Structure Design of Polysaccharides: Novel Concepts, Selective Syntheses, High Value Applications

Dieter Klemm*, Lars Einfeldt

Institute of Organic and Macromolecular Chemistry, University of Jena, Humboldtstraße 10, D-07743 Jena, Germany

SUMMARY: Using protecting silyl groups, catalyzed transesterification, and stereochemically controlled S_N2 mechanisms examples of well-defined 2-, 3-, 6-, and 2,6-ethers, esters, resp. amino derivatives of cellulose, starch, and related polysaccharides have been synthesized and characterized by two-dimensional NMR spectroscopy. Supramolecularly structured polymer matrices useful in biosensors could be developed by immobilization of enzymes like glucose oxidase and aromatic redox-chromogenic structures at 6-deoxy-6-(4-aminophenyl)-aminocellulose. Cellulose tubes "grown" biotechnologically from D-glucose in the culture medium of *Acetobacter xylinum* represent novel types of artificial blood vessels because of their unique highly-swollen nanofiber network-structure.

Introduction

Polysaccharides like cellulose and starch are important renewable biopolymers characterized by high functionality, strong hydrophilicity, remarkable structure-forming potential, chirality, and important biocompatibility.

To use these unique properties efficiently selective functionalization of the hydroxy groups and of subsequently introduced functionalities, high-performance analytical methods, and controlled formation of supramolecular structures are essential.

From this point of view the structure design of polysaccharides means a „tailor-made" modification in the whole scale from Angströms up to centimeters. As demonstrated schematically in Fig. 1 it starts with the regioselective functionalization within the repeating anhydroglucose units and along the polymer chains (molecular structure), leads to well-defined supramolecular films with recognition activity, and includes the formation and application of biotechnologically structured polymeric biomaterials.

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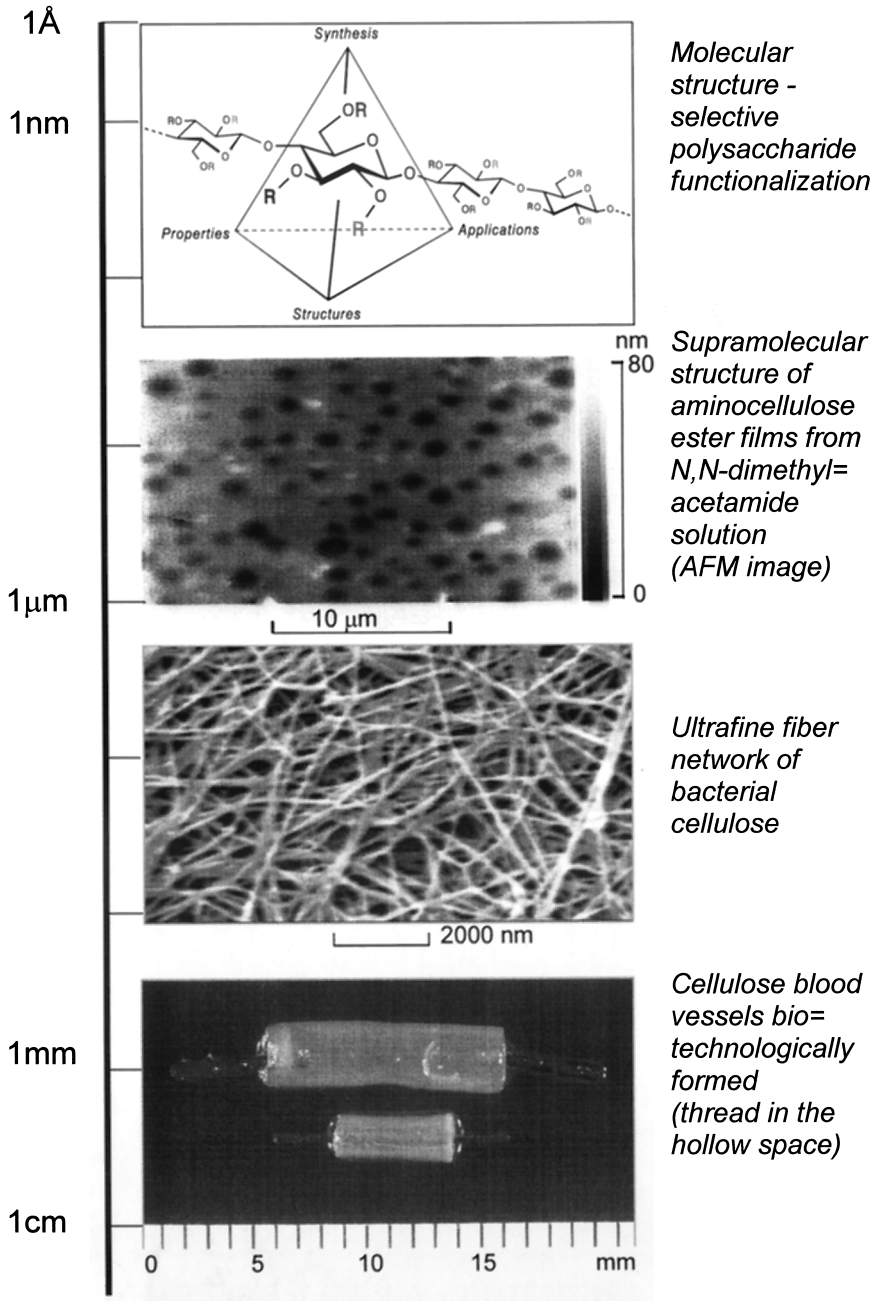


