Adsorption and activity of cytochrome c on mesoporous silicates†

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Cytochrome c (horse heart) has been adsorbed onto a range of mesoporous silicate materials with the extent of adsorption dependent on the silicate pore size; adsorption and activity profiles of the adsorbed protein are reported.

Mesoporous silicate materials (MPS) such as those which are formed by mediated pathways involving surfactants as structure directing reagents[1,2] are very suitable as hosts for large organic molecules[3] and proteins.[4–6] MPS exhibit highly ordered pore structures and very tight pore size distributions, as exemplified by the first type of these materials reported, MCM-41.[1] As a direct consequence of their mesopore structures, MPS possess large surface areas of the order of 1000 $m^2\ g^{-1}$. Owing to their silicate inorganic framework, they are chemically and mechanically stable[2] and are resistant to microbial attack. In addition, it is possible to chemically modify MPS with various functional groups, enabling electrostatic attraction or repulsion between MPS and the protein(s) of interest to be maximised.[7] Materials such as sol–gels display similar stability as MCS and have been used to encapsulate proteins for use as biosensors. However, sol–gels suffer from the disadvantage of possessing a highly variable pore size distribution.[8] More importantly, their preparation can involve the use of harsh conditions or reagents which are detrimental to proteins, resulting in denaturation of the protein.[8] With MPS, protein encapsulation occurs after synthesis of the support, avoiding this difficulty. MPS therefore hold great promise for use as supports to immobilise enzymes,[6,7,9] and may find applications in biosensors,[5] biocatalytic[9] and biomolecule separation[7] systems. For example, Stucky and coworkers[7] have used MPS to sequester and release proteins of similar size but varying charge, while Díaz and Balkus[9] have used MPS to immobilise cytochrome c. To date, there have been no reports on the adsorption isotherms obtained with MPS–protein systems, nor on the catalytic activity of a stable, adsorbed, protein–silicate system. In this report, we describe adsorption isotherms for cytochrome c onto four MPS materials covering a range of pore sizes, and peroxidative activity profiles of the adsorbed protein.

Cytochrome c is a small (12 384 Da) redox protein, with an approximate spherical diameter of 40 Å.[10] The cytochrome c (horse heart) (Sigma-Aldrich) used in this study was *ca.* 95% pure and was further purified using standard methods.[11] The MPS materials used consisted of a commercially available silicate (COS) (Fluka, Reidel-de Haën), a cyano-modified silicate (CNS), an MCM-41 material and an MPS material (MPS-F127) synthesised using a non-ionic triblock surfactant Pluronic-F127 as the structure directing agent. The physicochemical properties of the MPS materials are presented in Table 1 and the pore size distributions in Fig. 1.

Cytochrome c has been previously adsorbed onto MPS materials such as MCM-41,[9] SBA-15[14] (MPS of pore diameter 90–110 Å), Nb-TMS4[4] (MPS modified with Nb^{5+}). However, neither the adsorption isotherms nor the peroxidative activity of the protein were reported. The latter, in particular, is important if MPS are to be of use in biosensors and biocatalytic systems.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b0/b009478l/

Table 1 Physicochemical properties of mesoporous silicates used in adsorption studies

MPS	BET surface area/$m^2\ g^{-1}\ SiO_2$	Average pore size/Å	Mesopore volume /$cm^3\ g^{-1}\ SiO_2$	Cytochrome c maximum load/μmol $g^{-1}\ SiO_2$	Volume cytochrome c adsorbed[a]/ $cm^3\ g^{-1}\ SiO_2$
COS	470	55	0.46	3.8	0.3
CNS	379	130	0.61	10.2	0.7
MCM-41	1000	28	0.31	1.7	0.1
MPS-F127	537	50	0.38	6.8	0.5

[a] The volume of cytochrome c was calculated from the crystal structure to be 121 nm^3.[14]

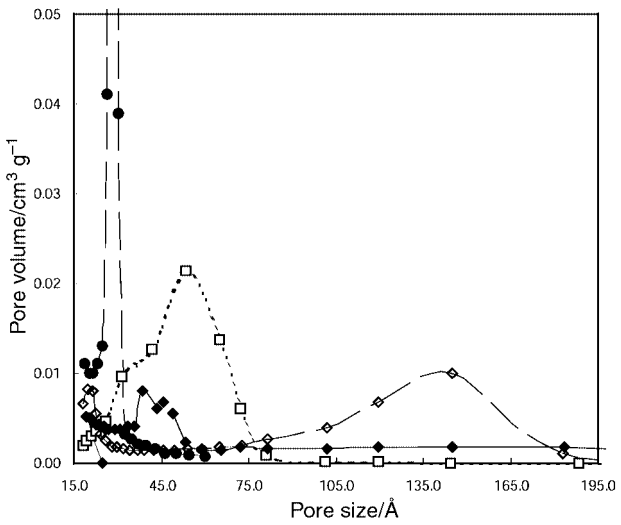

Fig. 1 Pore size distributions of COS (□), MCM-41 (●), CNS (◇) and MPS-F127 (◆).

