Pure & Appl. Chem., Vol. 56, No. 10, pp 1335-1344, 1984.
Printed in Great Britain.

0033-4545/84 $3.00+0.00
Pergamon Press Ltd.

ANTITHROMBOGENIC POLYMERS

Marcel JOZEFOWICZ and Jacqueline JOZEFONVICZ

Laboratoire de Recherches sur les Macromolécules, Université PARIS-NORD
GRECO 48 - ERA 607 CNRS - Avenue J.B. Clément F 93430 VILLETANEUSE
FRANCE

Abstract - Antithrombogenic materials may be designated as catalyst of the inhibition reactions of the coagulation process induced at the blood material interface. Analysis of the control mechanism of the coagulation cascade allows to propose a general approach to the preparation of antithrombogenic polymers. The different types of antithrombogenic materials which have been tailored are reviewed including the anticoagulant drugs releasing materials, those onto which anticoagulant drugs have been covalently bound, the heparin-like polymers. The latter are shown to be promising candidates for various medical applications.

INTRODUCTION

Blood coagulation is the primary effect which occurs when blood contacts any surface except the natural healthy endothelium wall of the blood vessels. Therefore, blood compatible materials, i.e., materials suitable for use as cardiovascular prosthetic devices, have to be primarily non thrombogenic or antithrombogenic ones. Indeed, blood coagulation will not be induced at the interfaces with non thrombogenic polymers while antithrombogenic polymers will prevent clot formation by use of the mechanisms which control the so-called coagulation cascade.

In order to show how antithrombogenic polymers may be designed and tailored, in the following we shall examine first the biochemistry of blood coagulation focusing attention on the control mechanisms. Then, we shall review the different types of antithrombogenic polymers which have been tailored successfully, namely :
- the anticoagulant drugs releasing materials
- the materials onto which anticoagulant drugs have been covalently bound
- the heparin-like polymers including insoluble and soluble materials.

THE COAGULATION OF BLOOD

Blood is a suspension of cells in an aqueous saline solution called plasma.
Blood cells are essentially erythrocytes, leucocytes and platelets. It is believed that only the latter play an important role in coagulation. Plasma contains several hundreds of proteins, lipoproteins and glycoproteins (Table 1). The biological activity of certain of these proteins, such as albumin and immunoglobulin, is essential, but it seems to be of less importance as far as the coagulation process is concerned. Since the beginning of the century, considerable attention has been paid to the biochemistry of the blood coagulation process and its control mechanisms which are now therefore relatively well understood.

The clot formation is the end result of a catastrophic event, called the coagulation cascade, a sequence of enzymatically catalyzed biochemical reactions in which both blood cells and plasma proteins are involved. With regard to their biological activity, these proteins are different. Some of them, zymogens, are able to undergo an activation process, i.e., a catalytic hydrolytic cleavage leading to the formation of enzymes. This is the case with the so-called coagulation factors with the exception of fibrinogen and factors VIII and V. Other proteins, such as fibrinogen, play the role of enzyme substrates. They are able to undergo an hydrolysis reaction by an enzymatically catalyzed process. A further category of proteins consists of the so-called cofactors. The presence of these proteins in the plasma in inactivated form is essential in so far as, when activated, they drastically increase the rate of enzymatically catalyzed reactions. To this category belong factors VIII and V.

On the other hand, the coagulation process is under control of inhibiting factors. Some of them are plasma enzyme inhibitors (Table 2) which are able to form specific inactive complexes with given enzymes. Other involve both membrane cell receptors and plasma zymogens which undergo activation by an enzymatically catalyzed process. In turn, the resulting enzymes are potent coagulation inhibitors.

TABLE 1. Normal values of protein concentration in human plasma

Protein	g/l
Albumin	35 - 55
Fibrinogen	2 - 4
Immunoglobulins	
IgG	9.2 - 14.8
IgA	1.4 - 2.6
IgM	0.9 - 1.8
Coagulation factors	
II	0.100
V	0.005 - 0.100
VII	0.001
VIII	0.010
IX	0.005
X	0.010
XI	0.003
Prekallikrein	0.050
High Molecular Weight Kininogen	0.070

TABLE 2. Normal values of some coagulation inhibitors in human plasma

Inhibitor	g/l
α_1 antitrypsin	2 - 4
α_2 antiplasmin	0.085
C_1 inactivator	0.250
α_1 antichymotrypsin	0.480
α_2 macroglobulin	3
Antithrombin III	0.150 - 0.300
Second heparin cofactor	-

The coagulation cascade

When blood is placed in contact with any surface, the following sequence of events appears.

Adsorption of proteins. In one minute or less, a competitive adsorption of proteins and glycoproteins occurs at the surface and forms a complex protein coating on the surface. Some of these adsorption processes are partially or completely reversible (Ref. 1-3). Depending on the nature of the surface, some of the deposited proteins may initiate different coagulation pathways (Fig. 1).

Platelet activation. First, the von Willebrand factor (a polymer of factor VIII) mediates platelet attachment onto the surface. Then, some internal constituents are released from aggregated platelets into the plasma. These transformations, which are under the control of prostaglandins, calcium ions, cyclic adenyl monophosphate and adenyldiphosphate concentration, are essential. They make available both specific proteins and platelet membrane phospholipids which are of prime importance for the acceleration and control of the coagulation process.