Fig. 1: Structure design of polysaccharides.

Concepts in regiochemistry of polysaccharides

To realize the site-selective functionalization of the OH-groups of polysaccharides three effective pathways have been developed up to now[1-4]:

- using bulky protecting groups, especially methoxy-substituted triphenylmethyl[5, 6] and different types of silyl groups[7, 8] (e.g., bulky 6- and 2,6-silyl ethers of starch and cellulose),
- selective activation of OH groups by special structures in solution combined with tailored reagents resp. catalysts (e.g., transesterification with carboxylic acid vinylesters to 2-O-esters of polysaccharides)[9-11],
- introducing of functional groups reacting site-selectively according to stereochemically controlled mechanisms (S_N2 reactions of cellulose sulfonates with diamines to 6-aminoderivatives)[12, 13].

Silyl starches

As demonstrated in Fig. 2 the homogeneous silylation of starch with bulky thexyldimethylsilylchlorosilane (TDSCl) in dimethylsulfoxide (DMSO)/pyridine leads to a high regioselective 6-O-silylation using equal amounts of the silylating agent.

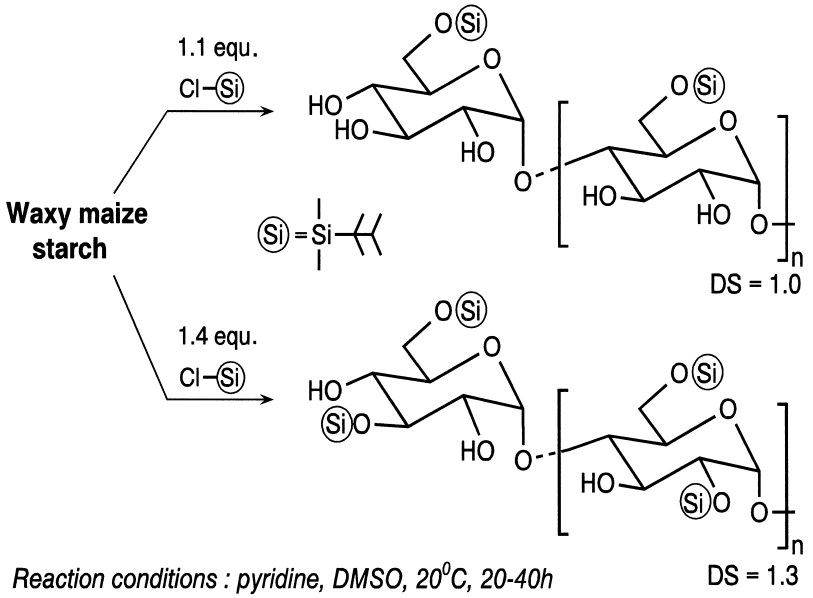


Fig. 2: Synthesis pathways to regioselectively functionalized silyl starches.

With higher equivalents of TDSCl a additional etherification of positions 2 have been obtained as known for the homogeneous silylation of cellulose[7, 8] and of cyclodextrins, too[14]. The further reaction of position 2 of the anhydroglucose unit (AGU) was proved exactly by two-dimensional ^{1}H-NMR spectroscopy as well as by the reductive cleavage method[14]. The [^{1}H^{1}H]-cosydqf-NMR spectrum of 2,6-di-O-thexyldimethylsilyl starch (DS 1.3) after complete peracetylation of free OH-groups is represented in Fig. 3.

Due to the branching structure, starch with high amylopectin content possess a high number of non-reducing terminal end groups (TEG). Suprisingly, in comparison to the AGU within the chains the silylation of the TEG showed a regioselective etherification of OH-groups in position 3 after 6-O-silylation[15].

2,6-di-O-silyl polysaccharides are useful tools for the synthesis of regioselectively substituted alkyl polysaccharides by subsequent etherification and desilylation with tetrabutylammoniumfluoride. Using 2,6-di-O-thexyldimethylsilyl cellulose uniformely substituted 3-O-methyl cellulose, DS = 1.0, has been obtained[16]. In contrast to these results, the subsequent methylation of the corresponding 2,6-di-O-thexyldimethylsilyl starch leads to the 2-O-methyl ether via migration of the TDS-groups from position 2 to 3[14]. Different from the AGU within the chains of starch the terminal end groups are methylated additionally in position 3 resp. 4 [17].

The described selective functionalization using protecting groups represents a suitable way to prepare novel types of functionalized polysaccharides with a well-defined molecular structure in a laboratory scale and to make success in basic questions of polysaccharide research. Typical examples of such a kind are detailed spectroscopic characterization, structure in solution, formation of supramolecular structures, properties at interfaces including interactions with enzymes, and structure-property relationships.

From the view-point of industrial important polysaccharide derivatives regioselectively one-pot synthesis pathways are of importance.

With respect to this situation and to avoid protecting groups we have been investigated the selective esterification of polysaccharides controlled by their structure in solution and by selected reagents resp. catalysts.

