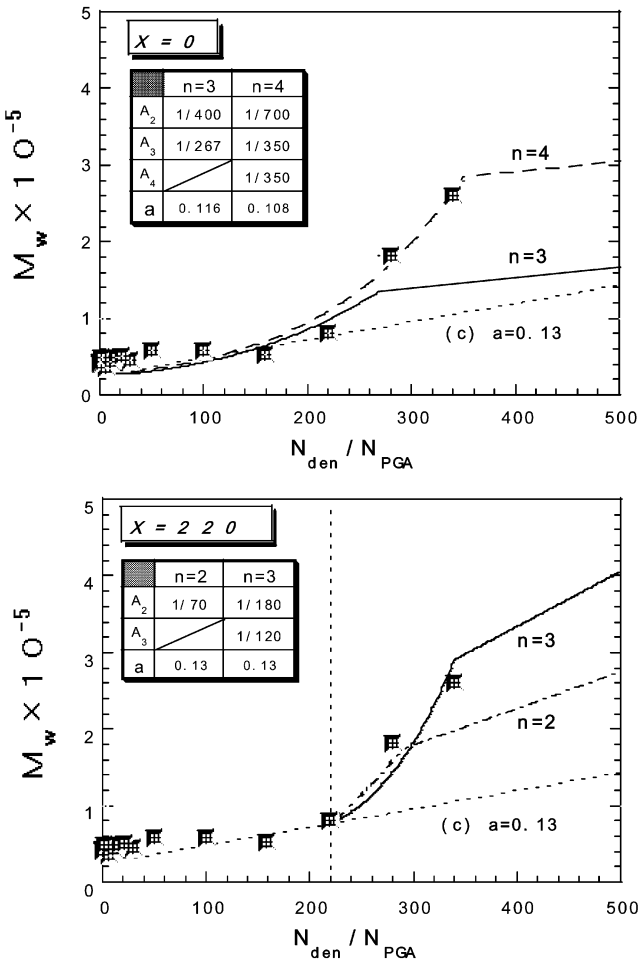


Figure 6. Calculation of M_w for mixed solutions of PAMAM dendrimer and NaPGA in 0.25 M NaCl as a function of N_{den}/N_{PGA} on the basis of the aggregation model. NaPGA concentration, 0.1 wt %. □, observed. The optimum parameters for the best fitting are included in the figures.

The weight-average molecular weights calculated in accordance with the aggregation model by using $X = 300$ for 0.01 wt % NaPGA or 220 for 0.1 wt % NaPGA are compared with experimental ones in Figures 5 and 6. For the comparison, the calculation was also carried out for $X = 0$, where the aggregation of complexes occurs along with the complexation of added dendrimer. The parameters A_i and "a" at the optimum fitting to the experimental data are shown in the figures. As seen in Figures 5 and 6, i-mers at least larger than trimer and dimer, respectively, are formed in 0.01 and 0.1 wt % NaPGA solutions. It is also apparent that the distribution of i-mer depends on the mixing ratio of dendrimer to NaPGA. Thus, at the same mixing ratio, larger i-mers are formed in more NaPGA concentrated solution.

TEM photographs were taken for specimens prepared from solutions of dendrimer−NaPGA mixture (Figure 7). Globular particles can be seen in the photographs. The particle size is larger for 0.1 wt % NaPGA solutions at $N_{den}/N_{PGA} = 335$ and 420 than at 168 and 250. This indicates that there is an increase in the aggregate dimension of dendrimer−NaPGA complexes. Similar difference in particle size was also observed between $N_{den}/N_{PGA} = 335$ and 420 in the case of 0.01 wt % NaPGA. This fact confirms that the aggregation of the dendrimer−NaPGA complexes occurs dominantly beyond the critical mixing ratio.

The structure of dendrimer−NaPGA complex is schematically illustrated in Figure 8. The aggregate consists of many dendrimers and a few PGA chains. Dendrimers interact electrostatically with functional groups of PGA macromolecule. When a dendrimer bound on a PGA chain interacts with another PGA chain, the dendrimer acts as a junction bridge, connecting two PGA chains. This leads to the cross-linking of PGAs by dendrimers to form a network.

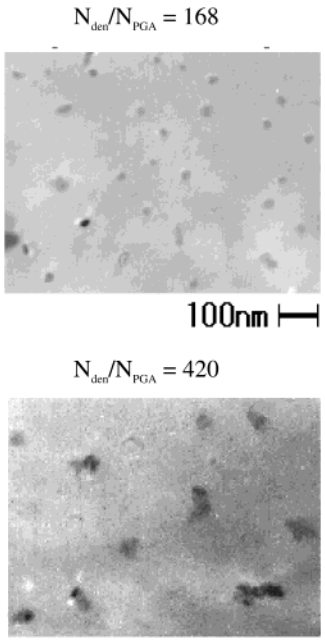


Figure 7. TEM photographs of specimens prepared from mixed solutions of PAMAM dendrimer and NaPGA in 0.25 M NaCl at $N_{den}/N_{PGA} = 168$ and 420.

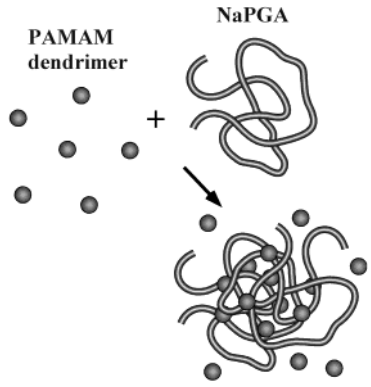


Figure 8. Schematic representation of aggregate formation of PAMAM dendrimer and NaPGA.

It is interesting to compare aggregates of PAMAM dendrimer and NaPGA with those of PAMAM dendrimer and hyaluronate studied before.[16] Both the linear polymers are anionic, interacting electrostatically with cationic dendrimers. In addition, there is a formation of hydrogen bonds between PAMAM dendrimer and hyaluronate that differs from the complex examined in the present work. Thus, the hyaluronate surrounded by bound dendrimers has the extended dimension. The complex formation was also studied for a dendrimer−DNA mixture. It was found that a DNA macromolecule surrounds a dendrimer of high generation, forming a nucleohystone-like complex with the dendrimer.

Conclusions

The binding of cationic PAMAM dendrimers on anionic NaPGAs was investigated in aqueous solutions. A complex of NaPGA with PAMAM dendrimers was formed with the addition

of dendrimer. At high mixing ratios, N_{den}/N_{PGA}, aggregation of complexes takes place. The formed aggregates consist of oligomers of complexes. Both the complexes and aggregates have globular structure, owing to the random-coiled structure of NaPGA.

The association of dendrimers with NaPGA differs from that of DNA and NaHA. Dendrimers interact with NaHA not only electrostatically but also through the formation of hydrogen bonds that results in the conformation change of NaHA from wormlike to rigid rod. On the other hand, DNA binds on high-generation dendrimer, surrounding it. Complexes of dendrimers with DNA further aggregate.[18] The established differences of binding behavior are caused by the structure and nature of polylelectrolytes.

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