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단백질흡착에 있어서 표면작용기의 영향

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The Effects of Surface Functional Groups to Protein Adsorption

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ABSTRACT

The adsorption characteristics of bovine serum albumin(BSA) on the modified carbon fiber and cellulose surfaces were investigated. In order to define the effects of solid surface characteristics on protein adsorption, surfaces of carbon fiber and cellulose were modified by physical and chemical treatment. The amounts of BSA adsorbed onto various solid surfaces were evaluated by batch method under various pH and ionic strength. The amount of adsorbed BSA was highly dependent on pH as well as surface functional groups.

INTRODUCTION

Recently protein adsorption on a solid surface became very interesting and important fields in relation with biological, medical, and biotechnological processes. Mammalian and bacterial cell adhesion to solid surface, initiation of blood coagulation and protein binding to cell surface receptors are related with protein adsorption as basic phenomena, and contact lens fouling, fouling of equipment in food processing industries and foaming of protein solutions are also related with protein adsorption in practical applications.

From the many previous studies[1-5], it was found that protein adsorption tendency onto solid surface depended on mainly three factors, i. e. the solution conditions such as pH and ionic strength, characteristics of solid surface such as surface functional group and hydrophobicity or hydrophilicity, and properties of protein in solution. Lee and Ruckenstein[6] showed that characteristics of solid surface is very influential to the amount of protein adsorbed onto solid surfaces in these three factors. On the other hand the properties of protein in solution was reported as decisive factor in determining of adsorption characteristics[7]. These conflicted results on protein adsorption onto solid surface are mainly due to difference of raw materials used in protein adsorption experiments.

In this experiment we used the carbon fiber and cellulose as solid surface which have homogeneous composition as basal plane. The carbon fiber and cellulose do not have any intraparticle pore. Therefore it is very simple and clear to interpret the influence

of surface characteristics in adsorption of BSA.

The objectives of this study are to define the influence of surface characteristics especially functional group of solid surface and hydrophobicity or hydrophilicity on protein adsorption through experi-mentally decided the adsorption equilibrium relations. We discuss here the effect of the surface total acidity, cationic of anionic surface on the BSA adsorption.

MATERIALS AND METHODS

Materials

The surfaces of carbon fiber and cellulose used in this work were listed in Table 1 and 2. Carbon fiber was provided from Japan Kynol Co. and 9 kind of cellulose samples from CE-A to CE-I were purchased from each maker and the other samples from CE-J to CE-L were prepared by the batch reaction method in laboratory as shown in Table 2. The raw material of carbon fiber is the phenol resin and its diameter is 11.5 μm. As a result seven kind of surface-modified carbon fibers and twelve kind of surface-modified celluloses as adsorbents were prepared for BSA adsorption experiment.

All the celluloses were washed by carbon tetrachloride in Soxlet apparatus for 3 hours and dryed at 80℃. After drying, the cellulose samples were washed by flowing deionized water in a column. Bovine Serum Albumin was purchased from Wako Chemical Co. Japan and used without further purification.

Table 1. Preparation method of modified-surface of carbon fiber.

Sample Name	Preparation Method
CF-A	Boiling at 100℃ for 2 hours
CF-B	Hydrogenation of CF-A at 1000℃ for 2 hours
CF-C	Hydrogen peroxide oxidation of CF-B at 100℃ for 2 hours
CF-D	Hydrogen peroxide oxidation of CF-B at 50℃ for 0.5 hours
CF-E	Hydrogen peroxide oxidation of CF-A at 100℃ for 3 hours
CF-F	Air oxidation of CF-A at 350℃ for 3 hours
CF-G	Amination of CF-A

All other chemicals were of analytical grade. Distilled and deionized water was used in the all experiments.

The surface acidity of the modified carbon fiber was determined by the method of Bohem[8]. Sodium bicarbonate($NaHCO_3$), sodium carbonate(Na_2CO_3), sodium hydroxide(NaOH), and sodium ethoxide ($NaOC_2H_5$) were used as titration bases. One gram of each sample was placed in a 100ml flask and 100ml of excess bases of 0.02N solution were added

Table 2. Preparation method of modified-surface of carbon fiber.

