Mutual Effect of the Interaction of Human Serum Albumin with Cellulose in Water

Nina E. Kotelnikova*, Olga V. Lashkevich, Evgeny F. Panarin

Institute of Macromolecular Compounds, Russian Academy of Sciences,
Bolshoy pr. 31, St. Petersburg, 199004 Russia

SUMMARY: Adsorption of human serum albumin to microcrystalline cellulose interface has been determined as functions of protein concentration and pH of the aqueous solutions. The maximum adsorption value is reached at the protein isoelectric point. The study of adsorption at several values of pH indicates that its interaction with the MCC interface is not controlled by the electrostatic effect. The FTIR, ^{13}C NMR data reveal that human serum albumin denaturates at its IEP and its macromolecules become extended. Resulting intercalates consist of microcrystalline cellulose, albumin, and solvent.

Introduction

The adsorption of proteins to solid interfaces and interactions between surfactants and proteins at liquid/solid interfaces are processes of significance to our daily lives and in such fields as medicine, food processing, biotechnology, etc. Protein adsorption is also scientifically intriguing.

The use of cellulose, a natural polymer, as a carrier for biologically active compounds is widely known (Ref. 1). However, pure cellulose has limited application as a matrix for proteins in spite of the fact that the combination of this natural polymer and some proteins can be promising for various medical applications.

Powder microcrystalline cellulose (MCC) is used for the manufacture of drug forms of many preparations as well as a dietary additive and as an independent agent in the treatment of some gastrointestinal diseases (Ref. 2). This is due to its valuable sorption properties. Moreover, controlled drug release from MCC provides a possibility of developing on its basis drugs with a prolonged activity and regulated discharge of the drug component.

Human serum albumin (HSA) is one of the most important transfer proteins. It is known that HSA adsorption from solutions to solid interfaces is a complicated process and adsorption value depends on many factors. The most important of them are surface properties of adsorbent, such

 CCC 1022-1360/00/$ 17.50+.50/0

as, its chemical composition, hydrophilicity or hydrophobicity, its surface charge and specific area, and porosity.

The goal of this paper is to obtain compatible polymer system on the basis of diffusion-adsorption interaction between MCC (adsorbent and matrix) and HSA. Our results on HSA adsorption from aqueous solutions to the MCC interface are presented at various values of pH and concentration of HSA solutions. The result of HSA capacity to be released from the interface appears to be also an important result of this study as for fundamental understanding of HSA adsorption to solid interface as to clarify our consideration on possible application of this system.

Experimental

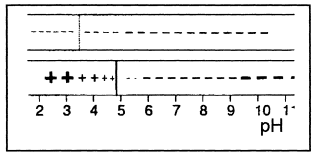
Microcrystalline cotton cellulose had particle size < 0.05 mm and was bone-dried up to moisture content not exceeding 1.0 wt %. The DP_v of MCC was 170. Pore volume, pore radius, specific area determined by mercury porosimetry were 2.16 cm^3/g, 20 mcm, and 0.5 m^2/g, respectively. MCC macromolecules are negatively charged at the range of pH 3.0-10.0 due to its carboxylic groups. Its isoelectric point (IEP) is lower than 4.0 (Ref. 3).

Human serum albumin was analytical grade and its MM is 68000. Its IEP is 4.8. HSA macromolecules are positively charged in solutions at pH<IEP, and negatively charged at pH>IEP (see scheme I).

Scheme I. Charge dependence on pH

Microcrystalline cellulose

Human serum albumin



Adsorption interaction of MCC with HSA in water at pH 6.2 (to keep the system as simple as possible, the use of buffer in the case of pH 6.2 was avoided) and in acetate buffer solution at pH 4.8 (HSA isoelectric point) and HSA desorption in water were carried out as follows. To obtain kinetic relationships, MCC feeds (0.5 g) were treated with HSA solutions (mass ratio 1:50) at different concentrations (0.3-9.7 mg/ml) under constant stirring for a time from 30 min to 24 h. Adsorption equilibrium was reached within 2 hours. The experiments were carried out at a temperature 15°C up to decantation of the supernatant obtained by vacuum filtration. An aliquot of the supernatant (0.5 ml) was taken to determine the equilibrium concentration of albumin solution after adsorption (C_{eq}). The initial HSA concentration before adsorption (C_0) as well as C_{eq} were estimated by the Lowry method (Ref. 4). The solid rests

were vacuum-dried to constant weight at 40°C. Adsorption values were calculated from difference between C_0 and C_{eq}.

