NUCLEAR MAGNETIC RELAXATION STUDY OF WATER AND PROTEINS ADSORBED IN MESOPOROUS ACTIVATED CARBONS

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Introduction

High resolution NMR technique is a unique method providing direct information about the mobility and environmental state of molecules in the adsorbed layer [1]. Particularly advantageous is the ability of NMR to study adsorbed molecules directly in the aqueous media. Proton relaxation times, both longitudinal, T_1 and transverse, T_2 respond to changes in the molecular environment and thus affected by the surface. The rate of the transverse relaxation, $R_2 = 1/T_2$ is very sensitive to the local gradients of the magnetic field, hence, it reflects the relaxation processes in the nearest vicinity of the surface and provides information on the structure of the adsorbed layer and the nature of an adsorbate-adsorbent interaction [2].

Experimental

Bovine serum albumin, BSA (MW 68 kD), Cohn fraction V (Sigma, USA) was recrystallised to obtain an essentially globulin-free protein. HPLC-purified mouse γ-globulin, IgG (MW 158 kD) was obtained from Palladin Institute of Biochemistry, Kiev, Ukraine. A carbodiimide coupling method was used for covalent attachment of protein molecules to the carbon surface [3]. A water-soluble carbodiimide, CMC was purchased from Sigma.

Adsorption experiments were carried out by shaking 0.2% aqueous solutions of individual proteins with a carbon sample at room temperature for 1 hour.

Activated carbon SCN was produced by a step pyrolysis of a mesoporous vinylpyridine-divinylbenzene (VP-DVB) copolymer containing 10% of DVB at 350-900°C and further steam activation at 900°C. Its physical characteristics are given in Table 1. SCN contains ca. 1% structural nitrogen.

NMR spectra were obtained with a WP-100SY high resolution NMR spectrometer (Bruker, Germany) at 100 MHz. A standard programme of spin-echo pulse sequence was used for measuring T_2. To decrease the contribution of bulk water in the transverse relaxation, a 'freezing-out' technique was used [4]. For this purpose relaxation measurements were performed at temperatures below the freezing point of water. The temperature was regulated by a VT-1000 probe (Bruker) with an accuracy of ± 0.5°C. The contribution of bulk ice to the NMR signal can be neglected and only the signal from unfrozen water in the adsorbed layer or in any other state affected by proximity to the surface is measured. The amount of bound water was determined from the ratio of signal intensities before and after freezing. The total amount of water in each sample was constant (2 ml).

Table 1. Some physical characteristics of the activated carbon SCN.

Burn-off, %	Density, g/cm³		Pore volume, cm³/g				S_{BET} m²/g	S_{me}, m²/g	r_{meso} nm
	ρ	d	V_Σ	V_{ma}	V_{me}	V_{mi}			
85	0.20	2.10	1.96	0.12	1.2	0.64	1500	300	35

ρ - bulk density; d - apparent density, determined by a liquid benzene replacement method; S_{me}, mesopore surface area. Analysis of porous structure was done using benzene vapour adsorption-desorption data and mercury porosimetry; r_{meso} - mean mesopore radius. SCN carbon has a narrow mesopore size distribution.

Results and Discussion

T_2 for water adsorbed on SCN carbon is low compared to T_2 in ice in the studied temperature range 200 - 250 K. Under these conditions, T_2 arises only from non-frozen molecules. They do not exchange with ice in the bulk or in the pores and slowly exchange between themselves. Existence of non-frozen water below 273 K is due to the steric reasons. Spatial distribution of water molecules inside narrow pores does not correspond to the ice lattice and water exists in a pseudoliquid state. The molecules inside pores form clusters. This exchange can be represented by the following scheme:

$(H_2O)_A \leftrightarrow (H_2O)^* \leftrightarrow (H_2O)_B$, where a water molecule exchanges between cluster A and cluster B in the matrix via transition state. Assuming a diffusion controlled monomolecular mechanism of exchange,

$T_2^{-1} = T_{20}^{-1} \exp(-E_a/RT)$, (1)

where: $T_{20}^{-1} = (kT/h)^{-1}$ for a first-order reaction. Rearranging equation (1) and taking into account that:

$E_a = \Delta H^* - T\cdot\Delta S^*$, thermodynamic parameters of the exchange processes can be estimated. Plotting T_2 dependence on temperature in the Arrhenius co-ordinates, ln T_2 vs. (1/T), several regions can be clearly distinguished for some of the systems studied (Fig. 1). Different exchange behaviour indicates presence of several types of water clusters in the porous matrix [5]. Covalent binding of protein molecules to the surface brings significant changes in the behaviour of both "SCN-

protein" systems. ln T_2 vs. (1/T) is linear without breaking points in the whole temperature range for the systems "SCN-BSA" and "SCN-CMC-IgG". It possibly means that in these systems adsorbed BSA molecules or covalently attached IgG molecules displace water at the surface. Different behaviour of BSA and IgG can be explained in terms of their structural difference. BSA is a molecule with a flexible structure and its physical adsorption on porous SCN is highly irreversible because of its multi-site binding. Amino acid residues of physically adsorbed BSA penetrate pores which are much smaller than the whole protein molecule irreversibly fixing it on the surface. Added carbodiimide binds protein molecules via amino groups with activated surface carboxylic groups. In addition, CMC cross-links protein molecules via their own amino -NH_2 and -COOH groups. Due to the covalent biding and cross-linking, BSA loses its flexibility and is not able to displace adsorbed water molecules. Contrary to BSA, IgG has a rigid structure which is not affected significantly by the surface. Hence, physically adsorbed IgG cannot substitute for pre-adsorbed water in micro- and narrow mesopores. Covalent binding results in a multi-site attachment of IgG to the surface via CMC-activated surface groups, which is more significant than cross-linking in the solution. It leads to the substitution of adsorbed water by IgG. Different regions on the ln T_2 vs. (1/T) plot reflect existence of several types of water clusters in the porous matrix, which may be ascribed to the water adsorbed in pores of different width [5].

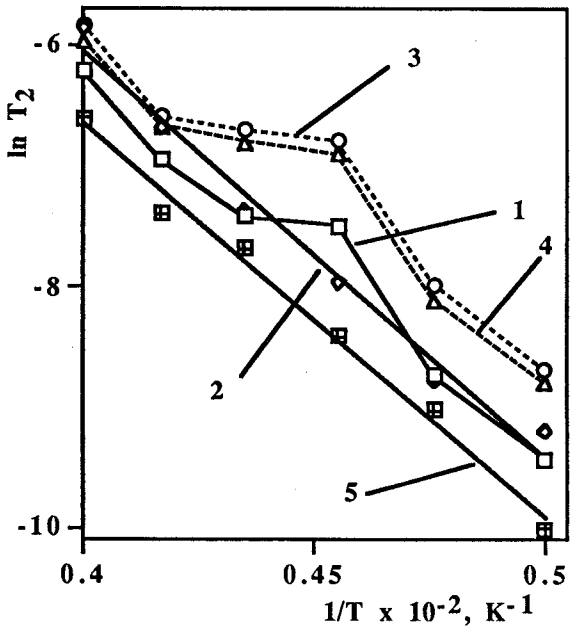

Fig. 1. Temperature dependence of T_2 for:
1 - SCN, 2 - SCN-BSA, 3 - SCN-IgG,
4 - SCN-CMC-BSA, 5 - SCN-CMC-IgG.

From the intensity of the water proton signal in the NMR spectra, concentration of bound water in the samples was determined (Fig. 2). Addition of carbodiimide reduced amount of water bound in the matrix, by 20-25%. Interestingly, in the SCN-BSA system the amount of bound water is slightly higher than in the pure SCN. This may be due to the presence of water bound to the BSA molecules. Adding CMC increases the freezing point of water in the pores by 10-15 K compared to "SCN-protein" system. It is possible to estimate the pore volume of SCN using these data. Assuming the molar volume of water in the pores is equal to the molar volume of liquid water, the pore volume is about 0.45 cm^3/g which is in a satisfactory agreement with the micropore volume determined by a different method (Table 1).

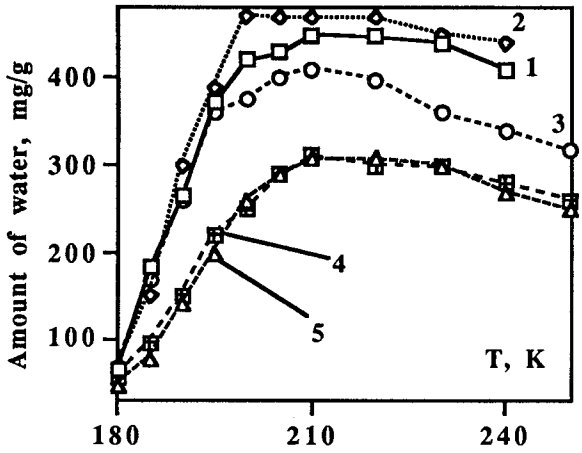

Fig. 2. Amount of bound water on different SCN samples. 1 - SCN, 2 - SCN-BSA, 3 - SCN-IgG, 4 - SCN-CMC-BSA, 5 - SCN-CMC-IgG.

Conclusions

Water proton magnetic resonance, in particular, measurement of the transverse relaxation time at temperatures below 273 K can be used for direct investigation of adsorbed protein layers on activated carbons.

References

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