Sample Name	Chemical Nomenclature	Maker
CF-A	Cellulose	Merk
CF-B	Acetylcellulose	Kanto Chemical
CF-C	Acetylbutyrylcellulose	Kanto Chemical
CF-D	Carboxylmethylcellulose	Serva Co.
CF-E	Cellulose phosphate	Sigma
CF-F	Diethylaminoethyl cellulose	Sigma
CF-G	Epichlorohydrine Triethanol-amine Cellulose	Sigma Sigma
CF-H	Polyethyleneimine Cellulose	
CF-I	Oxidation of CF-A by $NaIO_4$	
CF-J	Reduction of CF-I by $NaBH_4$	
CF-K	Oxidation of CF-I by $HClO_2$	
CF-L	Sulfonation of CF-I by $NaHSO_3$	

Table 3. The concentration of surface total acidity on modified carbon fiber.

Sample/Group Type	I	II	III	IV	Total
CF-A	—	0.012	0.002	—	0.014
CF-B	—	—	0.005	—	0.005
CF-C	0.010	0.037	0.020	0.003	0.070
CF-D	—	0.020	0.010	—	0.030
CF-E	—	0.020	0.010	—	0.030
CF-F	—	0.019	0.002	—	0.021
CF-G	—	—	—	—	—

I : Carbonyl Group
II : Carboxyl group that occurs as a lactone
III : Phenolic hydroxyl group
IV : Carbonyl group that reacts with the carboxyl group II to form the lactone(or lactol)

into the flask. The mixture was shaken for 24 hours in the thermostat(293K). Then 1ml of the supernatant was pipetted and neutralized with excess hydrochloric acid solution up to pH 5.0 and back-titrated with 0.005N NaOH solution. The analyzed results of carbon fiber surface acidity were listed in Table 3.

Methods

Adsorption isotherms were determined at 293K by treating each samples with various concentrations of BSA solution. Preliminary experiments showed that 5 hours was sufficient to reach equilibrium at pH 5.0. After reaching equilibrium state between solid surfaces and BSA solution, the sample solution was centrifuged at 10^5rpm. The concentration of BSA in the supernatant was determined by the UV method at 280nm. The amount of sample carbon fiber or cellulose weighed was placed in a flask. The amount of BSA solution weighed was added in the flask. The flask was shaken at 293K thermostat until it reached equilibrium. From material balance between initial and final concentration of solution, the amount of the adsorbed BSA per square meter was calculated.

Sodium phosphate and acetic acid buffer solution were employed for pH adjustments ranged from 6.0 to 8.0 and from 3.8 to 5.5, respectively.

RESULTS AND DISCUSSION

Adsorption Isotherms

Adsorption isotherms for BSA had been determined at various ionic strengths and pHs. Examples of isotherms for BSA on carbon fiber are shown in Fig. 1-3 and that of on cellulose in Fig. 4. In these figures the amount of BSA adsorbed(expressed in mg per square meters of adsorbent surface) was plotted against the BSA equilibrium concentration in solution. Ionic strength of solution was adjusted in three ways that is, 0.001, 0.01 and 0.1M and pH of solution was adjusted around 3.8, 4.8 and 6.0 for BSA adsorption measurement. From the results of adsorption isotherms of BSA, each isotherms shows a sharp initial rise. This means of a high affinity between the BSA and the adsorbent surface. The

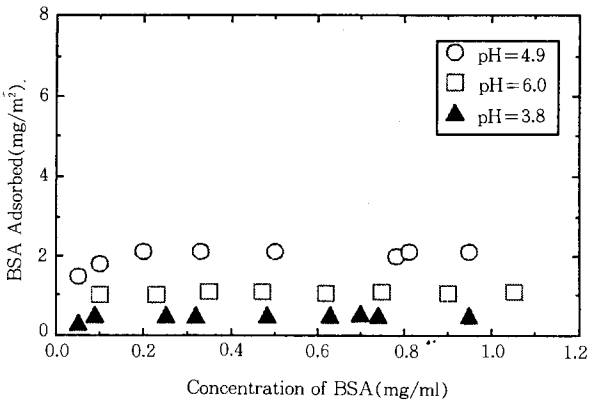

Fig. 1. Adsorption isotherms of BSA onto CF-A at IS= 0.01M and different pH(293K).

