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Interactions of biospecific sorbents with physiologically active substances

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The synthesis and application of hydrogel biospecific sorbents with immobilized physiologically active substances for creation of bio- and blood-compatible polymeric materials are given and discussed. The chemical nature and structure of affinic ligand, its availability and distribution in the matrix determine the action efficiency of these sorbents. A high blood-compatibility of polymers can be reached by chemical modification with several physiologically active substances acting at the different steps of thromboformation. The complex biotissue chemical modification permits the reduction of calcium and phosphorus accumulation in *in vivo* experiments by 10–100 times, compared with the control.

1. Introduction

The problem of bio- and blood-compatibility of polymeric materials which are used as bioprosthetic heart valves, blood vessel prostheses, devices for artificial blood circulation, etc., is now of great importance. It is generally accepted that beside the synthesis of specially aimed polymers, the chemical modification of known polymers by physiologically active substances (PhAS), selected according to their chemical nature and functioning biological purposes, and interacting according to the principles of affinic binding with other PhAS, may help to solve some problems in this area. It can be stated convincingly on the basis of our experimental [1, 2] and literature [3, 4] data that for creation of good bio- and blood-compatible materials, polymers can be modified using hydrogel systems with immobilized PhAS. Hydrogels on a synthetic polymer base, such as polyacrylamide, polyvinyl pyrrolidone, polyvinyl alcohol, etc., have dynamic properties, softness and elasticity similar to those of soft natural tissues of the human body. The activation of sorption and adhesion of platelets, and adsorption and inactivation of plasma proteins may be considerably decreased when such systems are used for temporary or permanent contact with blood.

The method of immobilization of PhAS in the hydrogel, and the preservation of PhAS activity during their chemical modification, are very important, because modification should not affect the PhAS-active centre, and "excess" modification not affecting the active centre or features of the PhAS immobilization can lead to the loss of biological activity, at the expense of either losing PhAS conformational mobility, or steric PhAS inaccessibility for the substrate.

Various chemical methods of immobilization with preliminary activation of the gel matrix by carbodiimide, cyanogen bromide, dialdehyde, and others, are known. However, the complexity of regulation of activation processes and increased non-specific sorption of other PhAS, make these methods virtually unacceptable for modification of biomaterials intended for implantation. Another possibility for chemical immobilization of PhAS into a gel also exists: the typical radical copolymerization of hydrophilic monomer, cross-linking agent and monomer-ligand (PhAS). Monomer-ligand must contain at least one double C=C bond in the molecule. This can be illustrated using the example of the macromonomer of heparin [5]. The special procedure of the reaction of *N*-desulphatation and further reaction of acylation with acryloyl chloride result in the formation of the macromonomer heparin. The double C=C bond is active in the radical homo- and copolymerization. Thus the polyheparin with a molar mass as high as 80 000 can be obtained (Fig. 1). The anticoagulant activity of polyheparin is higher than that of native heparin. Therefore, if the thrombin time in the presence of native heparin is 6 min, this parameter in the presence of polyheparin is 12–15 min. Heparin-containing polyacrylamide hydrogels can also be obtained. Heparin is chemically immobilized in a hydrogel and remains in the polymer matrix after thorough washing. Anticoagulant activity of the immobilized heparin is similar to the activity of native heparin [2].

PhAS-low-molecular synthetic inhibitors of thrombin (the derivatives of *p*-chlorobenzylamine can be used as ligands in biospecific sorbents for thrombin, serin protease) play an important role in blood coagulation. Thrombin affinity to the ligand is determined exclusively by the chlorobenzyl-radical structure and hydrophobicity of the spacer which binds the ligand to the matrix. The absorption characteristics of sorbents are estimated using the chromatography method and are presented in Fig. 2. When $n = 0$, thrombin is practically not bound by this sorbent. The ligand structure is such that during immobilization, the ligand is incorporated close to the macromolecules of the sorbent matrix and does not show an affinity to

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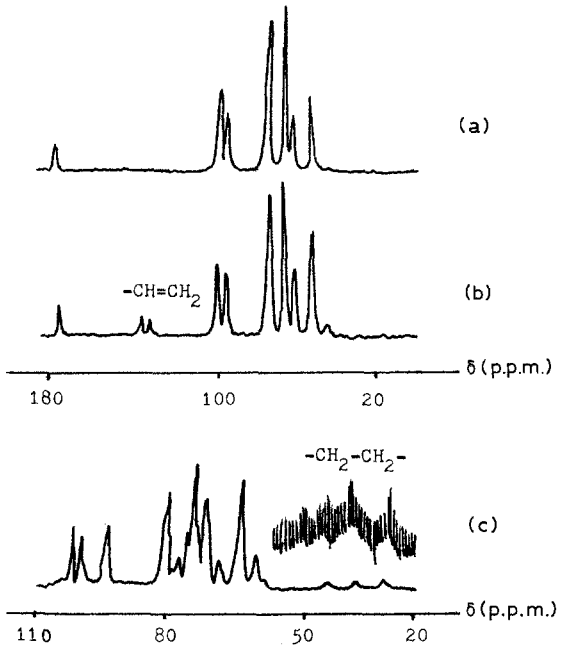


