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Double probe approach to protein adsorption on porous carbon surfaces

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ABSTRACT

Comparison of nitrogen adsorption isotherms of porous carbons before and after exposure to proteins yields information on the pore landscape that is unobtainable from small angle neutron scattering (SANS) [Carbon 2016; 106:142–151]. Two globular proteins, bovine serum albumin (BSA), and bovine pancreatic trypsin inhibitor (BPTI), are studied, with two different porous carbon substrates: a hydrophobic open structured carbon aerogel with basic surface pH (C1), and a hydrophilic medical grade microporous carbon with neutral surface pH (C2).

BSA and BPTI both interact more strongly with the hydrophilic carbon than with C1, but C2 adsorbs notably less protein. Both proteins are arrested at the micropore entrances. With increasing concentration in C1, these protein barriers, on drying, seal the micropores hermetically to nitrogen gas. Owing to the adsorbed protein, macropores that are otherwise too wide to be detected in virgin C1 shrink and become detectable by gas adsorption. In C2 the dry protein barriers are looser and remain permeable to nitrogen molecules, leaving the measured micropore and mesopore surface areas practically unaffected. This double probe approach corroborates and extends the earlier SANS findings, highlighting the role played by pore structure and the hydrophilic/hydrophobic character of the substrate in protein adsorption.

1. Introduction

The immense potential of porous nanostructured carbon materials in biomedical applications remains far from being realised. In biosensor preparations a fundamental step is to immobilise biomolecules, especially proteins, on carbon electrodes, by physicochemical means [1,2]. Nakanishi et al. [3] have drawn attention to the adsorption of proteins on nanocarbons (graphene, nanotubes and fullerenes) as a means of chemically functionalizing the substrate to serve as nanosensor devices. Another significant role is the removal of toxic proteins from body fluids. The effectiveness of these “medical carbons” in haemoperfusion treatment in cases of acute poisoning is well documented [4]. In this procedure toxins are removed from a patient's bloodstream by extracorporeal circulation through activated carbon [5,6]. Porous carbons are also promising candidates for eliminating biologically and chemically resistant pathological proteins, such as prions, from agricultural waste water. These proteins, which survive conventional water treatment processes, invade lakes and rivers [7].

The solid surface - protein interaction, which is of crucial importance in all these applications, involves different mechanisms including electrostatic, hydrophobic and van der Waals interactions, as well as formation of hydrogen bonds. Even in the case of flat surfaces the resultant effect is a combination of these. The wettability and chemistry (hydrophilic/hydrophobic nature) of solid surfaces have a decisive impact on the molecular interactions [8–10]. In porous systems size exclusion and confinement conditions also contribute to this picture [8]. Upon adsorption, proteins can denature or change their conformation, and hence their activity, and thus may present a health hazard [10,11].

For lysozyme, an enzyme of molecular weight $M_W = 14.6$ kDa, the maximum adsorption on untreated porous carbon surfaces occurs at its isoelectric point, around pH 11 [12]. Its high adsorption

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affinity is attributed to strong hydrophobic interactions between the non-polar side chains of the amino acid residues and the hydrophobic surface of the carbon. The surface area and the pore volume of ordered mesoporous carbon adsorbent control its adsorption capacity. Oxidative functionalization of the carbon enhances adsorption of the biomolecule through the anchoring capacity of the - COOH groups at the entrance of the mesopores, which can hinder desorption of the protein [13]. Yushin et al. found that the adsorption of cytokines does not result in denaturation of the protein, but that it is determined by the pore size distribution of the activated carbon substrate [14].

In this study of adsorption on porous carbons, we investigate two globular model proteins, bovine serum albumin (BSA) and bovine pancreatic trypsin inhibitor (BPTI). These were chosen for their widely different mass, size and pH response. BSA is a soft amphiphilic globular protein often used in model studies as a representative of biopollutants. Its loose structure and low isoelectric point (Table 1) make it pH sensitive. At low pH the resulting internal electrostatic repulsion denatures the protein, converting the molecular conformation from globular to an extended chainlike form [15].

The smaller protein studied, BPTI, also known as aprotinin, is a single polypeptide chain, folded in a stable, compact globular conformation [16,17]. Its compact structure, high isoelectric point and insensitivity to pH ensure its conformational stability throughout the physiological pH range.