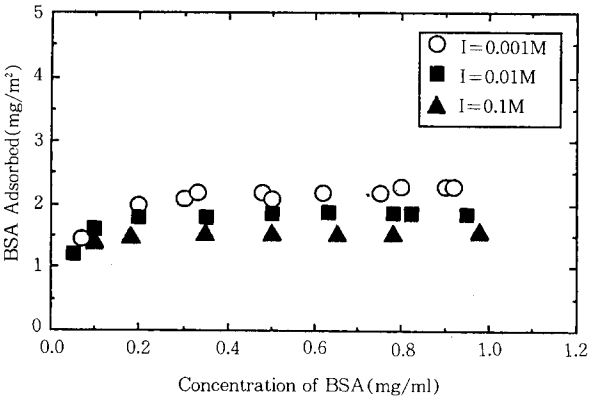

Fig. 2. Adsorption isotherms of BSA onto CF-A at pH= 4.9 and different ionic strength(293K).

adsorption tendencies of BSA onto carbon fiber and cellulose are very similar in spite of a big difference of surface characteristics. However amounts of BSA adsorbed onto each sample were greatly different. This means that the adsorption amount of BSA is strongly affected by the characteristics of solid surfaces as well as pH and ionic strength in solution.

The adsorption saturation values of BSA on all kind of solid surfaces reached around BSA concentration of 0.3mg/ml in solution. The saturation values of the sample surfaces are also strongly dependent on the pH and ionic strength. From the results of isotherm figures, the apparent adsorption isotherm relation shown as the Langmuir type, but it is very

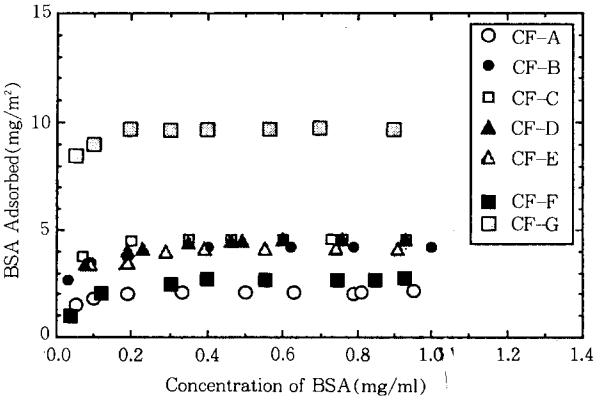

Fig. 3. Adsorption isotherms of BSA onto CF-A, B, C, D, E, F, G at IS=0.01M and pH=4.8(293K).

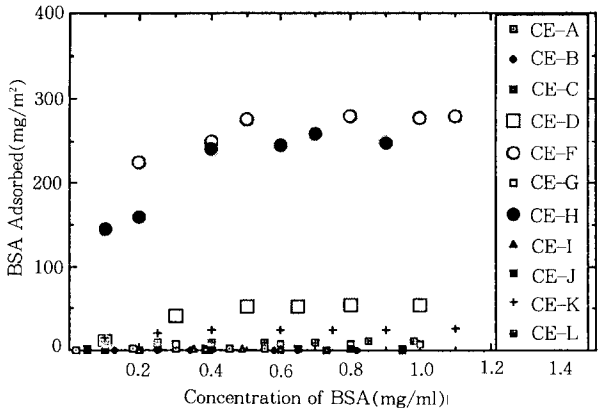

Fig. 4. Adsorption isotherms of BSA onto CE-A to CE-L at IS=0.001M and near pH=4.8(293K).

difficult to treat as Langmuir isotherm equation because of unsatisfying preconditions for the Langmuir equation.

pH and Ionic Strength Effects

Fig. 5 and 6 show the influence of pH and ionic strength on the adsorption of BSA onto surfaces of carbon fiber and cellulose, respectively. The value of pH is ranged from 3 to 9. The amount of BSA adsorbed show a maximum value near the isoelectric point of BSA. It is regarded as quite natural phenomena in protein adsorption[9]. The amount of adsorbed BSA in the alkaline region was increased with increasing ionic strength, which showed similar