Figure 1 ^{13}C NMR spectra (20, 115 MHz) of solutions of heparin in D_2O: (a) native lung heparin, (b) macromonomer of heparin, and (c) polyheparin.

thrombin because of steric hindrances. When $n = 6$–11, the sorbent's capacity is rather high. This extreme capacity is connected with the association of hydrophobic ligands at the sorbent swelling in aqueous media. Using such sorbents, thrombin with an activity of 2500 NIH clotting units per mg of enzyme, can be prepared [6].

Biospecific sorbents make it possible to select one influence of one of the components of complex biological mixture of substances of similar chemical nature. This can be illustrated by biospecific absorption with a system containing at least two immobilized PhAS-ligands, one of which enables selection of the separation of the required substance from the mixture, and

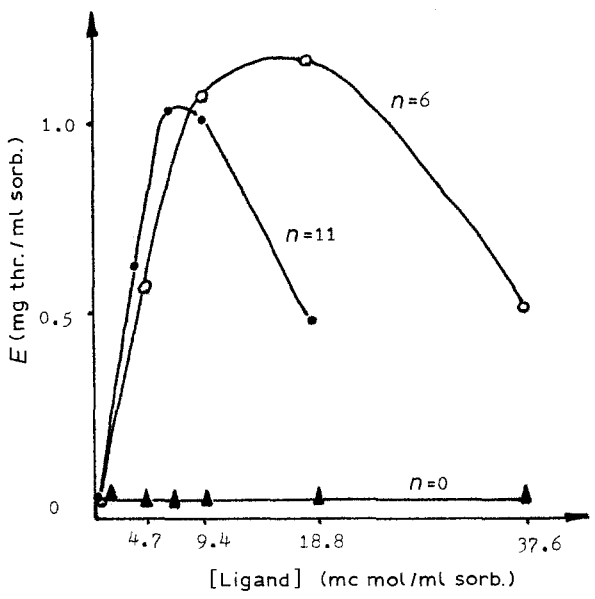


Figure 2 Capacity of affinic sorbents for thrombin as a function of ligand content in sorbents.

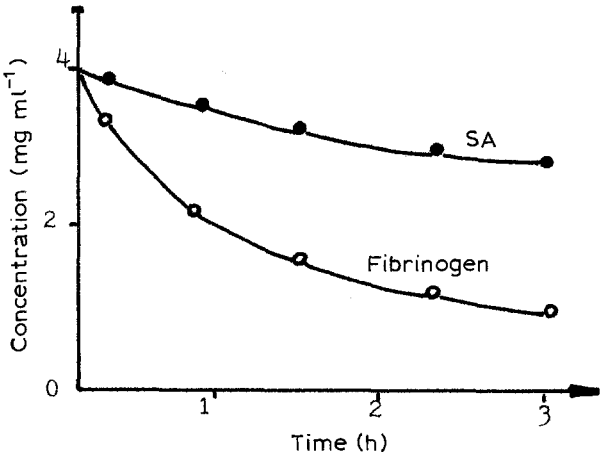


Figure 3 Hydrolysis of serum albumin and fibrinogen with trypsin–heparin-containing gel (0.46 mg trypsin and 1.43 mg heparin in 1 ml gel).

the other exerts an influence on the first. Both ligands, for example, chemically immobilized in hydrogel heparin and trypsin, work as a brigade of two [7].

Trypsin immobilized in a gel retains its proteolytic activity and lyses many proteins, in particular serum albumin and fibrinogen. The rate of hydrolysis for both proteins is practically equal; there is no selectivity. Fig. 3 shows the dependence of the amount of fibrinogen and serum albumin hydrolysed products versus the time of contact of the protein solution with the biospecific sorbent containing covalently immobilized trypsin and heparin. It is seen that the binary gel lyses fibrinogen more actively than a single-component trypsin-containing gel, because of the increasing concentration of fibrinogen in the vicinity of the trypsin active centre owing to the highly complex ability of the immobilized heparin with fibrinogen (heparin itself has no lytic activity, either in the native or the immobilized state).

Experimental data on the interaction of biospecific sorbents with PhAS have helped to a considerable extent to solve some important problems of the creation of blood- and bio-compatible polymeric materials. It is known that an increase of blood-compatibility of polymer materials can be achieved if the heparin is immobilized in a polymer gel. However, a much greater effect can be reached by chemical modification of the polymer with biospecific sorbents containing several PhAS, acting on the different steps of thromboformation, for example, with biospecific sorbents containing both heparin and trypsin [8], or heparin and anti-aggregants of platelets.

