CARBONS WITH SPECIFIC ADSORPTIVE ABILITY TOWARDS HEMOGLOBIN

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INTRODUCTION

At present the sorption methods for removing the harmful and toxic substances from organism enjoying widespread use for treating numerous severe diseases and poisonings [1]. Specially prepared carbons (carbon hemosorbents) known for their high sorptivity with respect to a broad range of toxic substances are preferably used then. As the method of the organism extracorporeal detoxification is being further improved, development of materials with a bioselective action in particular to extract free hemoglobin (Hb) from biological fluids have become an urgent problem of today.

In the present work we have tried to synthesize and investigate biospecific carbons grafting by certain bioactive low-molecular-weight compounds (amino-acids) to their surface. This is all the more interesting because aminoacid grafting to other porous materials - synthetic resins, dextranes (polysaccharides) etc. - has been reported [2,3] to yield bioactive adsorbents.

It is known, that sorbents with immobilized p-oxyphenylethylamine or α–aminoacetic acid have high sorptive activity towards Hb. We assumed, that aminoacid-containing carbon hemosorbents will be able to adsorb Hb too. Four aminoacids lysin, tyrosine, tryptophan and phenylalanine were immobilized on carbon surface.

EXPERIMENTAL

As a matrix we use the so-called "SCN" (nitrogen containing spherical carbonites) hemosorbents prepared on the basis of synthetic active carbons with regular spherical grains, which are free of mineral admixtures and have well-developed porosity and high biocompatibility. Carbon is inert material. For its further chemical modification it is necessary to create initial stable chemical groups on the carbon surface. Oxidization by nitric acid in fluid phase is simple and convenient method to prepare oxygen-containing stable groups with movable proton atom on the carbon surface. This determines the method of immobilization of aminoacids. They were chemically immobilized via carbon chloranhydride derivatives. Surface functional groups are analysed by pH-titration and X-ray photoelectron spectroscopy (XPS), "Varian IEE-15" spectrometer.

Structure-adsorptive studies were carried out as follows: a) Macro- and mezopores distribution by their radii was determined by the mercury porosimetry method (Porosimeter M 9300 "Micrometric" production. Threshold radius was 3 nm (limited pressure - 2500 kg/ccm); b) Determination of the sorption pores volume by the benzene vapours adsorption at 20 C taken off the isothermostatted vacuum adsorption unit supplied with quartz spring balance; c) Determination of specific surface area chromatographically by a nitrogen thermal desorption; d) Determination of adsorption capacity of water-soluble sorbates by spectrometric data.

RESULTS AND DISCUSSION

Chemical bonding of aminoacids to the carbon surface was proved by substantial alteration in the potentiometric titration curves and the pH-metric titration curves for the initial carboxyl-terminated carbons and those with aminoacids covalently bonded to their surface. Almost all modified carbons are ampholytes and their isoelectric point is shifted to a pH value of seven. As compared with the initial oxidized carbon, the modified ones have higher anion-exchange capacity (this excess reaches 0.6-0.8 mmole/g). Cation exchange capacity consequently decreases to the same extent. This reflects essential alterations in the carbon adsorbent's surface chemistry.

Establishing the chemical structure of the immobilized surface groups is a fairly complex problem since carbon sorbents are rather difficult for spectra investigations. X-ray photoelectron spectra can provide information on the energetic state of particular atoms. However, the surface groups contain the same atoms (C, O, N, H) as the SCN carbon framework, which is the main difficulty in spectrum interpretation.

As the energetic state of nitrogen atoms in the initial oxidized carbons has been studied well enough [4], we have analysed the state of nitrogen atoms in the modified carbons. The 2p-electron absorption band of nitrogen in XPS of modified carbons turns out to be 0.5-1 eV shifted to the low energies as compared with that of pyridine-like hybridized electrons in the initial carbon. This proves the appearance (as result of carbon chemical modification) of groups with nitrogen in a low energetic state, which is typical for nitrogen (III)-containing fragments of aminoacids, amines, etc.