The resulting samples containing adsorbed albumin were subjected to desorption. The adsorbate feed (0.2 g) was taken into water at pH 6.2 (mass ratio 1:50) and kept at 20°C for 24 h. It was established that desorption attains equilibrium during this time. Then the excess solutions were removed by vacuum filtration and the residue was vacuum-dried to constant weight at 40°C. An aliquot of solution was analysed by the Lowry method as in the case of adsorption. The amount of desorbed HSA was calculated in mass % to the content of adsorbed HSA. The weight method for estimation of adsorption and desorption values was also applied to compare with results obtained by the Lowry method.

The properties of the HSA and of MCC-HSA adsorbates in solid state were studied by FTIR and ^{13}C NMR spectroscopy as described elsewhere (Ref. 1). SEM study was performed to characterise the changes of the MCC surface after adsorption and desorption. Samples were previously covered by platinum and then observed in the raster electron microscope (Hitachi S 510).

Results and Discussion

Adsorption of HSA to the MCC interface

Kinetic dependencies of HSA adsorption values (AV, mg/g) on MCC at different concentrations of HSA initial solutions (Fig. 1.A) have a typical shape for adsorption processes taking place from solution and occurring on porous matrices and consist of two main stages: the first one passing at a high rate and the second one proceeding at a slow rate. The adsorption values depend on HSA concentration. The maximum AV at pH 6.2 ranges from 13 mg/g (at initial HSA solution concentration C_0=0.6 mg/ml) to 69 mg/g (at C_0 =9.7 mg/ml), e.g when C_0 increases 1.6 times, the maximum AV increases more than five times. The maximum AV at pH 4.8 ranges from 14 to 89 mg/g at the same concentration of initial solutions, e.g the maximum AV increases more than six times.

Dependence of AV on pH exhibits a hyperbola shape over all concentration ranges (Fig. 2). AV is markedly low at pH 3.56 (3.0 mg/g and 10.0 mg/g at C_0 1.8 and 4.8 mg/ml, respectively) and pH 8.0 (8.0 and 10.0 at C_0 1.8 and 4.8 mg/ml, respectively). Thus, the maximum AV is achieved at HSA isoelectric point. These results confirm some previously obtained data (Ref. 5, 6).

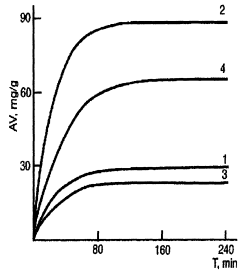


Fig. 1.A. Kinetic dependencies of HSA adsorption to MCC (1, 2 – pH 4.8, C_0=1.2 and 9.7 mg/ml, respectively; 3, 4 – pH 6.2, C_0=1.2 and 9.7, respectively)

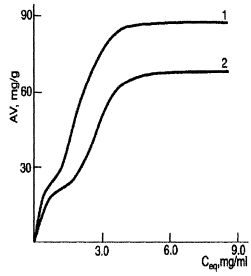


Fig. 1.B. Isotherms of HSA adsorption to MCC at pH 4.8 (1) and 6.2 (2)

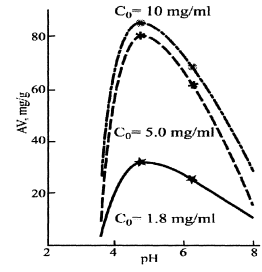


Fig. 2. Dependence of AV (mg/g) on the pH of HSA solutions

It was calculated that maximum AV values (at pH 4.8 and 6.2) corresponds to connection between ~ 30 moles of MCC and 1 mole of HSA at their adsorbates formation.