Platelet activation is also initiated by thrombin, adenyldiphosphate and other reactants. It can also be produced by turbulences of the blood flow and is a function of the flow rate.

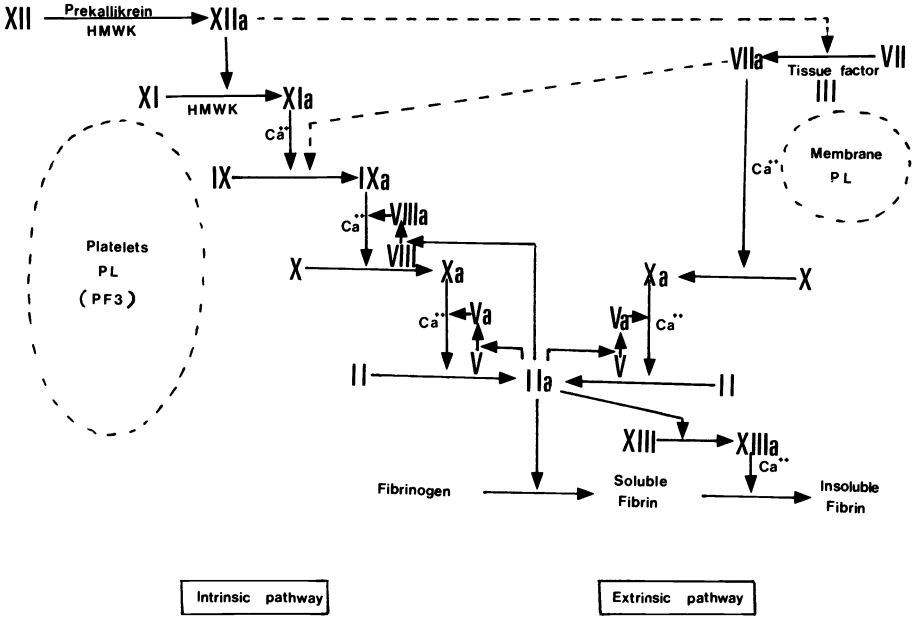

Fig. 1. The coagulation cascade

Intrinsic pathway. When artificial surfaces are placed in contact with blood, the activation of the coagulation process occurs through the so-called activation of the contact phase. This process involves several proteins, namely High Molecular Weight Kininogen (HMWK) prekallikrein, kallikrein, factor XII and XIIa and factors XI and XIa and their complexes, and results in increasing amount of factor XI_a attached to the surface. It is self accelerated in that the formation of the enzymes have feedback effect on the activation of the zymogens.

The latter enzyme initiates a sequence of enzymatic reactions which, in a short time, results in factor IIa (thrombin) formation (Fig. 1). The rate of the latter reactions is slow and calcium dependent. In contrast, these reactions are speeded up when platelets factor 3 (PF3) from the platelet membranes and cofactors VIIIa and Va are present.

Extrinsic pathway. The alternative pathway for the activation of blood coagulation, called "the extrinsic pathway", is involved when blood contacts natural surfaces excluding the normal vessel endothelium. In presence of tissue thromboplastin (factor III) and calcium ions, factor VII is activated and the resulting enzymatic activity causes factors IX and X activation. Then, thrombin formation occurs as in intrinsic pathway (Fig. 1).

Clot formation. Thus, whatever the pathway, the coagulation cascade leads to the formation of thrombin, which plays numerous roles. First, the thrombin is able to induce platelet aggregation and release. Therefore, this enzyme has a feedback effect on its own formation. Second, thrombin is able to activate fibrinogen in an hydrolysis reaction which leads to fibrinopeptides A and B and the soluble fibrin polymer. The crosslinking of soluble fibrin which occurs is catalysed by activated factor XIII forming the insoluble fibrin clot, and again thrombin is involved in the activation of factor XIII.

It is noteworthy that aggregated platelets, other blood cells and plasma proteins are entrapped in the crosslinked fibrin network.

Coagulation control systems

Considering the whole coagulation process, it appears that the coagulation should normally occur when blood contacts any surface, including the normal vessel endothelium. As this is not the case, it is obvious that the clot formation is under control of some inhibiting systems.

Endothelial cells controls. The mechanism by which endothelial cells achieve this control is not completely understood. However, two possible pathways have been recently proposed.

First, when exposed to blood, endothelium cells are able to release prostacyclin (PGI_2), a prostaglandin which is a very potent inhibitor of platelet aggregation (Ref. 4).

Second, endothelial cell membranes contain a receptor called thrombomodulin (Ref. 5). In the presence of the latter, thrombin enzymatically catalyses the hydrolysis of protein C. The resulting activated protein C ($\bar{C}_A$) is able to bind to platelet membranes. It is suggested that, when fixed onto the platelet membrane, C_A is, in turn, able to catalyse the hydrolysis of factors Va (Ref. 6) and VIIIa (Ref. 7). As a result, the rate of the activation of factors X and II is decreased in the vicinity of endothelial cells.