Long et al. [18] have studied the adsorption of BSA from buffer solutions in the pH range 2.35–10 on phenol-melamine-formaldehyde derived carbon aerogels with controlled particle and mesopore size. In this case, as also reported in many other studies, the highest protein uptake was achieved at the isoelectric point of BSA. The authors found that for optimum adsorption capacity the pore size must be slightly larger than the size of the protein. When the pore size is close to that of the protein, the preferred adsorption site is at the pore entrance, thus blocking access to the remainder of the pore that otherwise would be available for further BSA molecules. If the pore size is appreciably larger than the biomolecule, then the size no longer affects the adsorption capacity. In an investigation of adsorption of BSA and myoglobin ($M_w = 17.7$ kDa) on porous carbon close to physiological solution conditions (ionic strength 0.15 M, in pH 7.0 buffer) the uptake of the smaller protein was almost more than 3 times greater. The same study proposed that accumulation of the proteins in the macropores is due to both adsorption and self-association.

Adsorption of BSA in carbon nanochannels with controlled surface chemistry from buffered aqueous solutions (pH 7.8. and 9.6) was reported by Vijayaraj et al. [19] Although the adsorption mechanism relies mainly on hydrophobic interactions, uptake correlates with the amount of oxygenated surface groups. (The correlation is stronger if the surface area is small, otherwise mostly hydrophobic interactions prevail.) In spite of carbon samples with wider pores having a smaller surface area, the BSA uptake increases significantly with pore size, a clear demonstration of steric exclusion, with the possible accompaniment of pore blocking.

Table 1
Physicochemical characteristics of the model proteins.

	BSA	BPTI
Molecular weight, kDa	66.1	6.5
Elemental composition	$C_{2932}H_{4614}N_{780}O_{898}S_{39}$	$C_{284}H_{432}N_{84}O_{79}S_7$
Amino acid residues	583	58
Radius of gyration	27.6 ± 0.8 Å [21]	9.8 ± 0.5 Å [21]
Solubility in water	40 g/L [29]	>30 g/L [30]
Isoelectric point	4.8–5.5	10.5

In the literature, few investigations into protein adsorption on *porous* materials, including carbons, are reported, mainly owing to the limitations of such widely used and powerful methods as quartz crystal microbalance (QCM) or ellipsometry [20]. Observations of adsorption on porous carbon by optical methods, such as FTIR, are tributary to light attenuation, and hence open to the difficulty of discriminating between surface and bulk adsorption. Recently we reported results on the adsorption of BSA and BPTI on porous carbon materials with different pore size distributions and surface chemistry [21] as seen by non-destructive small angle neutron scattering (SANS) and small angle X-ray scattering (SAXS) techniques [22–24]. These techniques are unique in that they can detect the spatial structure and organisation of molecules adsorbed inside the porous medium [25].

For a full understanding of how proteins are adsorbed in porous carbons, the use of both scattering and adsorption approaches is essential. This paper, however, shows that the adsorption approach alone using a double probe (in this case, proteins and nitrogen gas) can reveal important additional information that is not accessible to scattering measurements. It focuses on the adsorption of the same proteins in the same two carbons as previously, of different pore size distribution, different surface chemistry and different hydrophobicity. The double probe approach measures not merely the total amount of target molecules adsorbed by the sample, but also how the adsorbed protein modifies the pore landscape of the carbon substrate.

2. Experimental

2.1. Materials

The two porous carbons studied are a resorcinol-formaldehyde based carbon aerogel (C1) possessing an open structure [22,24], and a commercial porous carbon made from phenol formaldehyde resin (C2) (MAST Carbon International, UK) [26–28]. The two probe proteins, bovine serum albumin BSA (Calbiochem) and BPTI (Sigma-Aldrich) have significantly different molecular weights, radii of gyration and isoelectric points (Table 1).

2.2. Methods

2.2.1. Characterization of the carbons

Nitrogen adsorption/desorption isotherms were measured at −196 °C, with a Nova 2000e (Quantachrome, USA) computer controlled volumetric gas adsorption apparatus. The samples were evacuated at 20 °C for 24 h. The apparent surface area S_{BET} was obtained from the Brunauer − Emmett − Teller (BET) model [31]. The total pore volume V_{tot} was calculated from the amount of nitrogen vapour adsorbed at relative pressure p/p_0 close to 1, on the assumption that the pores are then filled with liquid nitrogen. The pore volume at $p/p_0 = 0.95$, $V_{0.95}$, is also evaluated, where the corresponding pore width is 460 Å. The micropore volume (V_μ) was deduced from the Dubinin-Raduskevich (DR) model [32]. The pore size distribution (PSD) was calculated using quenched solid density functional theory (QSDFT) assuming slit shaped pores [33]. Transformation of the primary adsorption data and the (micro)pore analysis were performed by the NOVA2000e ASiQwin 3.0. Water vapour adsorption isotherms of this carbon were measured using a volumetric Hydrosorb apparatus (Quantachrome) at 20 °C, with vapour generated at 100 °C. The pH_{PZC} of these carbons was estimated by the standard pH shift method [34].

2.2.2. Protein adsorption

The adsorption isotherms of the two proteins were measured by batch method from their aqueous solutions (MilliQ water) at 20 °C,