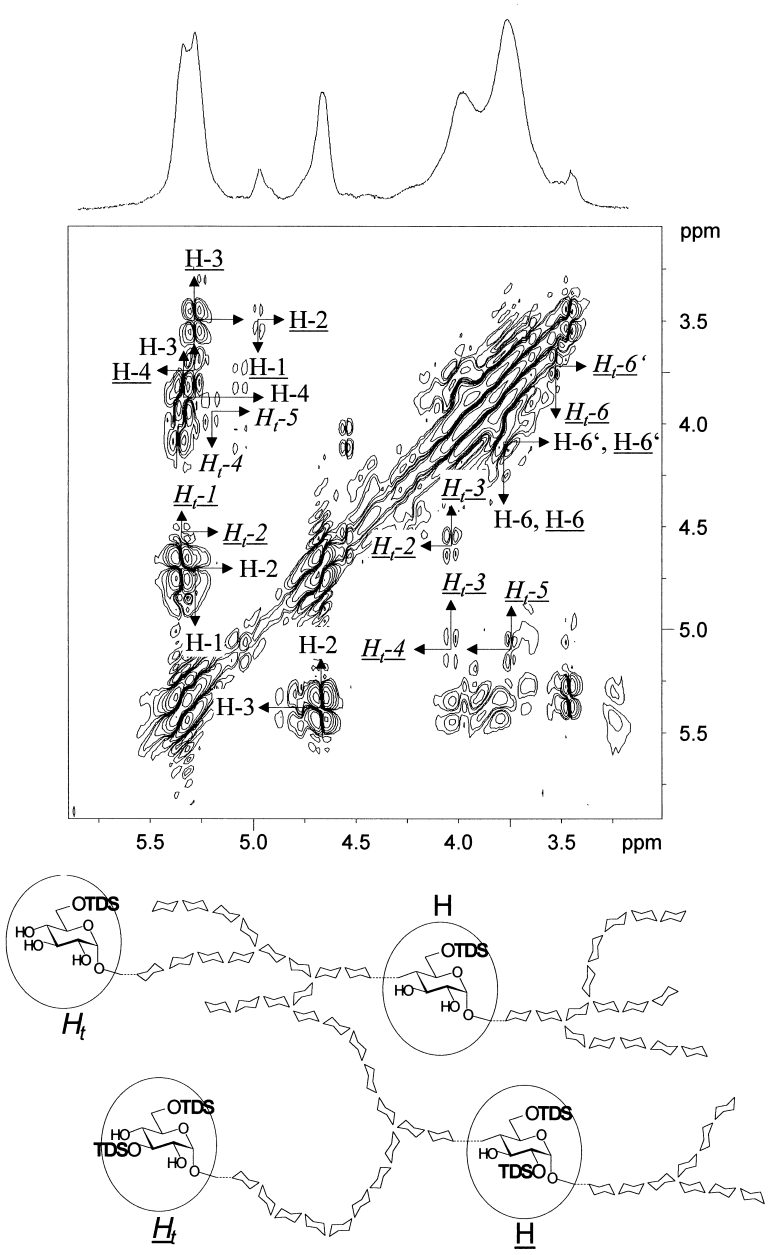
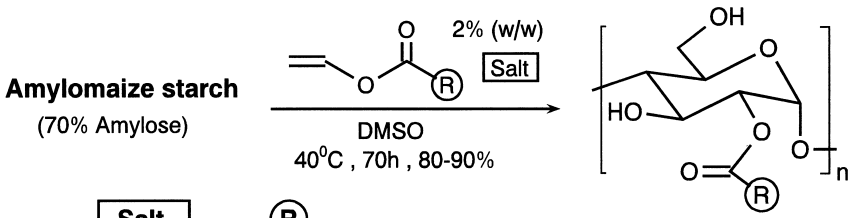


Fig. 3: Structure of peracetylated 2,6-di-O-thexyldimethylsilyl starch, DS_{Si} = 1.3. The cross-peaks of the [^{1}H,^{1}H]-cosydqf-NMR spectrum show the designed silylation of position 6 and than 2 in the AGU in contrast to position 6 and than 3 in the TEG. The arrows in the crosspeaks assign the chemical shifts of the methine and methylene protons of each AGU (H and <u>H</u>) as well as of TEG (H_t and <u>H_t</u>).

2-O-Esters of polysaccharides

The regioselective synthesis of 2-O-acyl polysaccharides can be realized by a transesterification method patented for different types of vinylester reagents, catalytic active salts, and polysaccharides[9, 10]. This procedure could be applied on different α-D-glucans like starch, nigeran, pullulan, and cyclodextrin, which are soluble in DMSO. Contrary, β-D-glucans like curdlan or cellulose showed no regioselectivity or low conversions if the polymer was insoluble in DMSO. An exception represents lichenan.

As shown in Fig. 4 aliphatic, aromatic, unsaturated, and additionally functionalized acyl groups can be introduced in position 2 of the polysaccharide molecules with high regioselectivity. Especially the synthesis of acrylates, methacrylates, and cinnemates should be emphasized, because known acylation procedures to unsaturated esters have often led to grafted polymers.