Both these pathways, and possibly others to be discovered in the future, are probably involved in the control of clot formation. Their failure probably contributes to atherosclerosis and, therefore, seems to be one of the possible way by which general thrombosis occurs as a consequence of this disease.

Fibrinolytic system. The activation of the fibrinolytic system results from the hydrolysis of plasminogen, enzymatically catalysed by factor XIIa. A potent enzyme, plasmin, formed during this reaction, promotes a sequence of reactions which lead to fibrinolysis and subsequent destruction of the fibrin clot. It is of interest to note that some foreign proteins, as for instance urokinase and streptokinase, have an enzymatic ability to activate plasminogen and to promote plasmin formation (Ref. 8).

Antiproteases and heparin. Plasma contains enzyme inhibitors (Table 2) that are able to play an important role in the control of the coagulation process. These inhibitors are generally able to form complexes with several proteases, at various rates. For instance, α_2 macroglobulin readily reacts with kallikrein and slowly reacts with thrombin.

The most potent plasma inhibitor is antithrombin III, which is able to form inactive stable complexes with serine-proteases including factors IIa, IXa, Xa, XIa and kallikrein. The reactions are irreversible and slow. They imply the formation of a chemical bond between the serin active site of the protease and an arginyl residue of the inhibitor, the exact nature of which is not completely clear. These reactions are more or less subject to catalysis by heparin and heparin analogs which might be present in some subendothelial or endothelial tissue. Commercially available heparin is a polysaccharide obtained from pig or beef mucosal linings of the lung and liver. In contrast with proteins, this copolymer is heterogeneous in molecular weight, chemical composition and sequences. It is mainly composed of alternating residues of sulfated glucuronic and iduronic acid and glucosamin derivatives (mainly N-sulfate and N-acetyl glucosamine) linked in the 1-4 position as shown in Fig. 2. Due to the

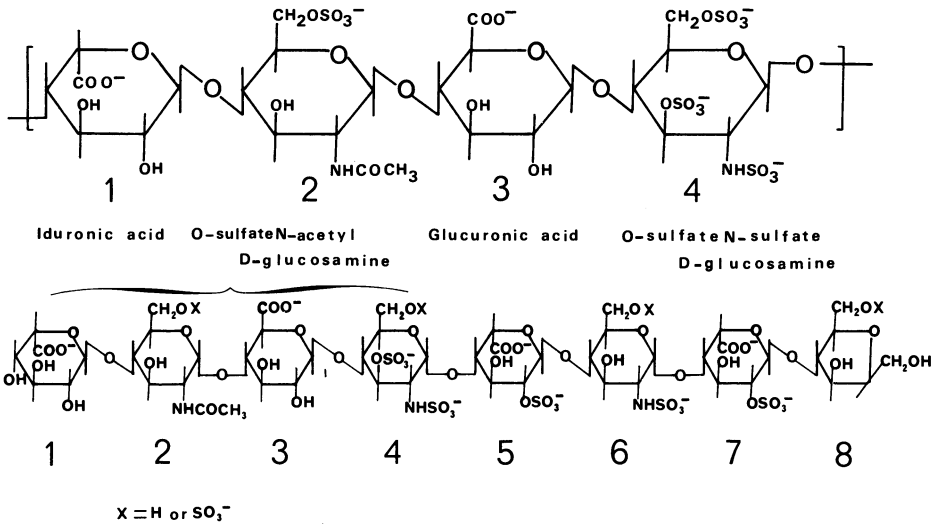

Fig. 2. Structures for the antithrombin III-binding tetrasaccharide or octasaccharide derived from heparin.

large variability in the molecule of heparin (Table 3) and to difficulties in chemical and catalytic activity measurements, the mechanism of the catalysed reaction and the exact chemical nature of the catalytic site are still, today, a matter of controversy (Ref. 9-12).

Indeed, several mechanisms have been proposed. The first one is based on the assumption that antithrombin III binds first to heparin and that the complex formed readily reacts with the

TABLE 3. Analyses of standard heparins

Iduronic acid % of total acid	Glucuronic acid % of total acid	Hexosamine %	Acetyl %	Sulfate %
47 - 75	30 - 64	20 - 26	0.4-2.5	25 - 34

thrombin. The second model proposes that the thrombin binds first to heparin and then a fast irreversible reaction occurs. A third model is based on the hypothesis that heparin constitutes a bridge between enzyme and antithrombin III.

Moreover, it appears possible that the same mechanism should not apply for each of the inhibition reactions of the different enzymes by antithrombin III, as shown for instance in case of factors IXa, XIa and IIa on one side and factors XIIa, Xa and kallikrein on the other side.

The nature of the sites involved in the binding of the proteins, i.e., inhibitor and proteases, to the catalyst is also a matter of controversy.

Conclusion

Clot formation appears to be the normal consequence of the contact between blood and any surface except that of the normal healthy vessel endothelium. It is initiated by the contact itself and depending on the nature of the surface, it may develop through different pathways, but the final result is thrombin formation which might well be the central event in coagulation.

Blood itself (and the natural endothelium of the vessel wall) is able to control the coagulation process. One of the potent control systems is the heparin catalysis of the complex formation between antithrombin III and the serin-proteases involved in the coagulation process.