Semilogarithmic adsorption anamorphoses confirm that this is two-stage process. Each stage is described by an equation of the pseudo-first-order reaction. The apparent rate constants of the first stage (K_1) are listed in Table 1. It can be seen that K_1 increases six times at pH 4.8 and four times at pH 6.2 when initial HSA solutions concentration increases sixteen times.

Isotherms for HSA adsorption at pH 4.8 and 6.2 are given in Fig. 1.B. The amount of adsorbed HSA (AV, mg/g) is plotted against protein equilibrium concentration in solution C_{eq}. Adsorption time was 120 min. In contrast to adsorption of HSA to some solid surfaces (Ref. 7, 8), the adsorption isotherms do not exhibit Langmuir's shape. In agreement with adsorption of other polymers and their complexes onto MCC (Ref. 1), they exhibit a pronounced S-shape (at both pH), which is known to indicate the stepwise character of adsorption. Both isotherms attain a limit at the same equilibrium concentration 2.0-2.5 mg/ml.

The isotherms can be assigned to type IV which characterises the combination of physical adsorption and chemisorption on the matrix and cannot be described by Langmuir's equation but can be satisfactorily described by Freundlich's equation (Ref. 1). Freundlich's constants are listed in Table 1. These constants show that the HSA affinity (coefficient 1/n) for adsorbent MCC is similar to those obtained under adsorption of high molecular mass PVP on MCC but relative ability of MCC to sorb HSA (coefficient K) is twice lower than in the case of PVP adsorption (Ref. 1).

C_0, mg/ml	Maximum adsorption values, mg/g		$K_1 \cdot 10^2$ min^{-1}		Freundlich's constants			
					pH 4.8		pH 6.2	
	pH 4.8	pH 6.2	pH 4.8	pH 6.2	1/n	$K \cdot 10^3$	1/n	$K \cdot 10^3$
0.6	14.0	13.0	0.7	1.0				
1.2	26.5	21.0	1.5	1.2				
2.4	55.6	32.0	2.5	1.8	0.78	1.36	0.70	1.28
3.6	73.0	52.0	3.3	2.2				
4.8	83.0	63.0	3.8	2.9				
9.7	86.7	69.0	4.3	4.1				

Table 1. Maximum adsorption values, apparent rate constants, and Freundlich's constants of HSA adsorption onto MCC

Explanation of the adsorption experimental results

Effect of pH on the shape and denaturation of HSA macromolecules

Adsorption of proteins (as well as of other polymers) to solid interfaces strongly depends on the shape of molecules. HSA molecules have a globular shape. Existence of globular structures is known to decrease contact capacities of macromolecules with surfaces and leads to preferable solvent adsorption. Interaction with surfaces increases when extended molecules are adsorbed. According to WAXS studies, near the IEP HSA molecules have a shape of compact globular particles. In acid media (lower than IEP) they partially acquire loose structure. This is accompanied by their partial denaturation and retention of additional solvent. The denaturation and additional water retention is also possible in alkaline media (higher than IEP). The change in pH and denaturation of HSA molecules can also lead to increasing HSA adsorption. Therefore, strong competition between MCC, HSA, and water molecules appears to proceed during adsorption. When the weight method was compared to the Lowry method, it was shown that the resulting compounds are the intercalates of MCC, HSA, and water. This is also due to high hydrophilicity of the cellulose matrix. Thus, changes occurring in the HSA solution are responsible for adsorption results. In many cases denaturation causes dissociation of the protein into smaller units. This can also favour higher adsorption values. Some IR and ^{13}C NMR spectra of HSA samples (Fig. 3) confirm these suggestions.