Fig. 2 shows protein adsorption isotherms for the cytochrome c–MPS systems. It can be clearly seen from Table 1 and Fig. 1 that CNS has the largest average pore size (130 Å) with MCM-41 exhibiting a pore size of 28 Å. The isotherms in Fig. 2 demonstrate that CNS has a much larger affinity for cytochrome c compared with MCM-41. The latter has a very tight pore size distribution at 28 Å (Fig. 1), a pore diameter too small for cytochrome c to penetrate, yielding a maximum adsorption of only 1.7 μmol g^{-1} MCM-41. The CNS material on the other hand, with a much larger pore diameter (130 Å), allows for a maximum adsorption of 10.2 μmol g^{-1} silicate. Clearly, the pore size of the material is a major factor in the adsorption process.[9] The pores in the COS material with an average diameter of 55 Å (Fig. 1) are also large enough to allow penetration of the protein molecule into the pores. With MCM-41, the amount of cytochrome c adsorbed does not change significantly over the range of concentrations studied from the initial 1.7 μmol g^{-1} SiO_2 level), indicating that adsorption is largely confined to the outside surface of the silicate.[9] The MPS-F127 allows for more protein to be adsorbed than in the

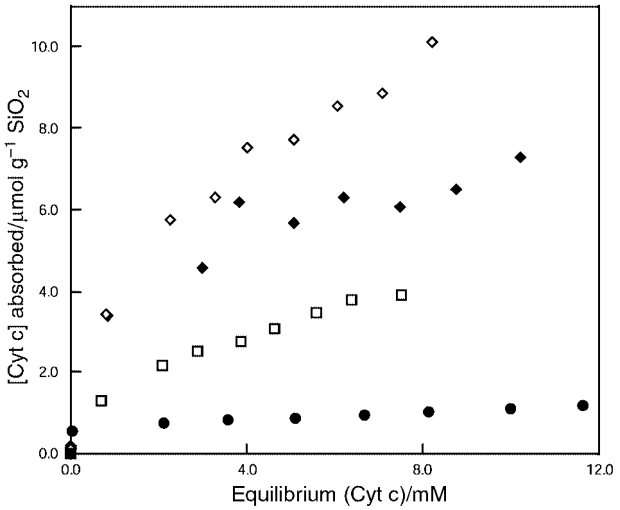

Fig. 2 Adsorption isotherms for pure cytochrome c on to COS (□), MCM-41 (●), CNS (◇) and MPS-F127 (◆).

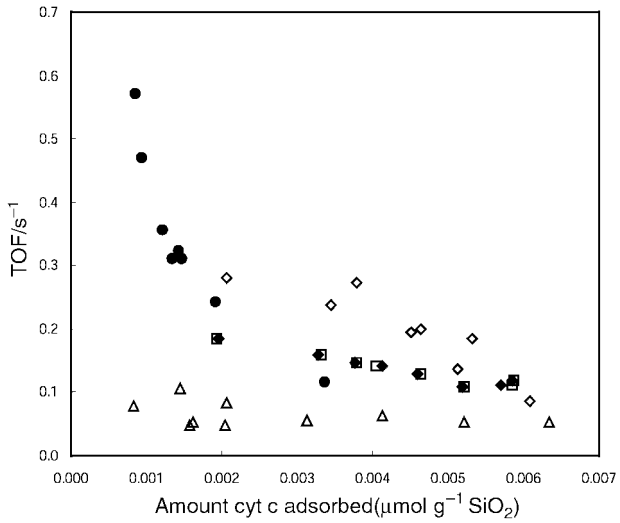

Fig. 3 Peroxidative activity (ABTS assay) profiles for cytochrome c adsorbed on to COS (□), MCM-41 (●), CNS (◇) and MPS-F127 (◆) and of aqueous cytochrome c (Δ). The standard deviations for the assays were 18, 24, 11 18 and 12%, respectively.

case of COS, despite having a similar pore size distribution; this is likely due to the presence of a small subgroup of mesopores with diameters in the range 60–200 Å in the former material (Fig. 1). In contrast to the adsorption of trypsin onto MCM-41,[9] where the enzyme leached out of the silicate, adsorbed cytochrome c was found to be stable to repeated washing in buffer (minimum of four washings). As with the adsorption of conalbumin onto MPS,[7] the ionic strength was found to strongly influence the amount of protein adsorbed, with little absorption occurring at high ionic strengths.[13]

Peroxidative activity profiles‡ over the range of adsorbed cytochrome c are shown in Fig. 3. Aqueous cytochrome c activity was constant over this range (TOF§ ≈ 0.075 s^{-1}). The similarity of the activity profiles of adsorbed protein on COS and MPS-F127 silicates may be a reflection of the similarity in the pore size distributions of these two materials. The general trend of higher TOF at lower amounts of adsorbed protein indicates that access of the ABTS substrate to cytochrome c molecules may be limited at higher protein loadings. This is seen clearly in the MCM-41 profile, where the TOF decreases to that of aqueous solution. From the data in Fig. 1, adsorption of cytochrome c onto MCM-41 appears to occur largely on the outside of the silicate, implying that diffusion of the substrate into the pores cannot be rate limiting. The high TOF values at the lower levels of protein adsorbed may be a result of assaying externally adsorbed protein, where increased substrate access would be expected. The CNS silicate activity profile is similar to the COS and MPS-F127 silicates, but with marginally higher TOF. This is possibly due to the larger pores of the CNS material allowing for faster substrate access. The TOF values obtained for the adsorbed protein are marginally higher for the aqueous protein. This is in marked contrast to the results obtained with trypsin where the activity of the entrapped enzyme was only 13% of that of the protein in solution.[9]

We have shown that a protein–mesoporous silicate system can be readily generated and that cytochrome c is readily adsorbed by such mesoporous materials, provided that the pore sizes of the mesoporous silicate are large enough to accommodate the protein structure and therefore allow access to the large internal surface areas of these materials. The activity of the adsorbed protein at the interface has also been clearly defined and is comparable with the activity of the protein in solution over the same concentration range.

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Notes and references

‡ The activity profiles for each of the silicates were adjusted to reflect only the activity of the adsorbed cytochrome c.
§ TOF is the turnover frequency defined as μmol of reduced ABTS s^{-1}/μmol cytochrome c on silicate.

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