It has to be emphasized that coagulation of blood is a complicated process. Its kinetics cannot completely be described by the models schematically mentioned above, the kinetics of the clot formation being a complex combination of each of the involved reactions. Moreover, these kinetics are strongly dependent on the flow rate of the circulating blood. This adds to the multiparameter system which controls the blood coagulation, one more parameter which is able to modify to some extent the whole feature of the clot formation process.

ANTITHROMBOGENIC POLYMERS

Considering the whole process of thrombus formation, it appears that there are two concepts, and two only, on which the tailoring of blood compatible biomaterials can be based. First, the designed biomaterial should not activate any of the coagulation pathways. Unfortunately, no materials of this kind are known. Moreover, it seems probable that even the endothelial cells membranes of the blood vessel wall are able to activate more or less the coagulation cascade. Second, the biomaterial surface, as any known material surface, will be blood coagulation activating, but in order to prevent thrombus formation at its blood interface, it should be able to inhibit the coagulation process.

Moreover, such biomaterials should not be inhibitors by themselves. Indeed, if it were the case, the biomaterial will be implied as a reagent in an irreversible inhibition reaction the result of which will be an irreversible transformation of the biomaterial surface which, in turn, will lose its inhibiting properties. Therefore, a suitable anticoagulant biomaterial should be a catalyst of inhibition reactions of the coagulation process. The surface of such a material should have a catalytic activity with regard to some of the control mechanism of the coagulation of blood.

Indeed, several possibilities exist to design such biomaterials and each of them has been the basis of an active research development during the past two decades. Historically, the first attempt to achieve antithrombogenic materials was the ionic binding of heparin onto a polymeric surface by Gott (Ref. 13) which resulted in a slow release of the anticoagulant drug into the blood stream. Since that time, the same strategy has been applied in numerous studies to different potent anticoagulant drugs.

Anticoagulant drugs releasing materials

It is the case, for instance, of biomaterials which release prostacyclin (PGI_2) entrapped in a macromolecular network (Ref. 14-15). When, placed in contact with blood, these biomaterials prevent platelet aggregation and release, and thus, control the coagulation process. Such materials are potent as long as they are able to release PGI_2. Unfortunately, PGI_2 is an expensive product and moreover unstable (its lifetime, after hydrolyses in biological conditions being less than one minute). Therefore, biomaterials of these kind have found no practical uses at the moment. Other platelet antiaggregating surfaces based on incorporation of antiaggregating drugs such as dipyridamole, for instance in polymers such as cellulose, cellulose triacetate, nylon and polyethylene-terephtalate, have been prepared (Ref. 16). The resulting materials have been claimed to be potent as antithrombogenic materials even when implanted in dogs.

The strong anionic character of heparin can be used to bind it ionically to cationic surfaces. Since the early work of Gott et al. (Ref. 13), numerous studies have been devoted to this aim. In all cases, polycationic polymers were prepared from polystyrene and its derivatives, cellulose, silicon rubber, epoxy resins, polyurethanes and from copolymers such as styrene or acrylonitrile and acrylate derivatives ; these polycationic polymers were treated with heparin in various ways. Heparin was also incorporated in crosslinked collagen etc.... An alternative route was to incorporate simultaneously heparin and prostaglandins in order to combine the respective thrombin and platelets aggregation inhibiting effects of the released drugs.

Whatever the techniques used, the resulting biomaterials have been proved potent as long as they release the ionically bound heparin into the blood stream. During the release, the concentration of heparin near the surface is sufficiently high to prevent thrombus formation for periods of at least several days. Unfortunately, after that period of time, the release decreases. Therefore, such biomaterials are only suitable for use in devices of short term application as for instance extracorporeal circulation and catheters, but do not allow their use as permanent implants[57-59]. They were, indeed, developed for such temporary use by Japanese, American, Swedish and French manufacturers, for the manufacture of catheters and peritoneovenous shunts.

An alternative approach to the ionic coating of heparin onto materials in order to get antithrombogenic surfaces involves the synthesis of polymeric gels in which heparin is entrapped. This can be achieved either by ionic binding of heparin to polymers in a first step followed by chemical or radiation induced crosslinking of the resulting network in a second step, or by trapping of heparin in a preformed hydrogel. The latter procedure was used in cases of polymeric gels prepared by radiation processing treatments involving vinyl alcohol or vinyl acetate and N-vinyl-2-pyrrolidone monomers. Cobalt-60 gamma rays were also used to graft chloromethylstyrene on polysilicone samples followed by quaternization with pyridine. As in the preceeding case, uptake of heparin from solutions occurs by exposure of the polymeric gel.

All these gel procedures have been proved to be potent, i.e., they allow the preparation of antithrombogenic biomaterials. Indeed, when compared to the former one the heparin release level appeared to be decreased but sufficient to prevent blood clotting in ex-vivo assays. Nevertheless, the potency of the devices is still insufficient to allow their use as permanent implants. Reviews of all these works were published recently and make the literature references easily available (Ref.17-20).