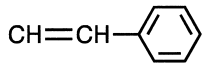
Salt	R	
Na_2HPO_4	CH_3	Acetate
		Benzoate
	$CH=CH_2$	Acrylate
	$C(CH_3)=CH_2$	Methacrylate
Na_2CO_3	$C(CH_3)_3$	Pivaloate
	CH_2Cl	Chloroacetate
	$CH=CH-CH_3$	Crotonate
		Cinnamate

Fig. 4: Regioselective synthesis of 2-O-acyl starches avoiding protecting groups.

A direct proof of the regioselective acylation could be made using two-dimensional NMR spectroscopy (HMBC- and HMQC-techniques) after peracetylation or perpropionylation of the 2-O-acyl polysaccharides. In dependence of the amount of acylating agent a controlled DS in position 2 could be achieved. Such 2-O-acetyl starches with DS = 0.3, DS = 0.8 and DS = 1.0 were used recently for the explaination of specific relaxation processes in the dielectric relaxation spectroscopy[18].

The pool of catalytic active salts includes further NaCl, NH_4Cl, $(NH_4)_2$ CO_3, Na_2HPO_4, $LiCO_3$, and K_3PO_4. Without any salt no regioselectivity could be obtained. The detailed mechanism of the catalyzed transesterification is now under investigation.

The regioselectivity is mainly controlled by the structure of polysaccharides in DMSO solution[19], the vinylester reagent forming acetaldehyde during reaction, and the type of catalytic active salt.

6-deoxy-6-aminocelluloses

An important way to introduce functional groups regioselectively in position 6 of cellulose consists in the nucleophilic substitution reaction of organo-soluble and film-forming p-toluenesulfonic acid esters of cellulose (tosylcelluloses) as intermediates[20, 21]. A typical example of this pathway is the synthesis of 6-deoxy-6-aminocelluloses using different types of diamines as nucleophilic reagents[12].

As shown in Fig. 5 tosylcellulose (DS 2.3) reacts with 1,4-phenylenediamine (PDA) under the described conditions to the corresponding 6-deoxy-6-(4-aminophenyl)-amino-2(3)-O-tosylcellulose ("PDA cellulose"). The best results have been obtained in presence of triethylamine with respect to buffering the p-toluenesulfonic acid formed and avoiding of staining the reaction mixture. A typical PDA cellulose with DS_{PDA} 0.75 and $DS_{tosylate}$ 1.30 which was used for subsequent reactions (see next part) was synthesized in presence of this amine at 100 °C within 3 h, employing 9 molar equivalents of PDA per AGU of the tosylcellulose. Under these optimized conditions the monoalkylation of PDA was possible, forming soluble polymers without detectable crosslinking. The substitution reaction of tosylcellulose and PDA follows a S_N2 mechanism. Because of the sterical proportions of tosylcellulose and PDA, the formation of the transition complex is obviously possible only at the C-6 position. Thus, the replacement of the tosylate groups by PDA proceeds exclusively at this site[12].

Based on these results a broad spectrum of PDA cellulose derivatives was synthesized after nearly complete acylation of the free OH groups at tosylcelluloses (DS 0.5-1.9) followed