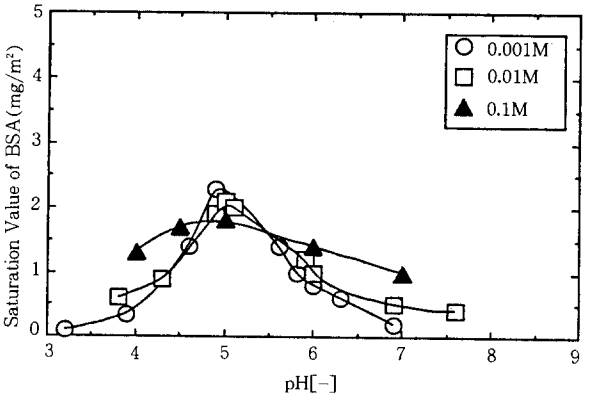

Fig. 5. pH dependence of saturation value of BSA on adsorption CF-A at different IS and equilibrium conc. 1mg/ml(293K).

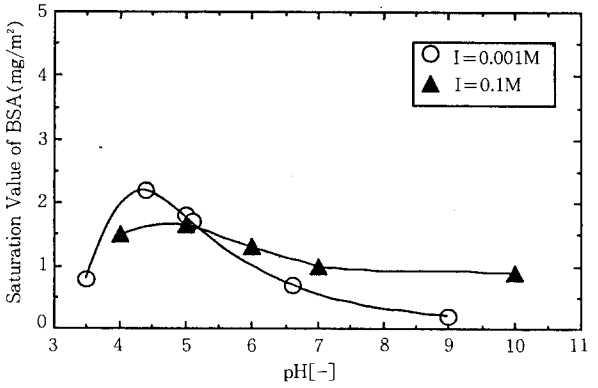

Fig. 6. pH dependence of saturation value of BSA adsorption on CF-A at different IS and equilibrium conc. 1mg/ml(293K).

feature in the acid region.

The carbon fibers are expected to carry a net negative charge at the pH ranged in these experiments. Carbon fiber surface is reported having several negative charged functional groups such as carboxyl, carbonyl and phenolic hydroxyl group[8]. Where the pH is below region of the isoelectric point of BSA, carbon fiber negatively charged and the BSA molecules are positively charged. Therefore, electric static attaction between the carbon fiber and the BSA molecules may cause increase the adsorption of BSA. But in the most cases reported in literatures the adsorption of BSA decreased with pH in this region

[1, 2]. This opposite adsorption phenomena can be explained due to expansion of BSA molecule. Therefore, wider surface area is required for adsorption of the expanded BSA molecule

In the region of pH greater than the isoelectric point, the carbon fiber would be negatively charged and the BSA molecules are also negatively charged, electric static repulsion force between the carbon fiber and the BSA molecules may cause decrease the adsorption of BSA. The experimental results showed the similar tendency in all 18 samples, but the saturation values were gratly different one another.

Effects of Surface Hydrophobicity or Hydrophilicity

The order of hydrophobicity of the modified surface of carbon fiber judged from the contact angle between sample surface and water is CF-B>CF-A> CF-D>CF-C. Fig. 7 showed in BSA adsorption onto the six kinds surface of carbon fiber listed in Table 1. In comparison of hydrophobicity among above 4 samples, the CF-B has the highest hydrophobic surface and CF-C has the highest hydrophilic surface. Generally it is reported that the hydrophobic surface is more favorable to adsorption of protein than the hydrophilic surface[10]. But in these experiments the amounts of adsorbed BSA showed almost the same value. Therefore the influence of hydrophobicity of carbon fiber surfaces on BSA adsorption would be hardly discernable. The similar phenomena can be found else where[11]. This means the hydrophobicity of the solid surface can not be so influential to adsorption of protein from solution for the same raw materials.

The effects of Surface Functional Group

Fig. 8 shows the relation between surface total acidity and the amounts of BSA adsorbed. The ionic strength is ranged from 0.001 to 0.1M. The changing tendency of saturation value of BSA against surface total acidity in each ionic strength value was very similar as shown in this figure. The amount of adsorbed BSA was increased up to 0.05me/g of surface total acidity in each ionic strength.

The amount of BSA at the saturation were 6.0, 4. 8 and 3.8mg/m^2 for ionic strength 0.001M, 0.01M and 0.1M, respectively. By assuming monolayer coverage adsoption on solid surface, each saturation value corresponded on solid surface, each saturation value corresponded to 18.5, 24.2 and 29.3nm^2/molecule, respectively. These results indicate that conformation of BSA molecule adsorbed on the solid surface can be changed with ionic strength in solution.