Covalent binding of anticoagulant drugs to polymers

The use of the fibrinolysis to control thrombogenicity of biomaterials has been studied. Indeed, Sugitachi et al., for instance, have attached urokinase or streptokinase onto polyvinyl chloride and silicone rubber tubes (Ref. 21). These surface treatments improve significantly the blood compatibility properties. Unfortunately, cleavage of the fixed enzyme may occur on exposure of the surface to blood, preventing long term uses of the biomaterials. However, short term uses are possible as shown by the fact that a Japanese company recently developed catheters based on urokinase coupled onto a polymer matrix ; but these materials are expensive and this may prevent their use for economical reasons.

Therefore, the most promising way to design antithrombogenic biomaterials has appeared to be the covalent binding of heparin onto polymeric materials. In principle, such biomaterials will not release their heparin content by exposure to the blood stream, if the covalently bound heparin remains active they will remain potent a longer time than it is the case for ionically bound heparin.

Covalent binding of heparin onto polymers can be achieved by different ways. First, heparin has been coupled to preformed hydrogels by the use of various classical activation techniques. Hydrogels derived from polyvinylalcohol on which heparin was fixed by acetal bridges were prepared. In other cases, heparin was bound to agarose and sepharose using the cyanogen bromide, the carbodiimide activation and so on. The same coupling reagents were used for the covalent binding of heparin either onto radiation grafted hydrogels derived from hydroxyethyl methacrylate and methacrylic acid or onto the same hydrogels radiation grafted on the surface of a silicon rubber.

In a second general procedure pathway, a polymer is chemically or radiochemically activated and, then allowed to react chemically with heparin. Such a typical procedure was developed to fix isocyanate groups onto polystyrene and then react the resulting polymer with heparin. The mucopolysaccharide was also bound to silicon in a two steps procedure. Analog technics were developed to bind heparin onto modified polyvinylalcohol hydrogels, elastomers, polyhydroxyethylmethacrylate-glycidyl-methacrylate copolymers or cellulosic membranes. Alterna-

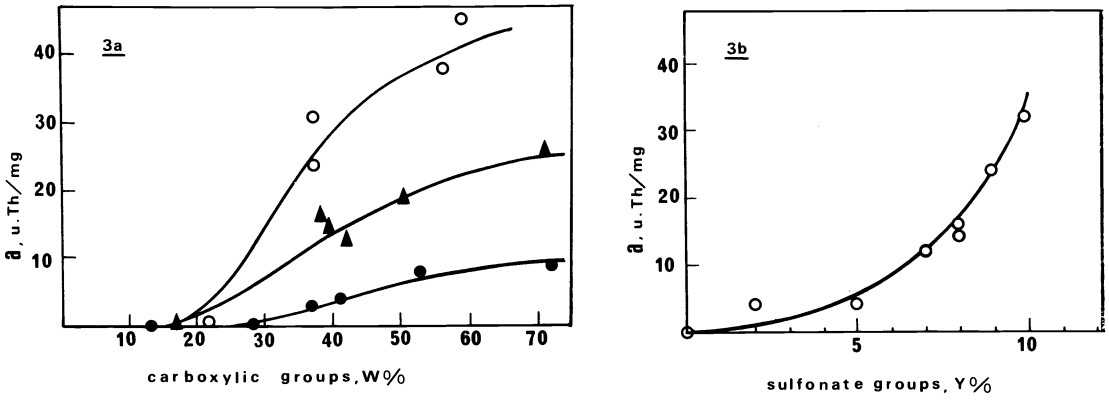

Fig. 3. Antithrombic activity of the modified Sephadex.

TABLE 4. Activity coefficient of some substituents linked to polystyrene resins

Substituent	Activity coefficient (meq^{-1}) for 1 NIH thrombin unit
$-SO_3^-$	50-70
$-SO_2NH$ butyl	0
$-SO_2^-$ 11-amino undecanoic acid	40-80
$-SO_2^-$ alanine	60-80
$-SO_2^-$ glycine	100-120
$-SO_2^-$ hydroxyproline	120-150
$-SO_2^-$ proline	120-150
$-SO_2^-$ methionine	120-150
$-SO_2^-$ threonine	120-150
$-SO_2^-$ N-benzyloxycarbonyl lysine	120-150
$-SO_2^-$ β-alanine	140-200
$-SO_2^-$ ε-amino caproic acid	300-350
$-SO_2^-$ glutamic acid	350-400
$-SO_2^-$ δ-amino valeric acid	400-450
$-SO_2^-$ γ-amino butyric acid	500-600
$-SO_2^-$ aspartic acid	500-600

tively, heparin may be bound to suitably prepared substrates to produce thromboresistant materials. This approach has been used by Sefton et al. (Ref. 22-23) to develop a high strength styrene-butadiene-styrene block copolymer. The same approach was used by Larsson et al. (Ref. 24) who adsorbed, onto sulphated polyethylene, colloidal particles composed of heparin and cetylamine hydrochloride and then reacted the mixture with glutaraldehyde. The authors claimed that in the resulting biomaterial heparin was covalently bound and that despite the fact that no release occurred the biomaterial was thromboresistant.