Accounting for the above-mentioned possibility that some adsorbents with immobilized low-molecular weight ligands are bioactive [2,3,5,6], it seems expedient to investigate the changes in biochemical indices of patients' blood serum after they have been treated with modified carbon adsorbents. Carbons with immobilized lysine and tryptophan [5,6] markedly decrease the concentration in serum of free hemoglobin, which is toxic and may strike the kidneys in some groups of patients.

For more details we have obtained isotherms of free hemoglobin sorption with aminoacid-containing carbons in a simulating solutions (0.15 M phosphate buffer solution with pH of 7.2 at 20 C for 4 hours). Their analysis shows that modified SCN carbons adsorb Hb. Lysine-containing carbon has the most pronounced selective hemoglobin affinity. Thus, in our experiments, its sorption capacity reaches 60 mg/g. Under the same conditions, initial carbon will asorb 7 mg/g of Hb, whereas lysine-modified carbon in the investigated range of hemoglobin concentration has a great reserve of sorption capacity.

The influence of structure-sorptive properties and degree of oxidization of initial carbon sorbents on sorptive capacity of lysine-containing hemosorbents towards Hb has been investigated. We have 5 carbon sorbents differing in specific surface area (from 1500 to 2100 sq.m/g) and sorption pore volume (from 0.55 to 1.25 ccm/g). All sorbents were oxidized to 4 different degrees (from 0.4 to 1.6 mg-eq/g). It was shown, that lysine-containing carbon prepared from hemosorbent with a specific surface area 1900 sq.m/g, sorption pore volume 1.25 ccm/g and oxidized to 0.4 mg-eq/g has reproduced sorptive capacity towards Hb (Fig.1).

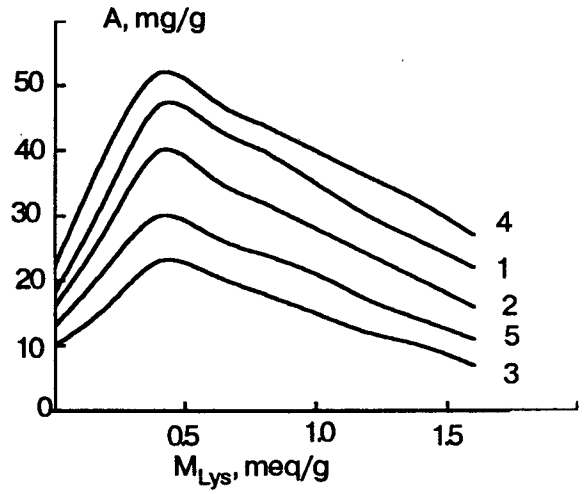

Fig.1. Dependence of adsorption of Hb by lysin-contaning carbons (differed in structural charac teristics Vs in ccm/g - S in sq.m/g) vis amount of immobilized lysine: 1 - (1.10 - 1830), 2 - (0.87 - 2030), 3 - (0.56 - 1600), 4 - (1.25 - 1930), 5 - (0.65 - 1500).

.CONCLUSION

Evidently, the obtained aminoacid-containing hemosorbents, which effectively recover free hemoglobin can be used in treatment of hemolytic diseases, as well as for the rehabilitation of the properties of stored blood and for the selective bonding of hemoglobin and its components in biotechnology.

REFERENCES

1. V.G.Nikolaev, V.V.Strelko. Hemosorption on active carbons.-Kiev: Naukova Dumka, 1979,- 216 pages (Russ).
2. Z.Yamasaki ea all. Abstracts of 29th Annual meeting of ASAIO. 12 45 (1983).
3. E.Behm et all. Abstracts of 7th Int. Symp. on Hemo perfusion.- Kiev: Naukova Dumka.-26 (1986)
4. V.Strelko et all. Sorption and Adsorbents.-11(1983) (Russ.).
5 V.V.Strelko et all. Sorption and Adsorbents. 11 (1983) (Russ).
6. T.Tsuchida et all. Proceedings.-Int.Symp. on Hemo perfusion, Adsorbents and Immobilized Reactants. 28 (1988).