Experimental Report

Proposal: **9-13-426** **Council:** 10/2011

Title: Adsorption of proteins by porous carbons

This proposal is resubmission of: 9-13-380

Researh Area: Soft condensed matter

Main proposer: **LASZLO KRISZTINA**

Experimental Team: SAVINA Irina
LASZLO KRISZTINA
GEISSLER Erik

Local Contact: GRILLO Isabelle

Samples: hydrochloric acid DCl
Carbon
heavy water D2O
bovine serum albumin BSA
bovine aprotinin
sodium azide NaN3

Instrument	Req. Days	All. Days	From	To
D11	2	1	19/11/2012	20/11/2012

Abstract:

The experiment is to determine the degree of penetration of two model proteins into mesoporous porous carbons, with a view to possible use in haemoperfusion or other clinical applications. Until now this therapy has been limited to small toxin molecules, which are readily adsorbed by microporous carbons. For proteins, carbons with larger pore sizes are needed. The good contrast match between the carbon substrate and D2O ensures high visibility for the SANS signal from the protein. Use of two types of carbon of different pore structure, and the inclusion of one of these carbons with modified surface chemistry (activated carbon), will contribute in discriminating between the steric and chemical effects of this adsorption. It is also important to compare the adsorption of the same molecule (albumin) under two different configurations.

Carbon Conference Rio de Janeiro 2013
ADSORPTION OF PROTEINS BY HIGH SURFACE AREA CARBONS WITH WIDE RANGE OF POROSITY

Krisztina László,[1*] Irina Savina,[2] Ajna Tóth,[1] Sergey Mikhalovsky,[2,3] Lyuba Mikhalovska,[2] Isabelle Grillo,[4] Erik Geissler[5]

[1]Department of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, H-1521 Budapest, Hungary
[2] School of Pharmacy and Biomolecular Sciences, University of Brighton, Lewes Road, Brighton, BN2 4GJ, United Kingdom
[3] School of Engineering, Nazarbaev University, Astana, Republic of Kazakhstan.
[4]Institut Laue-Langevin, BP 156, 6 rue Jules Horowitz, 38042 Grenoble Cedex 9, France,
[5]Laboratoire Interdisciplinaire de Physique CNRS UMR 5588, Université J. Fourier de Grenoble, BP 87, 38402 St Martin d'Hères cedex, France.
*klaszlo@mail.bme.hu

Introduction

Activated carbons have been widely employed for many centuries as general adsorbents. More recently they have been used notably for purifying various bio-fluids, including the treatment of acute poisoning by removing toxins from the bloodstream by adsorption. In such processes the size of the pores relative to that of the proteins to be removed is of crucial importance, since many toxins are large molecules that cannot penetrate into micropores [1].

Here we report measurements of the adsorption of proteins on two porous carbons of different pore size distributions, a resorcinol-formaldehyde based carbon aerogel (C1), possessing a very open structure [2, 3], and a porous MAST carbon made from phenolic resin (C2) [4]. The probe proteins, aprotinin (6.7 kDa, size ca 2.9 x 1.9 nm, Sigma-Aldrich) and bovine serum albumin BSA (67 kDa, ca 14 x 6 x 4 nm Calbiochem) are globular proteins and soluble in water at ambient temperature. Measurements using small angle neutron scattering (SANS) were made with the powdered carbon in contact with solutions of these proteins in D_2O, in the absence of any salt in order to reveal the distribution of these proteins in the liquid/carbon interface.

Experimental

The adsorption characteristics of the two dry carbons were obtained by nitrogen adsorption at 77 K. The pH of these carbons was also determined in aqueous solution with a standard method [5]. Table 1 lists the results of these experiments. In this table S_{BET} is the BET surface area, V_{tot} is the total pore volume, V_{μ} is the micropore volume and w is the average micropore width.