Chemical modification of polyethylene catheters and PETP vascular prostheses can be carried out by radiation graft copolymerization of hydrophilic monomer and macromonomers of trypsin and heparin. Estimation of blood-compatibility of such catheters being inserted in dogs' *vena cava* shows that the amount of thrombotic mass after 20 min blood contact with materials on the non-modified catheters, is equal to 10–14 mcg cm^{-2}, while for that modified with heparin and trypsin catheters, this parameter is 2–4 mcg cm^{-2} (Table I). The *in vitro* test of the relative time of blood coagulation and the relative index of

TABLE I Blood-compatibility of modified polyethylene tested *in vitro* and *ex vivo*

Quantity of PhAS on 1 cm^2 PE (mcg)		Relative time of blood coagulation	Relative index of platelet adhesion	Thrombotic mass (mcg cm^{-2})
Heparin	Trypsin			
0.31	0.16	2.1 ± 0.3	2.3 ± 0.7	2–4
0.30	0.26	3.2 ± 0.3	1.6 ± 0.7	
0	0.14	1.2 ± 0.1	1.3 ± 0.5	
1.50	0	10.0 ± 1.0	6.0 ± 1.2	
0.30	0	1.8 ± 0.1	3.9 ± 0.5	
0	0	1.2 ± 0.1	1.8 ± 0.1	10–14

glass is standard

platelet adhesion shows that a lowered adhesion of platelets and an increased time of blood coagulation exist in the case of polymer materials modified with a mixture of heparin and trypsin. Tests on blood with the coagulogram method before and after contact with such biospecific sorbents, show that there is a sharp decrease in fibrinogen concentration and a decrease in activity of the fibrin-stabilizing factor.

The chemical modification of biotissues with biospecific sorbents having immobilized PhAS helps to solve some problems in the prevention of calcification of pork aortic valves and bovine pericardium, which are used as bio-transplants in clinical practice. Biomaterials can be treated in accordance with alkaline proteases to complete the destruction of cell elements being removed from the tissue; then they can be cross-linked in 0.5% solutions of glutaraldehyde. During the stages of glutaraldehyde biotissue tanning, the modifying systems used were: *N*-vinylpyrrolidone (VP, a hydrophilic monomer for decreasing the biotissue porosity), acryloylsalicylic acid (ASA, an anti-aggregant of platelets), and acryloyl aminohydroxy propylidene diphosphonic acid (APDPhA, model derivatives of calcification inhibitors).

Modifying substances were immobilized by means of radiation graft copolymerization with a total dosage of 1 Mrad (surface and volume modification of materials occurs). The effect of calcification inhibition for modified and control materials can be studied *in vivo* using subcutaneous implantation of 30–40 mg of the sample under the dorsal skin of 3 week old rats with subsequent quantitative calcium (Ca) and phosphorus (P) determination after 21 and 100 days (10 animals in each series). Coagulation tests can be carried out *in vitro* by sample incubation with blood plasma of intact goats (7 tests in each series) and *in vivo* by suturing pork aortic valve cusps and pericardium with dimensions 20 mm × 20 mm, into the left *atrium auricula* and into the ascending aorta with the examination of blood clotting factors. Fig. 4 demonstrates the results of tests of the blood clotting system. It is seen that such a chemical modification does not impair material blood-compatibility, compared with the control. The data for accumulation of common Ca and P in the biotissues during subdermal implantation in young rats are represented in Fig. 5. It is seen that complex biotissue modification permits Ca and P accumulation to be reduced in *in vivo* experiments by

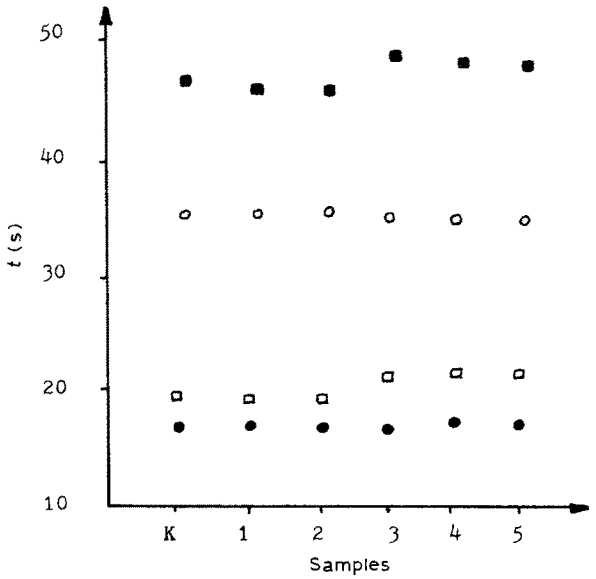
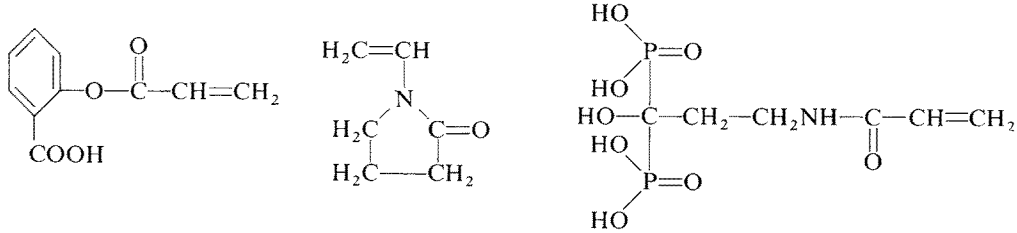