On the other side if the conformation change of BSA were not affected by the ionic strength near the isoelectric point of BSA and surface functional group as evaluated in total acidity, the saturation value would be independent on not only the ionic strength but also concentration of surface acidity.

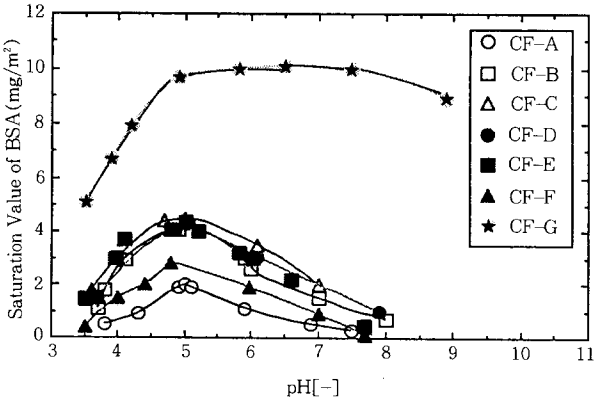

Fig. 7. pH dependence of saturation value of BSA adsorption on CF-A, B, C, D, E, F, G at IS=0.01M and equilibirum conc. 1mg/ml(293K).

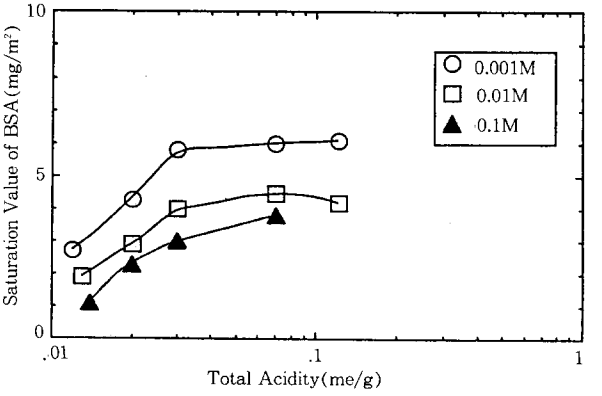

Fig. 8. Corelation between total acidity and saturation value of BSA adsorption at different IS and equilibrium concentration 1mg/ml(293K, pH=4.8).

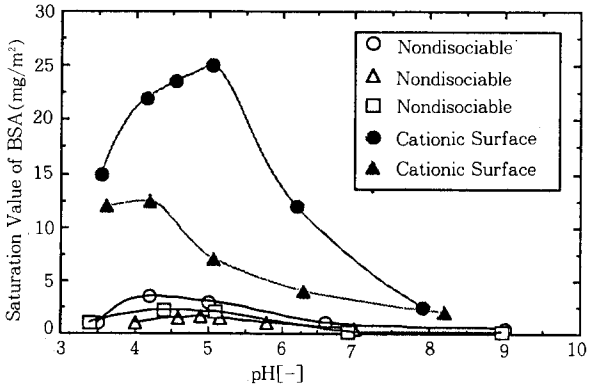

Fig. 9. pH dependence of saturation value of BSA adsorption onto cellulose derivatives at IS= 0.01M293K).

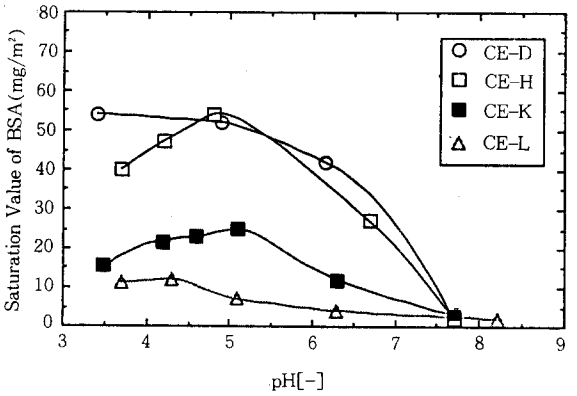

Fig. 11. pH dependence of saturation value of BSA adsorption on CE-D, H, L, K at IS=0.01M (293K).

