ADSORPTION OF HUMAN SERUM ALBUMIN ONTO MESOPOROUS ACTIVE CARBON AND CARBON BLACK

S.V.Mikhalovsky*#, M.P.Levchenko*, E.A.Fesenko*, R.G.A.Faragher#, A.W.Lloyd#

*Institute for Sorption and Problems of Endoecology, 32-34, Prospect Palladina, Kiev-142, Ukraine
#Department of Pharmacy, University of Brighton, Brighton BN2 4GJ, U.K.

INTRODUCTION

Polymer-pyrolyzed active carbons (PPACs) are produced by pyrolysis of synthetic polymers and resins. If the precursor polymer has a fixed meso- or macroporous structure,the final PPAC can retain a very similar structure. Thus active carbons with an unusual pore-size distribution have been synthesized [1]. In addition to having a well defined porous structure, these materials have high mechanical strength and excellent blood compatibility. This combination of features makes PPACs widely applicable in the treatment of diseases by hemoperfusion. The biocompatibility of materials is a complex phenomenon involving many different steps. However, it is believed that adsorption of proteins at the blood-biomaterial interface is of prime importance for the biocompatbility of the material [2]. Human serum albumin (HSA) is the main protein constituent of blood, hence, the adsorption of HSA onto mesoporous PPAC has been studied [3]. So far, the adsorption kinetics of albumin have been studied and it was shown that adsorbed protein molecules undergo conformational changes. Nevertheless, little is known about the structure of the surface layer of adsorbed HSA on active carbons.

EXPERIMENTAL

HSA of 99% purity and rhodamine-labeled HSA, R-HSA, were supplied by "Serva", p-chloroaniline, pCA ("Aldrich"), was purified by recrystallizing from water three times. PPAC was made from styrene-divinylbenzene-vinylpyridine copolymer [1] in the form of spherical beads 0.63-1.0 mm in diameter. It has V_{micro} = 0.61 cm^3/g, total pore volume=0.97 cm^3/g, S_{meso} = 200 m^2/g, mean r_{meso}= 35 nm (by mercury porosimetry). Acetylene carbon black (CB) was produced at the Institute of Colloid Chemistry, Kiev. All adsorption tests were carried out at room temperature as batch experiments. Adsorbed amounts were calculated by the absolute decrease in the optical density of solutions at 280 nm (HSA), 570 nm (R-HSA), and 238 nm (pCA). Before contact with the protein solution the active carbon was conditioned with the corresponding buffer solution for 2 weeks. CB samples were used without pre-conditioning. The duration of the adsorbent contact with HSA was 6 h. The adsorbent was subsequently separated from the protein solution and placed in a solution of pCA for 24 h. R-HSA has been used to prove that pCA does not cause desorption of protein from the surface as both adsorptives can be determined in the solution simultaneously. It has been found that after having been brought into contact with pCA, no desorption of protein occured from the adsorbent preloaded with R-HSA even at high initial concentrations of pCA (0.7-0.8 mg/ml).

RESULTS AND DISCUSSION

Adsorption isotherms of HSA on CB and PPAC are distinctively different (Fig. 1). From the results of the experiments with consecutive adsorption of HSA and pCA, the average area occupied by a HSA molecule (s°) can be estimated. Adsorption isotherms of pCA are linear if $\log_{10}(\alpha v^*)$ is plotted vs $\{\log_{10}(C_s/C_e)\}^2$, according to the Dubinin-Radushkevich (DR) equation (Fig.2) [4]: $\log_{10}(\alpha v^*) = \log_{10} v_\alpha - D\{\log_{10}(C_s/C_e)\}^2$

Here D is a combination of characteristic parameters and constants and is constant itself; α - adsorbed amount of pCA, mmol/g, C_e - equilibrium concentration, mmol/l, C_s - solubility, v^*- molar volume of pCA, and v_α - total pore volume of the adsorbent. v^*= 0.109 $cm^3/mmol$, C_s = 24.0 mmol/l. Values of the specific surface area of CB, S_{sp}, calculated from the DR plot, are given in Table1. The s° of pCA is assumed to be constant and equal to 0.503 nm^2, the thickness of the adsorbed pCA layer =0.37nm [4]. The s° calculated for HSA on CB from this data is in good agreement with the value of 44 nm^2, reported for the side-on orientation of the adsorbed HSA molecule, retaining the native conformation of the ellipsoid of rotation with axes 14x4 nm [5]. The maximal adsorbed amount of HSA, a_m, on CB is about 85 mg/g, i.e. 0.65 mg/m^2, which is 4-5 times less than a_m of HSA on polymeric and inorganic non-porous adsorbents [6,7]. Without taking into account values of free surface area, determined by pCA adsorption, the apparent s° of HSA is 170 nm^2 . This is not realistic as s° determined

by STM on another non-porous carbon, pyrographite, is only 70 nm^2. This suggests that even in the plateau region of the adsorption isotherm HSA forms an incomplete coating of the CB surface. Results obtained on PPAC significantly differ from CB, probably due to the high micro- and mesoporosity of the former. While adsorbed in the mesopores, HSA molecules reduce access to the micropores. Substantial fraction of the inner surface remains free, and protein molecules can slowly penetrate pores by intraparticlediffusion and conformational rearrangement which probably explains absence of a plateau on the adsorption isotherm on PPAC (Fig.1).