Table 1. Characteristics from nitrogen adsorption (77 K) and pH

	S_{BET} m^2/g	V_{tot} cm^3/g	V_{μ} cm^3/g	V_{tot} - V_{μ}	w Å	pH
C1	847	1.69	0.34	1.35	15	6.6±0.3
C2	1248	1.27	0.50	0.77	16	9.6±0.3

Protein adsorption

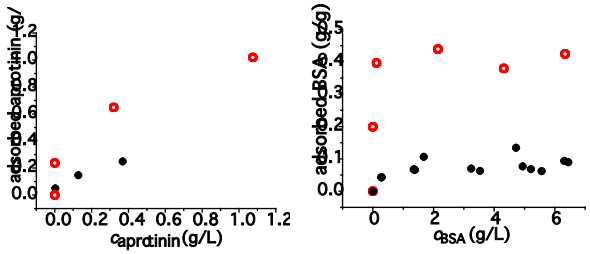
The adsorption isotherms of the two proteins were measured from their aqueous solutions (MilliQ water) at 20 °C. The equilibrium concentrations were detected by Ultra Performance Liquid Chromatography (UPLC) (Waters) using a photodiode array (PDA) detector at 280 nm.

SANS

The SANS measurements were made on the small angle scattering D11 instrument at the Institut Laue-Langevin, Grenoble, at incident wavelength λ=7.8Å, and wavelength spread Δλ/λ=0.1. Measurements were also made at wider angles on the D16 instrument at wavelength 4.74Å. The carbon samples were ground to powder and placed in flat quartz cells with a 2 mm path length (for the solutions and carbons containing D_2O solution). Owing to the lower density of the aerogel powder, the mass of carbon contained in the cells was 5 times smaller for C1 (20 mg) than for C2 (100 mg). The resulting loss in scattering intensity from the adsorbed proteins in C1 is thus partially compensated by its greater adsorption capacity for proteins.

Results and discussion

Protein adsorption isotherms



a b

Figure 1: Adsorption isotherms from aqueous solutions of a) aprotinin, and b) BSA on carbon aerogel C1 (o) and on carbon C2 (•).

Figure 1 shows the adsorption isotherms from unbuffered aqueous solutions of the two proteins for each carbon. At saturation C1 adsorbs ca. 1.1 g/g aprotinin and 0.41 g/g BSA. In C2, the corresponding values are 0.27 and 0.09 g/g, respectively. The shape of these isotherms reflects a much stronger interaction between proteins and C1 than with C2 carbon. The steric hindrance encountered by large molecules in microporous systems is illustrated by the fact that although the BET surface area of C2 is about 50% greater than that of C1, its adsorption capacity for either of the two proteins is less than one quarter of that of C1.

SANS low q response

Penetration of the proteins from aqueous solution into the two nanoporous carbons was measured by SANS. In both systems the D_2O solvent matched the signal of the carbon in the micro- and mesoporous q range. At small q (<0.01 $Å^{-1}$), however, the signal of the D_2O-filled samples displayed strong residual scattering with power law behavior, $I(q) \propto q^{-m}$, where $m \approx 4.8$ in C1 (Figure 2) and $m \approx 3.1$ in C2 (Figure 3). These responses are the signature of surface scattering. (The finding that $m>4$ could be due to a concentration gradient normal to the surface.) The intensity of this surface scattering feature stems from a discontinuity in contrast at the carbon interface of the largest pores due to a layer of low scattering length density, either a material layer with high proton density, or a void layer due to poor

wetting of the carbon by the D_2O. In the present case, where the surface of C1 is strongly hydrophobic (the basic behavior stems from the delocalized electrons [6]), the latter interpretation is physically more plausible. When proteins are present in the solution, this scattering feature disappears or is diminished: by acting as surfactants and adhering to the walls of the larger pores, the proteins reduce the contrast discontinuity at the interface. With the reduced scattering length density contrast, the power law behavior shifts to lower q, out of the SANS observation range. With BSA (Figure 2b), however, some surface scattering persists (slope steeper than -3), suggesting that not all the carbon surface is accessible to the larger BSA molecules.