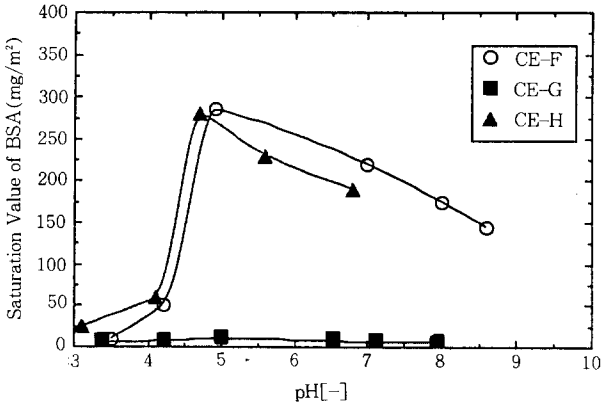

Fig. 10. pH dependence of saturation values of BSA adsorption onto CE-F, G, H at IS=0.01M.

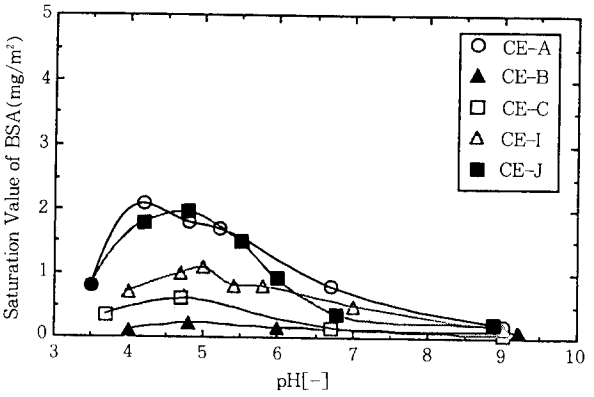

Fig. 12. pH dependence of saturation value of BSA adsorption for nondissociable surface functional group at IS=0,001M(293K).

However as shown in Fig. 6 the saturation value was highly dependent on surface acidity as well as the ionic strength. This fact can be illustrated as conformation of BSA is highly affected by surface functional group. Another experiment results which indicated the similar tendency as Fig. 8 were shown in Fig. 9. This figure showed the effect of functional group of cellulose surface to BSA adsorption. Each cellulose surface was modified by each functional group as listed in Table 2. The order of saturation value of BSA adsorption at pH 4.8 equivalent to isoelectric point of BSA was given -COOH(CE-K)>-SO_3H(CE-L)>-CHO(CE-I), =-OH(CE-J). Consequently protein adsorption can be greatly affected by functional group of solid surface as shown in this results.

Fig. 10 and 11 shows the effects of surface cationic or anionic groups on the adsorption of BSA, respectively. In case of anionic group on the surface, the amount of BSA adsorbed was increased greatly up to 280mg/m². As shown in these two figures, saturation values of BSA is greatly affected by the kind of functional group on the surface of cellulose such as cationic or anionic.

Fig. 12 shows the effects of dissociability of sur-

face functional group on the adsorption of BSA. Each sample CE-A, B, C, I and J indicated this figure does not have dissociable functional group. In comparison with the adsorption experiment results of Fig. 10 and 11 which have dissociable functional group on the surface such as cationic or anionic, the amount of adsorbed BSA was very low. From this difference of adsorbed amout of BSA we could say that dissociability of surface functional group influenced crucially to adsorbability of BSA on the solid surface.

국문요약

단백질흡착에 있어서 흡착제 표면의 작용기의 영향을 알아보기 위하여 송아지혈청알부민(BSA)을 모델단백질로 선택하여 흡착실험을 하였다. 흡착제로는 표면이 균일하게 이루워진 탄소섬유와 셀루로즈를 선택하였다. 표면의 작용기를 표면개질반응에 의해 여러 가지 형태로 변화시켜서 이에 따른 흡착량의 변화를 조사하였다.

본 실험결과에 의하면 용액의 성질 즉, pH, 이온강도 등에 의해서도 영향을 받지만, 흡착제의 표면성질, 즉 소수성과 친수성, 표면의 작용기의 종류에 따라서 단백질의 흡착량이 크게 영향을 받는다는 것이 본 연구에 의해 정량적으로 밝혀졌다.

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