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Temperature-programmed desorption as a tool for quantification of protein adsorption capacity in micro- and nanoporous materials

R. Gadiou[a,*], E.A. dos Santos[a,b], M. Vijayaraj[a], K. Anselme[a], J. Dentzer[a], G.A. Soares[b], C. Vix-Guterl[a]

[a] Institut de Science des Matriaux de Mulhouse/CNRS LRC 7228, 15 rue Jean Starcky, BP 2488, 68057 Mulhouse, France
[b] Lab. de Biomateriais para Engenharia Óssea, COPPE/UFRJ, CP 68505, Rio de Janeiro 21941-972, Brazil

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ABSTRACT

The protein adsorption capacity of porous sorbents is generally obtained by measuring the concentration of proteins desorbed from the materials after treatment by a detergent, or by measuring the decrease of protein concentration in the solution. These methods have some drawbacks and often lead to a low precision in the determination of the adsorption capacities. We describe in this paper a new method that allows to directly quantify the amount of proteins adsorbed on porous materials. This method is based on the quantitative analysis by mass spectrometry of some low mass gaseous species which evolve from the biomolecules during the heat treatment of a temperature-programmed desorption analysis (TPD-MS). The method has been applied to bovine serum albumin and cytochrome C adsorbed on an activated carbon. The adsorption uptake of the proteins on the carbon material could be measured by this direct analysis. A comparison with the depletion method was done, it shows that the two methods are complementary. The depletion method allows a determination of the total adsorption capacity, while the TPD-MS method focus on irreversible capacity.

1. Introduction

The adsorption properties of carbon were already recognized by the Egyptians and Sumerians in particular for medicinal purposes, and activated charcoals were used to adsorb ingested poisonous substances [1,2]. The potential of carbon materials for medical applications concerns also their use as biosensors [3–6] or as nanovectors for drugs [7,8]. Otherwise, activated carbon materials are used for blood cleaning by hemoperfusion. For this last application, the carbon granules are coated with cellulose or heparin hydrogel to improve their hemocompatibility and to avoid any complication due to the release of fine carbon particles. However, the coating of carbon particles dramatically reduces the efficiency of hemoperfusion and limits their use to the treatment of protein-free solutes of low molecular mass. Consequently, some research have been developed on the possibility to use uncoated medical adsorbents [9]. Among them, the development of new materials with well-defined porous structure has lead to new possibilities for the stabilization of enzymes [10]. Inorganic mesoporous adsorbents such as silica and oxides of titanium or zirconium have been considered. It has been shown that they have a great adsorption potential for biomolecules because of their well-organized and interconnected porosity [11–14]. They display also good performances for separation of biological molecules [15,16]. It has also been observed that mesoporous carbon materials synthesized by negative replication of these mesoporous silica templates display several advantages for protein adsorption [12,17,18].

In all these applications, the direct quantification of the adsorbed proteins inside the mesopores is a problem. A lot of techniques, as listed by Chan et al. [19], have been developed for monitoring protein adsorption on membranes or on flat and dense materials (SEM, ATR-FTIR, ellipsometry, AFM, radio-labelling, etc.). Nevertheless, few of them are really quantitative, and none, except the radio-labelling, can be applied on porous materials [20]. The disadvantages of the radio-labelling are that this method is not easily applicable in all laboratories and that it can modify the adsorption potential of the native biomolecule. Therefore, to evaluate the adsorption potential of materials, researchers use an indirect approach by quantifying the concentration of proteins in the supernatant solution before and after immobilization on materials (depletion method) [4,3,17,18,21–23] or in the case of enzymes, by quantifying their activity after adsorption [4,5,13,22,24]. As the indirect approach is imprecise and the radio-labelling often difficult to apply, we propose a new approach based on temperature programmed desorption coupled with a mass spectrometer (TPD-MS) for quantifying protein adsorption in a porous material. This analysis method is based on the quantitative analysis of some specific molecules which evolve from a material as a function of temperature during a thermal treatment at a constant heating rate. Such

* Corresponding author. Tel.: +33 389608719; fax: +33 389608799.
E-mail address: roger.gadiou@uha.fr (R. Gadiou).

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an approach has already been used to study the chemical functions which are present at the surface of carbon materials [25–27], and it was also applied previously for studies on amino-acid adsorption on metallic substrates [28,29]. However, to our knowledge, it is the first time that this approach is proposed for the quantification of proteins adsorbed on a porous material.

For protein analysis, we have mainly focussed our study on sulfur containing species. This element is present in most proteins which are involved in enzymatic catalysis processes or more generally which are of biologic interest. Sulfur is present in two aminoacids: cysteine and methionine and can be involved in disulfide bridges (cystine structures). As pointed out above, the pyrolysis of biological materials has been already used for the analysis of amino-acids. In most cases, the experimental method was based on flash pyrolysis with a Curie point pyrolyzer, coupled with an analysis by gas chromatography–mass spectrometry (Py-GCMS) [30,31]. For sulfur containing amino-acids such as methionine, the main sulfur containing species detected were H_2S, CH_3SH and the dimethyl sulfide CH_3SCH_3(DMS). For cysteine or cystine, H_2S is the main molecule detected. When the methionine is included in dipeptides, cyclization has been observed during the pyrolysis which yield to the formation of diketopiperazines [32,33]. Nevertheless, this cyclization has not been detected by all authors [34]. Either the pristine dipeptide or the cyclic one can then undergo cleavage by electronic ionization, and the main peak related to sulfur containing species correspond to DMS ($m/z = 61$) [32,35]. Nevertheless, it must be noticed that in many studies, the formation of low molecular weight species like H_2S was not investigated as it is the case in this study.