In a third general procedure, a chemical or radiochemical treatment of heparin induces the formation of a macroradical which, in turn, induces the polymerization of a monomer resulting in a copolymer in which an heparin moiety is covalently bound to a synthetic polymeric sequence. This procedure was first achieved by Labarre et al. by use of cerium (IV) peroxidation of heparin followed by reaction with acrylic and methacrylic monomers (Ref. 25-26). Baquey et al. (Ref. 27-28) described a similar method, using the radicals resulting from γ ray irradiation of heparin instead of cerium (IV) oxidation and used this method for the production of acrylic acid or dacron copolymer.

The properties and antithrombogenic activity of such covalently bonded heparin surfaces have been proved (Ref. 29-30) to be similar to those of soluble heparin. An alternate pathway consisting in modification of heparin so that it should be able to polymerize or to copolymerize has been proposed. These biomaterials, as it was the case for the preceding one, were shown to be anticoagulant (Ref. 31).

The above methods were reviewed recently (Ref. 19-20). They allow the preparation of biomaterials where heparin is covalently bound onto the surface. However, depending on the method used to fix heparin to the polymer matrix, they may or not have an anticoagulant heparin-like activity. It is also noteworthy that surfaces on which heparin is covalently bound activate platelets when exposed to blood or platelets suspension. However, preincubation of these surfaces with either antithrombin III aqueous solutions or plasma, passivated the surface with regard to platelets activation.

As a conclusion it was suggested that biomaterials on the surfaces where heparin is covalently bound might be thromboresistant because of their heparin-like anticoagulant activity and/or because of their ability to be passivated by binding antithrombin III toward platelets activation. Nevertheless, the use of such biomaterials may be limited for two reasons. The first one may be economic ; the second one may result from the fact that an exposure to blood may degrade readily heparin under the action of heparinases. If it is the case, the use of such biomaterials as long term implants might be hopeless. This is probably the reason why people started early to synthesize anticoagulant and heparin-like materials.

Heparin-like materials

As early as 1951, Lovelock and Porterfield (Ref. 32) described the anticoagulant properties of polystyrene sulfonates, resulting from a reaction of polystyrene tubings with sulfuric acid. Since that time, the anticoagulant properties of sulfonated soluble polymers, specially polystyrene and polyethylene sulfonates, have been described by Gregor. (Ref. 33). Furthermore Machovich (Ref. 34) has shown that polymethacrylic acid has an anticoagulant activity in aqueous solution.

Later on, anticoagulant materials were obtained by reaction of N-chlorosulfonyl isocyanate with an unsaturated polymer followed by a suitable basic hydrolysis. For instance, a soluble polyelectrolyte derived from 1,4-cis polyisoprene was shown to possess an anticoagulant activity which was 10 % of heparin (Ref. 35). Finally, Jozefowicz et al. (Ref. 36-45) have obtained resins with significant antithrombogenic activity by binding sulfonate, carboxylic, aminoacid sulfamide or amide groups onto crosslinked polystyrene, polysaccharides or polystyrene-polyethylene graft copolymers. They have proved this activity to be antithrombic and anti Xa, and to involve a plasmatic component by use of tests performed on platelet-poor plasma suspensions of the polymers.

This activity has also been proved to be located on the surface of the polymers. Moreover, by direct kinetic studies performed on suspensions of the polymers in aqueous solution of antithrombin III and proteases, they were able to show that the anticoagulant activity is heparin-like, i.e., the polymers act as heterogeneous catalyst with regard to the AT III - proteases inhibiting reaction. Thermodynamic studies of the adsorption of proteins on the surface of the resins allowed the authors to propose a mechanism for these catalytic reactions (Ref. 42).

The anticoagulant activities of the materials are strongly dependent upon the content and the nature of the substituting groups which were shown to have either a cooperative or an additive effect. Given these observations, it was possible to correlate anticoagulant activities and compositions of the heparin-like resins as shown in Table 4 and Fig. 3.

Based on these results, it was suggested that the isolated functional groups borne by polysaccharide chain of heparin, rather than the secondary or tertiary structure of this mucopolysaccharide, are responsible for its interaction with AT_{III} or protease. Moreover, the comparison and discussion of the estimates of activity parameters substantiate the hypothesis that carboxylic functions are essential for heparin-like activity.

Ex vivo animal experiments were performed on surface-treated small-diameter tubing made of polystyrene-polyethylene copolymers. They showed that no significant platelet adhesion and aggregation could be observed on the wall of the tubing, when pretreated with either plasma or AT_{III}.

The same principle was applied by the authors (Ref. 45-47) to modify soluble dextrans. The resulting products have shown to be heparin-like with regard to inhibition of the coagulation and of the alternative pathway of complement (Fig. 4 and Table 5). Thus, these polymers appear