It can be seen that IR and ^{13}C NMR spectra of the initial HSA (1) and of that one dissolved at pH 6.2 and subsequently lyophilised (2) are similar. However, spectrum of HSA dissolved at

pH 4.8 and lyophilised (3) has specific features. The new bands at 1411 cm^{-1} and 1580 cm^{-1} appear in the IR spectrum. This can be attributed to the conformational rearrangement in HSA

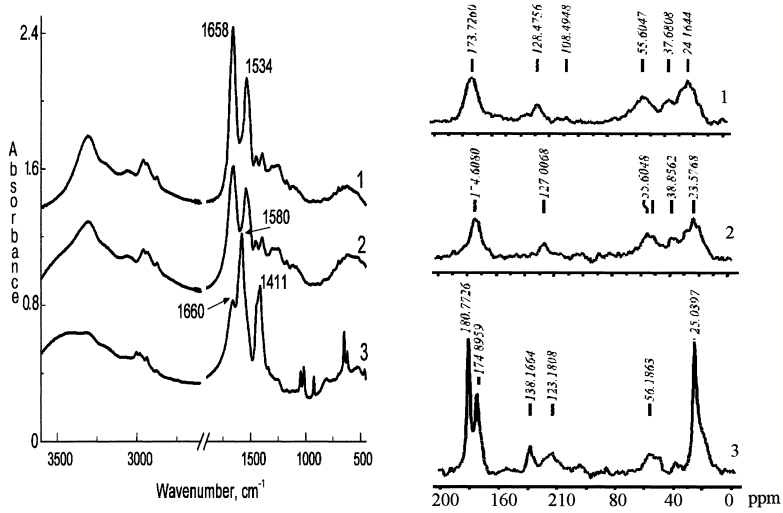
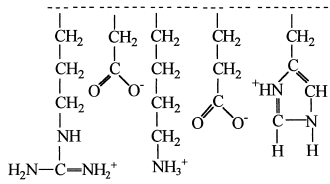


Fig. 3. IR (left) and ^{13}C NMR (right) spectra of initial HSA (1), HSA dissolved in water at pH 6.2 (2) and at pH 4.8 (3) and subsequently lyophilised.

molecules which causes the intensity decrease of the amide I (1660 cm^{-1}) and shift of the amide II (1534 cm^{-1}) bands. The NMR spectrum of the same sample greatly differs from that of the initial HSA. These phenomena usually occur during proteins conformational changes and denaturation. Thus, it is the dissolution of HSA at pH 4.8 that leads to its denaturation and, hence, to unfolding of macromolecules. Adsorption at pH 4.8 proceeds with already uncoiled HSA macromolecules and results in higher degree of adsorption.

Effect of the charge and chemical interaction of macromolecules

HSA molecules in an aqueous solution have positive charge in the pH range from 2.5 to 4.8, and MCC molecules are negatively charged at all pH range (3.5-9.0) (scheme I). Hence, it can be suggested that adsorption should be preferable in the pH range lower than HSA IEP. In the range where HSA and MCC molecules have negative charges, adsorption value should be low. In contrast, maximum AV is achieved at pH 4.8, and is still high at pH 6.2. A protein molecule in an aqueous solution tends to concentrate its hydrophobic moieties in a nucleus surrounded by more hydrophilic moieties. The hydrophilic moieties can exchange protons and counter-ions with the medium. In an aqueous solution, side chains fragments of the HSA molecule can be represented by the following scheme II:



When HSA is adsorbed to the MCC interface negatively charged in an aqueous solution, the repulsion of negatively charged residues of aspartic and glutamic acids from this interface takes place. However, the high hydrophilicity of the MCC surface enriched with OH groups favours multipoint ionic interaction between the amino- and OH-groups (RNH^+, RN and ROH). As a result, stable bonds ($=CO(H)\cdots H^+NR$ and $=CO^-\cdots H^+NR$) can be formed.

On the basis of these data, two main ways can be suggested for MCC-HSA adsorbates formation: stable bonds between MCC and HSA are formed due to interaction of HSA amine groups and OH surface groups and/or end aldehyde groups of MCC. The first reaction will lead to formation of new H-bonds, and the second one to aldimine bonds formation. Thus, our above suggestion about the binding of ~30 moles of MCC to 1 mole of HSA appears to be possible because there are a great variety of the MCC aldehyde and OH groups that can be bound to HSA amine groups.