The aim of this work was to develop a new method for the quantitative analysis of some proteins based on their sulfur content. This method should be suitable either for solutions of proteins in a classical liquid medium like the phosphate buffer solution (PBS), and for proteins adsorbed on a solid porous materials. To achieve this goal, we choose to use temperature-programmed desorption (TPD) coupled with a quantitative analysis of hydrogen sulfide by mass spectrometry. The proteins used in this study were the bovine serum albumin (BSA) and the cytochrome C (Cyt-C). These proteins were chosen because they are typical of two different kinds of biomolecules: the BSA is a big protein with a relatively large number of sulfur atoms in disulfur bonds while the Cyt-C is a smaller molecule with only four carbon atoms and no disulfur bridge. Moreover, the Cyt-C has an iron atom in a heme and is typical of proteins involved in catalytic phenomena. The sorbent was a commercial activated carbon material supplied by PICA (France).

2. Materials and methods

2.1. Proteins

Bovine serum albumin as well as human serum albumin (HSA) are known to adsorb on several kinds of biomaterials [36–38]. Moreover, they are the most abundant protein in serum (60%). The BSA is a small heart shaped protein (8.4 nm × 8.4 nm × 4.8 nm) which includes 41 amino acids with sulfur atoms: 35 cysteines and 6 methionines. The molecule exhibits 17 disulfur bonds. The albumin was obtained from Sigma–Aldrich (ref. A7956). A master solution of BSA in phosphate buffer (PBS) with a concentration of 2 mg/mL was produced, sterilized and stocked at 4 °C.

Cytochrome C is a small heme protein (molecular weight 12,500 Da), it is roughly ball shaped with a diameter of 3.5 nm. The adsorption of this protein on several kinds of materials has been studied in the fields of enzymatic catalysis or biosensors [12,39]. The molecule has 4 sulfur atoms, 2 cysteines and 1 methionine are close to the iron atom of the heme. The last sulfur atom is in a methionine group far from the heme. The horse-heart cytochrome C was obtained from Sigma–Aldrich (ref. C7752). As for BSA, a master solution of Cyt-C in sodium bicarbonate buffer solution (BBS) was prepared, sterilized and stocked at 4 °C.

2.2. Sorbent

The carbon material used as protein sorbent was a microporous activated carbon from PICA. The textural properties of the sorbent have been obtained from the gas phase adsorption. The adsorption isotherm of N_2 at 77 K was obtained with a Quantachrome Autosorb A1-LP apparatus. Compared to highly activated carbon materials such as Maxsorb, this carbon material has a lower specific surface area of 1220 m^2/g. The nitrogen adsorption isotherm is of type 1 in the IUPAC nomenclature, this indicates that its pore size distribution includes micropores (with a size below 2 nm) and a very low amount of mesopores (size between 2 and 50 nm). The volume of micropores was obtained by using Dubinin–Radushkevich analysis on N_2 isotherm [40], the corresponding volume is $V_{micro} = 0.62\ cm^3/g$. This value is very close to the total pore volume $V_P = 0.67\ cm^3/g$. From these data, the volume of mesopores can be obtained by difference between V_P and V_{micro}, as expected from the shape of the isotherm, the result is a very low value: $V_{meso} = 0.05\ cm^3/g$. The α_s method was used to obtain the external surface area, that is the surface of macro- and large-mesopores. The value obtained was $S_{ext} = 43\ m^2/g$, this very low surface area is in agreement with the type 1 shape of the isotherm. Compared to a mesoporous sorbent, this carbon material is of course no the best one to achieve high adsorption capacities with large molecules like proteins. Nevertheless, it has the advantage that the driving force of the adsorption is the direct interaction between the carbon surface and the biomolecule and not a pore filling. Therefore, we have chosen this material to study carbon–protein interaction and not to reach high adsorption uptakes.

2.3. Adsorption of albumin on carbon sorbent

The carbon material was packaged as particles with a size between 1 and 1.6 mm. Prior to the adsorption, the sorbent was heat-treated up to 900 ° C using a heating rate of 2 K/min under argon to clean the carbon surface. Then, adsorption experiments were performed by immersing 100 mg of sorbent in 1 mL of protein solution for 1, 4, 24 and 96 h at 23 ° C under constant shaking. For the two proteins, the concentration of the solution was 250 μg/mL. As it was observed that the equilibrium was reached after a 4 h, most experiments were done with this duration. The supernatant solution was then discarded for quantification of the protein by the depletion method (see below). The solid was then filtered, washed three times with the buffer solution (PBS or BBS) and dried before the quantification of the BSA or Cyt-C by TPD-MS experiments.

2.4. TPD-MS experiments

TPD experiments were conducted in a vacuum device equipped with a line-of-sight detection quadrupole mass spectrometer. The gas phase molecules which evolve during the heating are detected and analyzed by the mass spectrometer. As the protein undergo a significant degradation during the experiment, TPD-MS experiments are not *stricto sensu* desorptions. The typical frequency for data acquisition was two mass spectra per degree, and the mass range used was 1–100 mass units. To allow a precise quantification of the main species, a calibration of the spectrometer was performed using known quantities of four gases: hydrogen, nitrogen, carbon dioxide and hydrogen sulfide. For the analysis of H_2S, the signals at $M/Z = 33$ and $M/Z = 34$, corresponding, respectively, to SH and H_2S, were used. Before each experiment the spectrometer sensibility was verified by performing a calibration with N_2 and the calibration of the other gases was adjusted by this procedure.