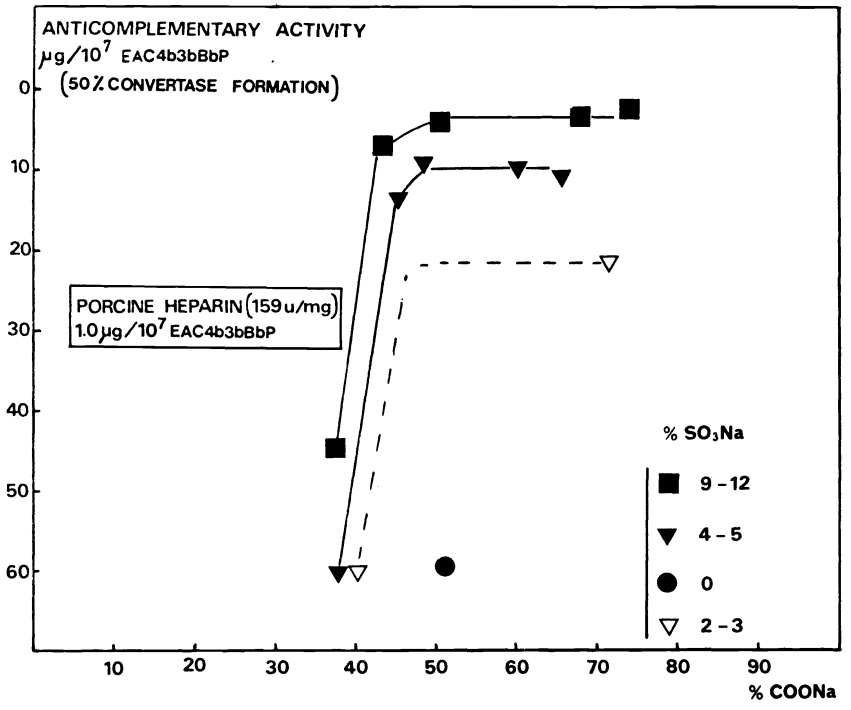

Fig. 4. Inhibiting activity of the C3 convertase (complement alternative pathway).

to be promising candidates as soluble constituent of plasma expanders.

CONCLUSION

The available cardiovascular prosthetic devices, are made of thrombogenic biomaterials. Therefore their use implies the delivery of a permanent anticoagulant therapy to the host patient.

Improvements of these devices can result from either better mechanical compliance of the biomaterials or surface treatments designed to improve their antithrombogenicity.

Surface treatments based on the binding of natural coagulation inhibitors such as platelet aggregation inhibitors, plasminogen activators, or heparin were proposed. Among them, the heparin releasing biomaterials appear to be the most promising for the making of cardiovascular devices for short-term uses, as for instance, catheters.

In turn, surface treatments achieved to endow heparin-like properties to biomaterials with suitable mechanical compleance will probably allow, in the next future, to make cardiovascular devices of short-and long-term uses.

Moreover, this review ot the published data suggests that in order to tailor blood compatible biomaterials, it is necessary to take into account the reactions that occur at the blood-

TABLE 5. Effect of substituted dextrans on coagulation tests

TEST		Michaelis buffer	Inactive dextran derivative 325 µg/ml a = 0 uTh/mg	Active dextran derivative 325 µg/ml a = 25 uTh/mg	Heparin 0.6 µg/ml 150 uI/mg a = 3500 uTh/mg
Thrombin time (in plasma)	(s)	23	23	> 300	> 180
Thrombin time in presence of protamine chloride	(s)	21.4	-	24	21
Reptilase time	(s)	20.4	20.2	21.4	20.4
Prothrombine time	(s)	13.8	14	13.4	14
Activated partial thromboplastin time (APTT)	(s)	80	76	131	110
Thrombin time (fibrinogen 2g/l)	(s)	20	24.2	62.8	26
Heparin-like antithrombic activity (UI/mg)		0	limit of the sensibility	0.83	150
Thrombin Generation Test					
Plasma inhibition (%)		0	30	82	82
Plasma time of the peak value		5 min 15s	7 min 45s	11 min 15s	7 min 15s
Euglobulin* inhibition (%)		0	limit of the sensibility		
Euglobulin* time of the peak value		12 min	12 min	22 min	25 min

*Fraction of plasma with factors and without inhibitors.

material interface. Then it will be possible to design materials which are able to control the coagulation process initiated at the interface.