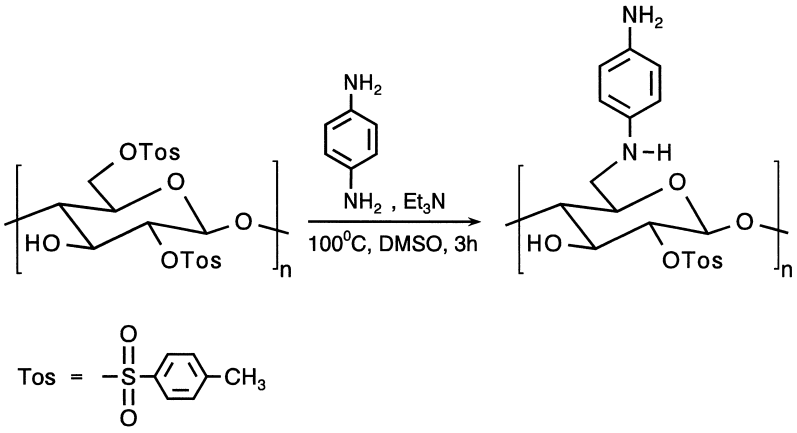


Fig. 5: Reaktion of tosylcellulose (DS 2.3) with 1,4-phenylenediamine.

by the substitution reaction with PDA. Typical ester groups are benzoates and carbanilates. It could be shown that the substitution of the tosylate groups by PDA occured up to more than 90 % after 6 h avoiding a remarkable cleavage of the secondary ester groups[13].

Supramolecular cellulose architectures with recognition activity

With the aim to develop polymer matrices useful in enzyme sensors the design of polymer supports with the following typical properties was important: solubility in organic solvents, formation of transparent films, anchor groups for immobilization of enzymes and redox-chromogenic structures, enzyme compatibility, and stabilization of redox-chromophors.

As presented schematically in Fig. 6 the aminocelluloses described in the previous part are convenient starting materials to prepare this type of polymers.

All PDA cellulose esters are soluble in DMSO and N,N-dimethylacetamide (DMA) after isolation. During storage, the solubility decreases caused by formation of hydrogen bonds between the amino groups of the PDA unit and residual OH-groups of the cellulose backbone and by crosslinking reactions of the NH_2-functions with tosylate residues resp. by autoxidation and subsequent oxidative coupling of the PDA groups[13]. The longest stable derivatives were the PDA cellulose carbanilates (S in Fig. 6: C_6H_5-NH-C(O)O).

The PDA cellulose esters form transparent films from the solutions. The supramolecular structure of the films (typical example see Fig. 1) is controlled by the ester groups, the solvent, and the film-forming temperature.

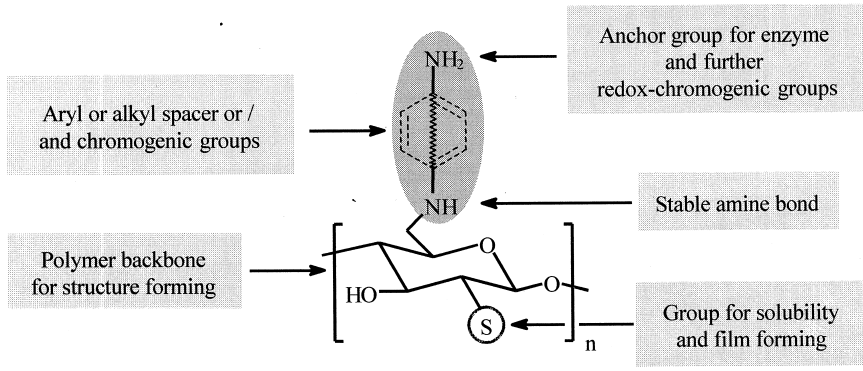


Fig. 6: 6-deoxy-6-Aminocelluloses tailor-made for analyte-sensitive reagent phases.

Onto PDA cellulose derivative films enzymes like glucose oxidase were immobilized[13] using the well-known surface activation by means of glutardialdehyde reaction[22] , diazo coupling[23] , and the novel ascorbic acid reaction[24]. Fig. 7 shows these and additional procedures used in our investigations.