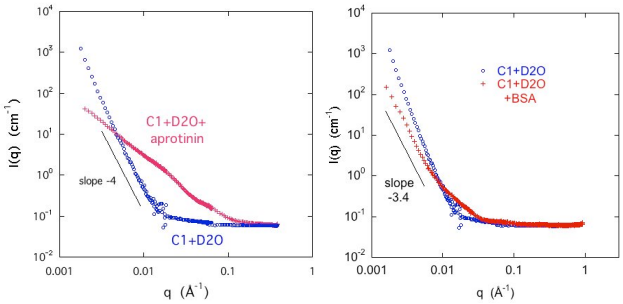


Figure 2. SANS signals from carbon aerogel C1 with D_2O alone (o) and same sample with D_2O and protein (+): (a) aprotinin, (b) BSA.

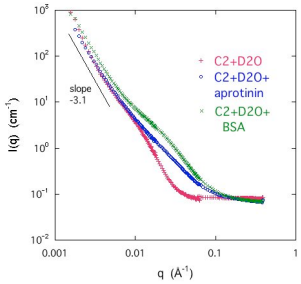


Figure 3. SANS response of sample C2 containing pure D_2O (+), aprotinin in D_2O (o), and BSA in D_2O (×).

Figure 3 shows the corresponding scattering responses for the C2 carbon. In this case the low q behaviour of C2 in D_2O alone also displays surface scattering, but the slope (-3.1) is the sign of a high degree of surface roughness. It is noticeable that the same curve also exhibits weak residual scattering from the micropores, which appears as a deviation from the power law behavior with extra intensity at $q \geq 0.01$ Å^{-1}. These findings imply that 1) not only do the large-scale interfaces remain unaltered by the D_2O, but 2) also that the density of the D_2O in the micropores is slightly smaller than that of the bulk liquid. The low q responses of the samples with either aprotinin or BSA also differ qualitatively from those of sample C1: the protein signal simply adds to that of the carbon-water system. Thus, unlike C1, the scattering length density mismatch at low q in C2 is not due to poor wetting, but rather to the presence of surface chemical groups. The surface composition of the two carbons is therefore different, as is confirmed by their notable difference in pH (Table 1). This conclusion is further supported by the substantially larger incoherent scattering intensity at high q in the dry C2 sample (0.011 cm^{-1}), which reflects the hydrogen content of the functional groups.

SANS intermediate q response

Figure 4 shows the SANS responses of the free solutions of the protein in D_2O. For BSA, ionization of the dissolved polymers in the absence of added salt causes electrostatic repulsion between the molecules that gives rise to the correlation peak at around 0.05 Å^{-1}, similar to that found in polyelectrolyte solutions [7]. No such peak is observed in the case of aprotinin under these conditions. The continuous line through the 10 mg/mL aprotinin data is the Debye expression for the scattering intensity of particles with radius of gyration R_G,

$$I(q)=A\ \exp[-(qR_G)^2/3] \qquad (1)$$

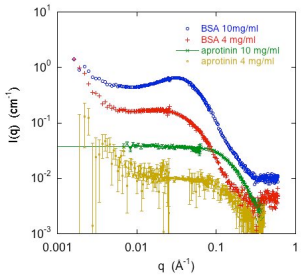


Figure 4. SANS responses of the protein solutions in D_2O. The maxima in the signals from the BSA solutions are due to residual electrostatic repulsion in the salt free solutions. Continuous line through the 10 mg/mL aprotinin data is the fit to eq 1.