REFERENCES

1. J.L. Brash and D.J. Lyman, J. Biomed. Mater. Res. 3, 175 (1969).
2. D.J. Lyman, J.L. Brash, S.W. Chaikin, K.G. Klein and M. Carini, Trans. Am. Soc. Artif. Intern. Organs 14, 250 (1968).
3. G.A. Boffa, N. Lucien, A. Faure, M.C. Boffa, J. Jozefonvicz, A. Szubarga, M.J. Larrieu and P. Mandon, J. Biomed. Mater. Res. 11, 317 (1977).
4. B.B. Weksler, A.J. Marcus and E.A. Jaffe, Proc. Natl. Acad. Sci. U.S.A. 4, 3922 (1977).
5. C.T. Esmon, N.L. Esmon, J. Sangstab and W.G. Owen, Pathobiology of the Endothelial Cell, p. 121, N.L. Wossel and H.J. Vogel Eds. (1982).
6. W. Kisiel, W.N. Canfield, L.H. Ericson and E.W. Davie, Biochemistry, 16, 5824 (1977).
7. G. Vehar and E. Davie, Biochemistry 19, 401 (1980).
8. E.J.P. Brommer, P. Brakman, F. Haverkate, C. Kluft, D. Traas and G. Wijngaards, Recent Advances in Blood Coagulation, vol. 3, p. 125, L. Poller Ed. Churchill Livingston (1981).
9. J. Choay, J.C. Lormeau, M. Petitou, P. Sinaÿ and J. Fareed, Ann. N.Y. Acad. Sci. 370, 644 (1981).
10. G.M. Oosta, W.T. Gardner, D.L. Beeler and R.D. Rosenberg, Proc. Natl. Acad. Sci. U.S.A. 78, 829 (1981).
11. L. Thunberg, G. Bäckström and U. Lindahl, Carbohyd. Res. 100, 393 (1982).
12. R. Machovich, G. Blasko and L.A. Palos, Biochim. Biophys. Acta 379, 193 (1975).
13. V.L. Gott, J.D. Whiffen and R.C. Dutton, Sciences 142, 1297 (1963).
14. J.C. Mac Rea and S.W. Kim, Trans. Am. Soc. Artif. Intern. Organs 24, 746 (1978).
15. G.A. Grode, J. Pitman, J.P. Crowley, R.I. Leininger and R.D. Falb, Trans. Am. Soc. Artif. Intern. Organs 20, 38 (1974).
16. W. Marconi, Makromol. Chem. Suppl. 5, 15 (1981).
17. R.I. Leininger, Chem. Technol. 5, 1/2 (1975).
18. A.S. Hoffman, Int. Nat. Atom. Energ. Agency, Vienna, p 279 (1982).
19. C. Fougnot, D. Labarre, J. Jozefonvicz and M. Jozefowicz, Macromolecular biomaterials and Applications, Chapter 9, C.R.C. press Boca Raton, U.S.A. (in press).
20. J. Jozefonvicz, M. Mauzac, N. Aubert and M. Jozefowicz, Biocompatibility of Natural Tissues and Their Synthetic Analogues, C.R.C. press Boca Raton, U.S.A. (in press).
21. A. Sugitachi, M. Tanaka, T. Kawahara, N. Kitamura and K. Takagi, Third Annual Meeting of the Intern. Soc. for Artif. Organs, Paris, (July 1981).
22. M.F.A. Goosen and M.V. Sefton, J. Biomed. Mater. Res. 13, 347 (1979).
23. M.V. Sefton and M.F.A. Goosen, Chemistry and Biology of Heparin, p. 463, Elsevier, New York (1981).
24. R. Larsson, P. Olsson and U. Lindahl, Thromb. Res. 19, 43 (1980).
25. D. Labarre, M.C. Boffa and M. Jozefowicz, J. Polym. Sci., Symp. 47, 131 (1974).
26. M. Jozefowicz, J. Jozefonvicz, C. Fougnot and D. Labarre, Chemistry and Biology of Heparin, p. 475, Elsevier, New York (1981).
27. Ch. Baquey, Ch. Darnez and P. Blanquet, Rad. Res. 70, 82 (1977).
28. Ch. Baquey, A. Beziade, D. Ducassou and P. Blanquet, Innov. Tech. Biol. Med. 2, 379 (1981).
29. D. Labarre, M. Jozefowicz and M.C. Boffa, J. Biomed. Mater. Res. 11, 283 (1977).
30. M.C. Boffa, D. Labarre M. Jozefowicz and G.A. Boffa, Thrombos. Haemostas. (Stutgart) 41, 346 (1979).
31. N.A. Plate, L.I. Valnev and F.K. Gumirova, Dokl. Akad. Nauk. S.S.S.R. 244, 1505 (1979).
32. J.E. Lovelock and J.S. Porterfield, Nature (London) 167, 39 (1951).
33. H.P. Gregor, Polym. Sci. Technol. U.S.A. 7, 51 (1975).
34. R. Machovich and I. Horvath, Thromb. Res. 11, 765 (1977).
35. T. Beugeling, L. van der Does, L. Bantjes and A. Bantjes, Biocompatibility of Implant Materials, p. 187, Sector, London (1976).
36. C. Fougnot, J. Jozefonvicz, M. Samama and L. Bara, Ann. Biomed. Eng. 7, 429 (1979).
37. C. Fougnot, M. Jozefowicz, M. Samama and L. Bara, Ann. Biomed. Eng. 7, 441 (1979).
38. M. Mauzac, C. Fougnot and J. Jozefonvicz, Artif. Organs, 5 (Suppl.) 504 (1981).
39. M. Mauzac, N. Aubert and J. Jozefonvicz, Biomaterials 3, 221 (1982).
40. C. Fougnot, M. Jozefowicz, L. Bara and M. Samama, Thromb. Res. 28, 37 (1982).
41. C. Fougnot, M.P. Dupillier and M. Jozefowicz, Biomaterials 4, 101 (1983).
42. C. Fougnot, M. Jozefowicz and B. Rosenberg, Biomaterials 4, 294 (1983).
43. C. Fougnot, M. Jozefowicz and B. Rosenberg, Biomaterials (in press).
44. C. Fougnot, J. Lindon, L. Kirshner, E.W. Salzman and M. Jozefowicz, Biomaterials (in press).
45. M. Mauzac and J. Jozefonvicz, Biomaterials (in press).
46. F. Maillet, M. Mauzac, J. Jozefonvicz and M.D. Kazatchkine, Second World Congress on Biomaterials, April 27 th, Washington, D.C. (1984).
47. M. Mauzac, J. Jozefonvicz and M. Jozefowicz, French Patent n° 83 19110 (1983).