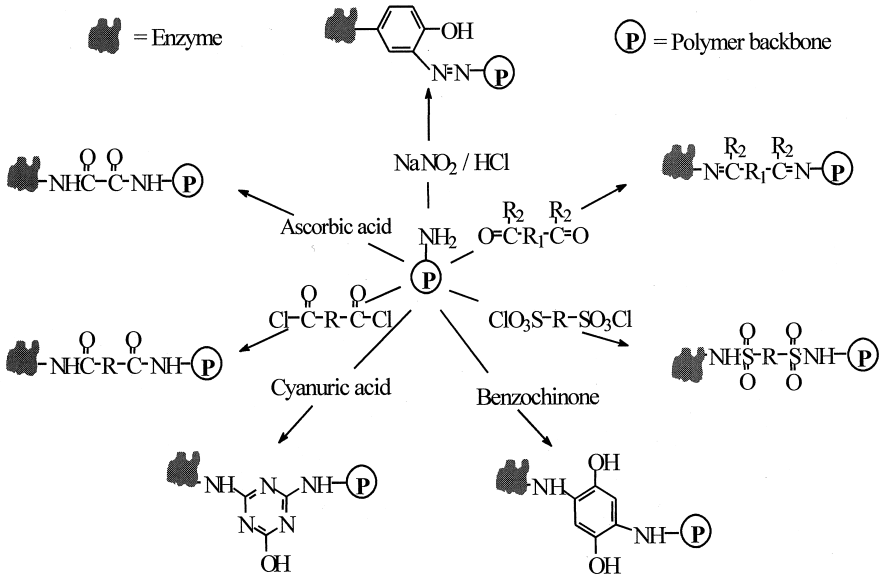


Fig. 7: Covalent enzyme immobilization on aminocelluloses (see Fig. 6) by different types of bifunctional reagents.

In summary, the PDA cellulose esters are suited excellently for enzyme immobilization. The enzymes remain fixed on the films in all cases. No enzyme activity is lost within several days. A typical immobilized enzyme activity is 145 mU/cm^2 for coupling with ascorbic acid onto PDA cellulose carbanilate[13].

With respect to the use of PDA cellulose esters as supports for analyte-sensitive reagent phases in fiber-optical biosensors, the redox-chromogenic properties were demonstrated by oxidative coupling reactions of phenols onto the PDA groups in presence of H_2O_2 and peroxidase (Fig. 8). The best coloring results were obtained by using PDA cellulose carbanilates.

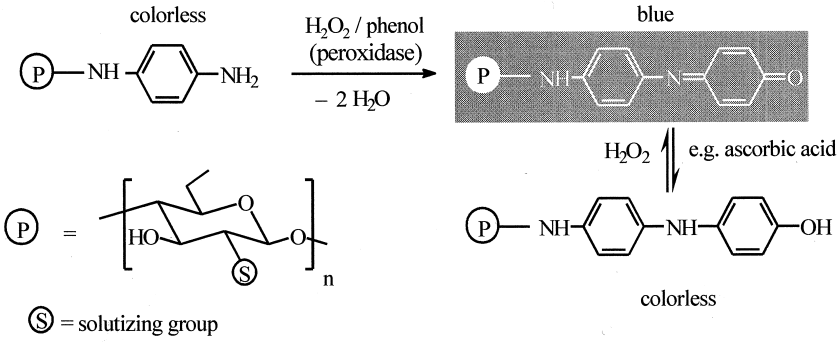


Fig.8: Oxidative coupling of phenol onto PDA cellulose esters (see Fig. 5).

Biotechnological formation and application of cellulose biomaterials

In addition to plants, bacteria like *Acetobacter xylinum* are able to produce cellulose in the form of pellicles at the air/liquid interface of a static culture medium[25-27]. In contrast to plants, the bacterial cellulose biosynthesis starting from D-glucose offers the very important possibility to control the cellulose formation[27, 28], especially the design of alternative supramolecular structures. Another important advantage of the microbial cellulose is their mouldability in situ.

Based on this biotechnological process we developed the novel biomaterial BASYC-BActerial SYnthesized Cellulose[29, 30].