Table 1. Surface area (S) of carbonaceous adsorbents with and without preadsorbed HSA (a) and average area (s°) occupied by the HSA molecule as determined by pCA adsorption.

Adsorbent	pH	a, mg/g	S, m2/g	s°, nm2
CB	7.1	0	140	-
CB	7.1	43	120	46
CB	7.1	70	120	32
CB	5.1	0	140	-
CB	5.1	50	120	48
CB	5.1	80	105	46
PPAC	7.1	0	670	-
PPAC	7.1	11	640	290
PPAC	7.1	32	620	180

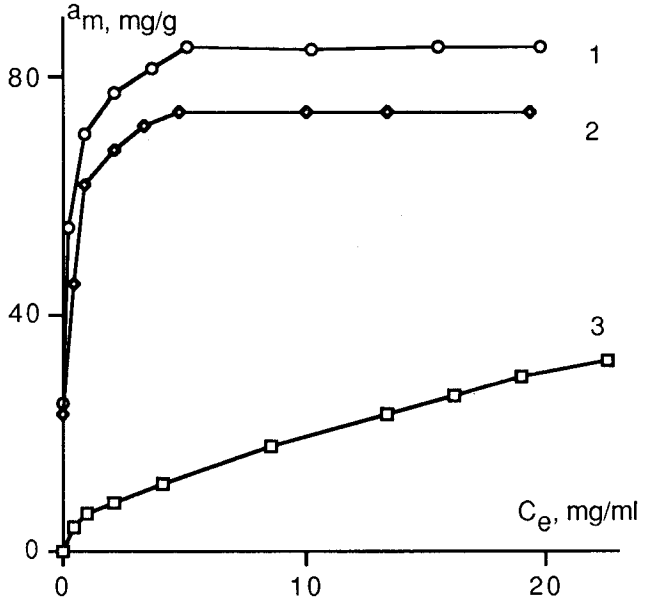

Fig. 1. Adsorption isotherms of HSA on CB (1, 2) and PPAC (3) at pH = 5.1 (1) and pH = 7.1 (2,3).

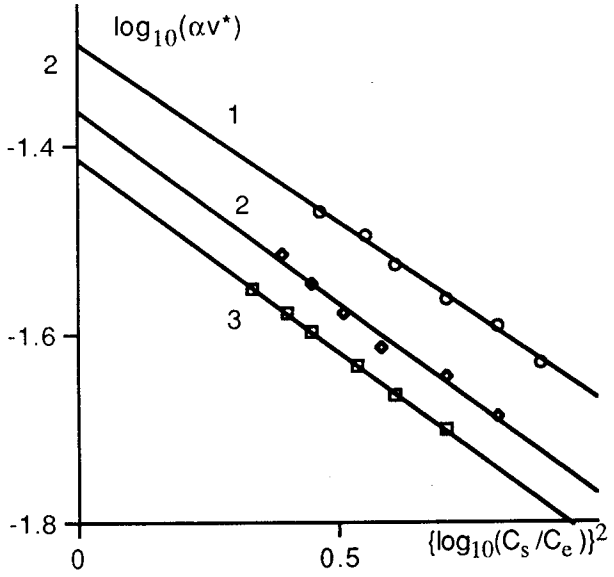

Fig. 2. Adsorption isotherms of pCA on CB with different preadsorbed amount of HSA.
1 - pure CB; 2 - 50 mg/g HSA; 3 - 80 mg/g HSA. pH=5.1

CONCLUSIONS

The surface layer of HSA adsorbed onto non-porous (CB) and porous (PPAC) carbons is incomplete. A substantial fraction of the surface remains uncovered even at high equilibrium concentrations of HSA.

REFERENCES

1. N.T.Kartel, A.M.Puziy, V.V.Strelko. In: Characterization of Porous Solids II. Eds. F.Rodriguez-Reinoso et al. Amsterdam, Elsevier, 1991, pp. 439-447.
2. Blood in Contact with Natural and Artificial Surfaces. E.F.Leonard, V.T.Turitto, L.Vroman, eds. The Annals of the NY Academy of Sciences, NY, v. 516, 1987, pp.1-688.
3.S.Mikhalovsky, M.Levchenko, I.Larionova, V.Strelko. In: Proceedings Carbon'92, Essen, Germany, 1992, pp.286-288.
4.A.M.Koganovsky,T.M.Levchenko, V.A.Kirichenko. Adsorption from solutions (Russ.). "Naukova Dumka" Publ., Kiev, 1977, pp.1-223.
5.D.C.Carter, X.M.He, S.H.Munson et al. *Science*, 152, 1195 (1989).
6. C.A.Bornzin, I.F.Miller. *J.Colloid and Interface Sci*, 86, N2, 539 (1982).
7. P.G.Koutsoukos, W.Norde, J.Lyklema. *J.Colloid and Interface Sci*, 95, N2, 385 (1983).