Mutual effect of HSA and MCC morphological structure during HSA adsorption (SEM data)

The transformation of native protein under denaturation in the absence of specific reagents can be brought about not only by heat but also by the effect of surface, organic solvents, such as alcohols, etc. (Ref. 9) . The strong effect of HSA adsorption on the MCC morphological structure was confirmed by SEM study of HSA samples and MCC-HSA intercalates (Fig. 4, A-C).

After HSA adsorption to MCC all samples reveal remarkable surface disordering compare to the initial MCC fibre. It can also be concluded that HSA deeply penetrates into the fibres. SEM micrographs show that treatment of MCC by HSA solutions at different pH gives various effects, e.g. the higher pH, the higher the destructive effect of this treatment. Thus, fibres of the MCC sample treated with HSA solution at pH 6.2 exhibit very loose shape with destroyed fibril structure on the surface. This is especially noticeable in the intercalate samples after partial desorption of adsorbed HSA. This corresponds to higher release of HSA under desorption. Destructive effect is even more marked at pH 8.0. MCC fibres are totally destroyed and do not reveal surface fibril structure. Consequently, this effect is mutual. On the other hand, some HSA properties (for instance, its solubility) depend on adsorbent

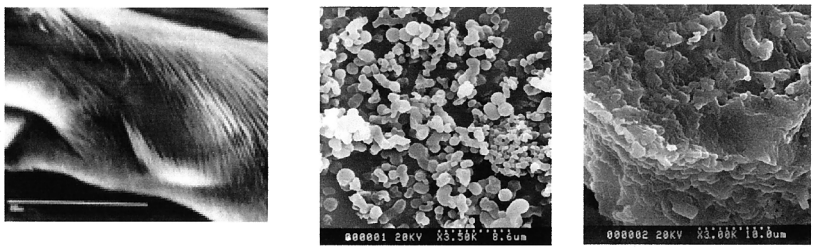


Fig. 4.A. SEM micrographs of MCC fibre (left), HSA (middle), and HSA dissolved at pH 4.8 and lyophilised

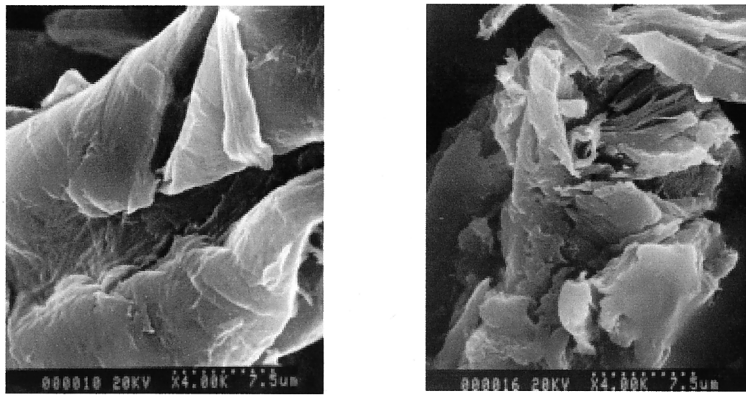


Fig. 4.B. SEM micrographs of MCC-HSA intercalates prepared at pH 6.2. Left- after HSA adsorption to MCC, right – after HSA desorption from the intercalate

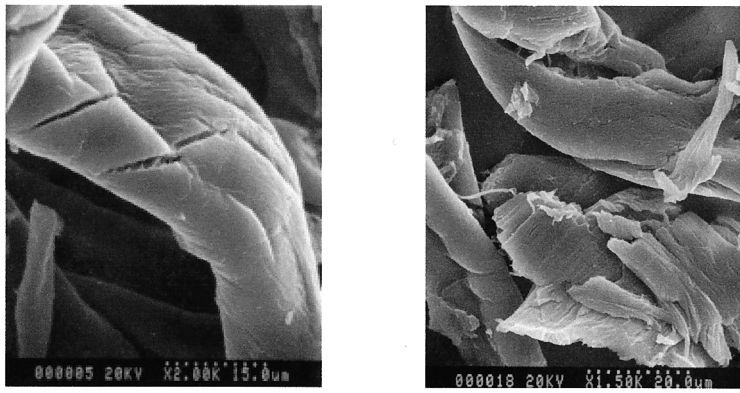


Fig. 4.C. SEM micrographs of MCC-HSA intercalates. Left- prepared at pH 4.8, right – prepared at pH 8.0

surface. Thus, HSA becomes partly insoluble near its IEP after adsorption at pH 4.8.