With a special technology it is possible to prepare miniaturized tubes (hollow fibers) with inner diameters of about 1 mm during the cultivation and without application of physical styling methods (see Fig. 1, bottom). That means, the tubes are “growing” from dextrose

directly in the aqueous culture medium. This process is demonstrated schematically in Fig. 9.

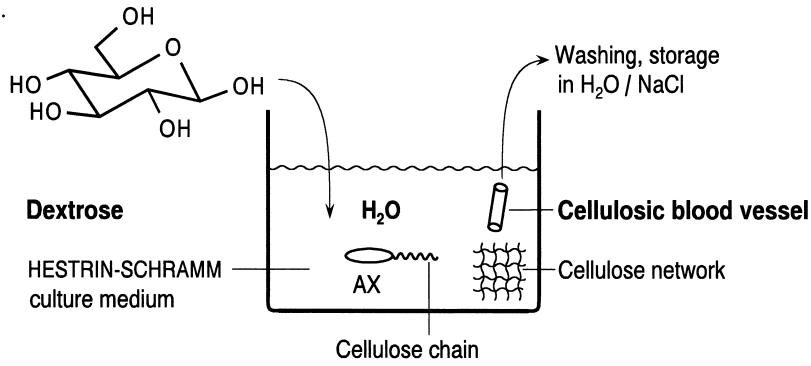


Fig. 9: Schematic representation of direct "growth" of artificial blood vessels in the culture medium of *Acetobacter xylinum.*

The BASYC biomaterial shows an unique supramolecular structure and properties not available starting from plant cellulose or using regeneration processes. The wall of the tubes is formed by a swellable network of nanofibers (see Fig. 1) resulting in a good mechanical strength in the wet state and in a very low roughness of the inner surface.

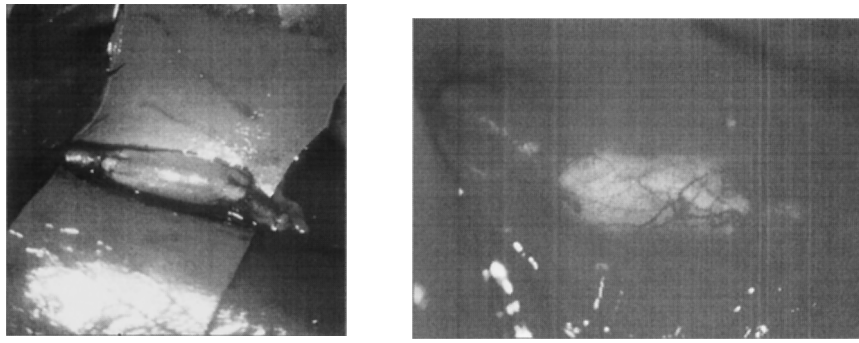


Fig. 10: Carotid artery of the rat with a BASYC interposition immediately after operation (left) and 4 weeks after operation (right).

As demonstrated in animal experiments (rat) the BASYC tubes are useful as "nature-like" blood vessels in microsurgery. The cellulose vessels are biocompatible, not rejected and do not increase the risk of thrombosis. A "vitalization" of the biomaterial (complete overlay of the inside wall with endothelial cells) takes place during a period of about 4 weeks. Connective tissue grows over the outside wall of the cellulose tube integrating it into the body. Fig. 10 shows a BASYC interposition implanted in the carotid artery of the rat[31)].

Conclusion

New concepts in regioselective functionalization, in formation of supramolelcular structures, and in biotechnological preparation enable the "tailor-made" modification of polysaccha- rides in the whole scale from the molecular up to the morphological/ macroscopic level.

Based on these results, new insights in basic questions of polysaccharide research and high value applications, e.g., polymers with recognition activity and novel types of biomaterials are accessible.

Acknowledgement

We are grateful for the financial support of the Deutsche Forschungsgemeinschaft granted within the Schwerpunktprogramm "Cellulose und Cellulosederivate – molekulares und supramolekulares Strukturdesign".

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