Figure 4 Tests on the blood-clotting system: K, control blood; 1, pericardial control sample; 2, pork aortic valves control sample; 3, pericardium modified with ASA and VP; 4, pork aortic valves modified with ASA and VP; 5, pork aortic valves modified with ASA–APDPhA-VP. (■) Recalcification time of full blood (BRT), (○) activated partial thromboplastin time (APTT), (□) thrombin time (TT), (●) prothrombin time according to Quick.



(ASA) (VP) (APDPLA) (1)

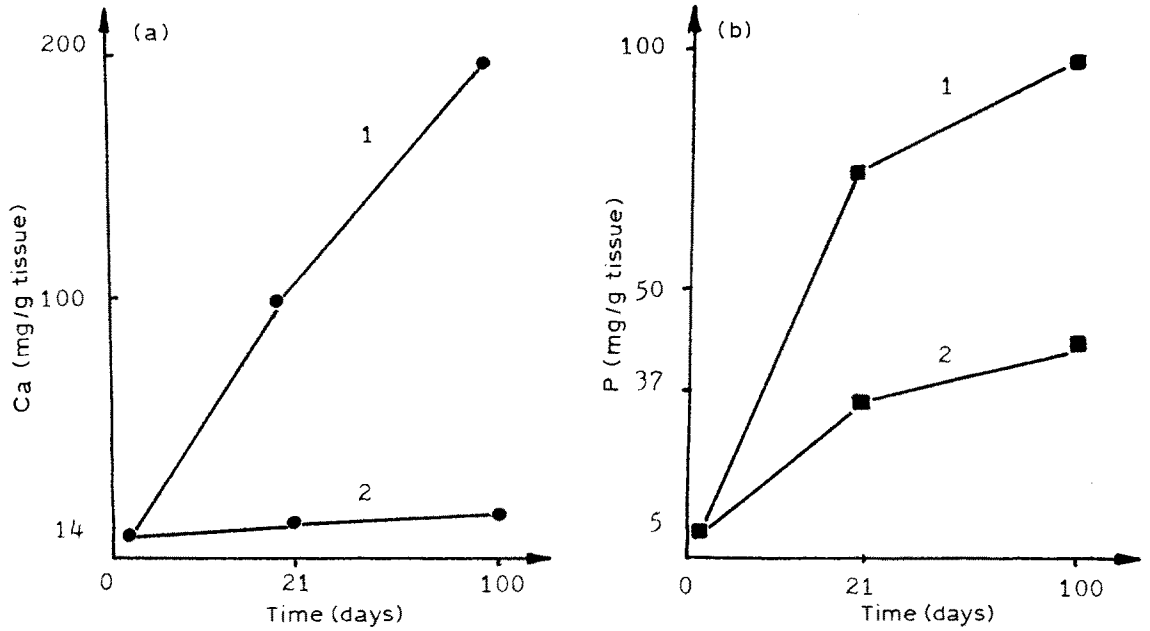


Figure 5 Accumulation of common calcium and phosphorus in the bioprostheses of pork aortic valves during subdermal implantation in young rats. 1, Control samples of biotissue; 2, modified with ASA–APDPhA-VP samples of biotissue.

10–100 times, compared with the control. Ca and P accumulation is essentially affected by the quantitative proportions of modifying substances in the mixture. In the case of modification using ASA–APDPhA–VP, inhibition of calcification for component proportions 1:1:1 and 1:1:2, differed by twice. Such a relationship for the effectiveness of calcification inhibition may be connected with the different composition and distribution of the groups in the grafted polymer chains, which in turn are determined by the monomer reactivity and composition of the initial modifying mixture.

Thus the study of interactions of biospecific sorbents with physiologically active substances and chemical modification of synthetic and natural polymers with PhAS made it possible to solve some problems of the creation of bio- and blood-compatible materials.

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