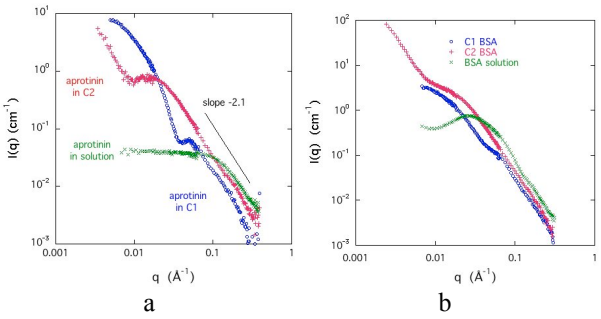
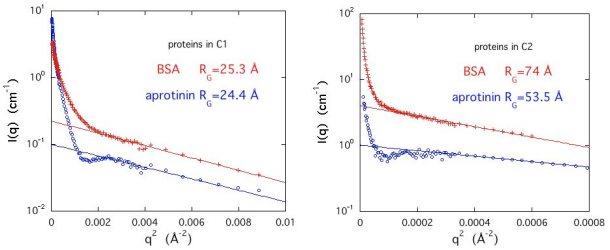


Figure 5. SANS analysis of (a) aprotinin in carbon (after subtraction of wet carbon background); (+): aprotinin in C1, (o) aprotinin in C2, and aprotinin in free solution (×), (b) BSA in carbon; (o): BSA in C1, (+) BSA in C2, and BSA in free solution (×).

with R_G=9.4 Å. An identical result is obtained for R_G in the 4 mg/mL solution. This value is about 1 Å smaller than that measured by Appavou et al [8]. For the BSA solutions, the values of the radius of gyration, measured beyond the correlation peak, were found to be identical in both samples, R_G=27.6 Å. This value is consistent with those found in the literature, which range, for example, from 26.6Å [9] to 28.5Å [10]. (In the latter measurement, from SAXS, the contribution from the hydration shell has been removed.)

In Figure 5a the scattering responses of aprotinin in free solution is compared with that in the two carbon matrices, i.e., the difference signal betwee{n the protein-containing carbons and that of the wet carbon. The appearance of a plateau at low q in the C1 carbon is an artefact due to the subtraction of the wet carbon signal, which, as noted earlier, displays extra scattering from interfaces that are not contrast matched. The maximum in this signal at q≈0.05 Å^{-1} is the polyelectrolyte peak of the aprotinin solution inside the pores of the carbon. In C2, a similar peak, located at q≈ 0.015 Å^{-1}, is an order of magnitude more intense. Analogous behaviour is found with BSA (Figure 5b), but the correlation peak is poorly resolved. In C1 both aprotinin and BSA display a pronounced shoulder at q≈0.05 Å^{-1}, while in C2

the corresponding shoulder occurs for both at $q \approx 0.015$ Å^{-1}.



a b

Figure 6. Guinier plots of the scattering intensity in the q-region beyond the correlation peak. (a) BSA and aprotinin in the aerogel carbon C1, (b) the two proteins in the C2 carbon.

Figure 6 shows the same difference signals as in Figure 5, plotted in the Guinier representation $\log[I(q)]$ *vs* q^2, where the data are taken from the q region just beyond the correlation peak. The radius of gyration R_G of both proteins in C1 is practically identical (25 Å), while in C2, R_G is two or three times larger. The intensity of the signal of both proteins in C2 is correspondingly an order of magnitude greater than in the carbon aerogel C1, which is consistent with their greater size. This finding implies that the correlation peak is determined by the carbon matrix rather than by the protein. In the free solutions, a correlation peak is observed only for BSA, and its position (at $q \approx 0.03$ Å^{-1} for polymer concentration c=10 mg/mL) is intermediate between that in C1 and in C2. The different pH of the two carbons reflects the different chemical environments at the interface.