Desorption of HSA from the intercalates

The release values (RV) of HSA under desorption depend on HSA concentration in the adsorbate. RV have a limit which is reached at an adsorption value of about 70 mg/g. The maximum release is ~20 mass % and is higher for the intercalates obtained at pH 6.2. It is of interest that the HSA retention over all the AV range does not depend on pH of HSA solution during adsorption. It was also established that RV determined by the weight method are much higher than those calculated by the Lowry method and attain ~ 90-100 wt%. This means that the HSA retention in the MCC-HSA intercalates depends on adsorption conditions and adsorption values. The HSA retention linearly increases with AV.

The important conclusion of this study is that strong competition between MCC, HSA, and water exists in the adsorption. The resulting compounds are the intercalates of MCC, HSA, and water. Their composition is: 80 wt% MCC, ~ 8.0 wt% HSA, and ~ 20% water.

Conclusions

1. Adsorption of human serum albumin to microcrystalline cellulose was determined as functions of protein concentration and pH of its aqueous solutions. The maximum adsorption value is attained at the HSA isoelectric point.
2. The isotherms of HSA adsorption are S-shaped. The study of adsorption at several pH values indicates that interaction of HSA with the MCC interface is not controlled by the electrostatic effect.
3. The FTIR and ^{13}C NMR data reveal that HSA macromolecules are denaturated at IEP and become uncoiled. This results in high adsorption.

Acknowledgements

The authors acknowledge Mrs. A. Bobasheva performing IR measurements and Mr. M. Mokeev performing ^{13}C NMR measurements.

References

[1] Kotelnikova, N. E., Panarin, E. F., Shchukarev, A. V., Serimaa, R., Paakkari, T., Jokela, K., Shilov, S. V., Kudina, N. P., Wegener, G., Windeisen, E., Kochetkova., I.S., The effect of quaternary ammonium base adsorbates on the molecular and morphological structure of microcrystalline cellulose. Carbohydrate Polymers, 38(3):239-246 (1999)
[2] Veinshtein, S.G., Julkevich, I.V., Kotelnikova, N.E., Petropavlovsky, G.A., Defensive properties of microcrystalline cellulose in experimental diabetes mellitus of rats. Bull. Experim. Biolog. Mediciny (russ). 2:167-168 (1987)
[3] Sidorova, M.P., Ermakova, L.E., Kotelnikova, N.E., Kudina, N.P., Electrosurface properties of microcrystalline cellulose of various natural origins in 1:1 solutions of charged electrolites. Colloid. J. (russ), submitted
[4] Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall R.J., Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275 (1951)
[5] Bresler, S.E., Kolikov, V.M., Katushkina N.V., et al., Adsorption of proteins on the macroporous glass. Colloid J. (russ). 36(4):748-751 (1974)
[6] Norde, W., Lyklema, J. J., The adsorption of human plasma albumin and bovine pancreas ribonuclease at negatively charged polysterene surfaces. I. Adsorption isotherms. Effects of charge, ionic strength, and temperature. Colloid Interface Science. 66(2):257-265 (1978)
[7] Lindau, G., Rodius, R., Die physikalish-chemishe Analyse adsorbierter Eiweissmolekule in der Grenzflache fest-flussig. Z. phys. Chem., A 172:321-347 (1935)
[8] Elensky, A.V., Iampolskaya, G.P, Ismailova, V.N., Adsorption of lipase and other global proteins on the hydrophobic surfaces. Colloid J. (russ), 69(1):143-144 (1987)
[9] M.L. Anson. Protein denaturation and the properties of protein groups. Advances in Protein Chemistry, v. II, 361 (1945)