In the open aerogel structure of C1, the concentration of both proteins is enhanced with respect to the free solution, as indicated by the appearance of a correlation peak in the aprotinin signal and by the shift to larger q in the position of the correlation peak of BSA. Furthermore, the radius of gyration of aprotinin in C1 increases significantly over that in dilute solution (from 9.4 Å to 24.4 Å), showing that the small molecules form oligomers. For BSA, on the other hand, the size of the molecule decreased marginally with respect to the free solution (from 27.6 Å to 25.3 Å). The change in concentration of the BSA in the gel with respect to the free solution can be estimated by comparing the position of the correlation peak: it moves from $q \approx 0.027$Å^{-1} in free solution to ~0.050 Å^{-1} in the aerogel, which implies an increase in concentration by a factor of roughly $(0.05/0.027)^3 \approx 6$. (At low polymer concentrations, the position of the correlation peak varies as the one-third power of the concentration [11].) In the more predominantly microporous carbon, C2, large protein oligomers form, which, owing to their size, scatter strongly. This result is striking, since the clusters are significantly larger than the micropore width in this material (w=16 Å), from which they are therefore excluded, thereby limiting the adsorption capacity of this carbon. It seems unlikely that the weak correlation feature at $q \approx 0.015$ Å^{-1} is of electrostatic origin; it probably corresponds to separation distances between mesopores of approximately $d = 2\pi/q \approx 400$Å.

Conclusions

Aprotinin and BSA are both readily adsorbed by the carbon aerogel C1, which has higher mesopore content. The SANS data indicate that both proteins adhere to the walls, and the concentration of BSA in the pores of this carbon is enhanced over that in free solution at concentration 10 mg/mL by a factor of about 6. Importantly, the radius of gyration of both proteins inside this carbon (about 25 Å) is greater than the micropore width (15 Å) determined by nitrogen adsorption. In the commercial MAST carbon, in which the large scale surfaces are rough and have neutral pH, proteins form oligomers with a radius of gyration of 50-70 Å, which is very much larger than the micropore width (16 Å) of this carbon. The comparison of these two systems suggests that the greater proficiency of C1 in adsorbing these proteins is attributable to its broader pore size distribution. The increased adsorption capacity of C1 with respect to C2 is almost the same for both proteins. The shape of the adsorbed protein however is strongly influenced by its own pH sensitivity and the acid/base properties of the surface.

Acknowledgements

We are grateful to the Institut Laue-Langevin for access to the neutron scattering instruments D11 and D16. We thank the help of G. Bosznai and P. Jenei in the adsorption measurements. The financial support of the Marie Curie International Research Staff Exchange Scheme (ENSOR, Grant No. 269267), the Marie Curie Reintegration grant, PERG08-GA-2010-276954, and OTKA 101861 is acknowledged. AT recognizes the support of TÁMOP 4.2.4. A/1-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system". The project was subsidized by the European Union and co-financed by the European Social Fund.

References

[1] Gun'ko VM, Mikhalovska LI, Savina IN, Shevchenko RV, James SL, Tomlins PE. Mikhalovsky SV. Soft Matter 2010; 6(21): 5351-5358.

[2] László K, Czakkel O, Dobos G, Lodewyckx P, Rochas C, Geissler E. Carbon 2010; 48:1038 –48.

[3] László K, Czakkel O, Demé B, Geissler E. Carbon 2012; 50:4155 – 62.

[4] Tennison RS. Applied Catalysis A 1998; 173: 289-311.

[5] Lopez-Ramon MV, Stoeckli F, Moreno-Castilla C, Carrasco-Marin F. Carbon 1999; 37:1215-21.

[6] Leon y Leon CA, Radovic LR. Interfacial chemistry and electrochemistry of carbon surfaces. In: Thrower PA, editor. Chemistry and Physics of Carbon, vol. 24, Marcel Dekker; 1994 p. 213-310.

[7] Nierlich M, Williams CE, Boue F, Cotton JP, Daoud M, Farnoux B, Jannink G, Picot C, Moan M, Wol C, Rinaudo M, de Gennes PG. J. Physique 1979; 40:701-4.

[8] Appavou MS, Gibrat G, Bellissent-Funel MC. Biochimica et Biophysica Acta 2006; 1764:414-23.

[9] Das A, Chitra R, Choudhury RR, Ramanadham M. Pramana - Journal of Physics, Indian Academy of Sciences 2004; 63:363-8.

[10] Santos SF, Zanette D, Fischer H, Itri R. J. Coll. Interf. Sci. 2003; 262:400-8.