Film-forming aminocellulose derivatives as enzyme-compatible support matrices for biosensor developments

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Abstract

Based on 6(2)-O-tosyl celluloses and 6(2)-O-tosylcellulose derivatives, it has been possible to synthesize a novel soluble aminocellulose type, P-CH2-NH-(X)-NH2 (P = cellulose, (X) = alkylene, aryl, aralkylene or oligoamine) with diamine or oligoamine residues at C6 and solubilizing groups (S) such as acetate, benzoate, carbanilate, methoxy and/or tosylate groups at C2/C3 of the anhydroglucose unit (AGU). Depending on the nature and degree of substitution (DS) of (S), the aminocelluloses are soluble either in DMA and DMSO or in water. They all form transparent films from their solutions. In the case of water-soluble aminocelluloses, for example, an enzyme-specific pH value can be adjusted by protonation of the NH2 end groups at C6. The aminocelluloses apparently form aggregates (on a scale of nanostructures) according to a structure-inherent organization principle. The nanostructures could be imaged on the aminocellulose film surface by atomic force microscopy (AFM) in the form of characteristic topographic structures – as a result of the aggregation of the aminocellulose derivative chains and their interaction with the functionalized film support. In this way, structural and environment-induced factors influencing the nanostructure formation were found. The aminocellulose films can be covalently coupled with biomolecules by bifunctional reaction via NH2-reactive compounds. With the aid of analytically relevant enzymes, e.g. glucose oxidase (GOD), horseradish peroxidase (HRP) and others, it was found that the enzyme parameters can be modified by the interplay of the aminocellulose and coupling structures. A number of new bifunctional enzyme coupling reactions, e.g. via L-ascorbic acid or benzenedisulfonyl chlorides, forming amide or sulfonamide coupling structures led to efficient enzyme activities and long-term stabilities in the case of GOD and HRP coupling to PDA celluloseoxylylate films.

Abbreviations: ADA – alkenylenediamine; AFM – atomic force microscopy; AGU – anhydroglucose unit; APTES – 3-aminopropyltriethoxysilane; BDA – butylenediamine; DDA – dodecylenediamine; DETA – diethylenetriamine; DMA – N,N-dimethylacetamide; DMAP – N,N-dimethyaminopyridine; DMF – N,N-dimethylformamide; DMSO – dimethylsulfoxide; DPTA – dipropyleneetriamine; DS – degree of substitution; EDA – ethylenediamine; GOD – glucose oxidase; HDA – hexylenediamine; HRP – horseradish peroxidase; LOD – lactate oxidase; ODA – octylenediamine; PDA – 1,4-phenylenediamine; TEA – triethylamine; TETA – triethylenetetramine; THF – tetrahydrofuran.
Introduction

Our investigations are aimed at enzyme-compatible support matrices for biosensor development on the basis of film-forming aminocellulose derivatives (support materials for biosensors; see e.g. Mizutani and Asai 1990; Senda and Ikeda 1990; Ulman 1991; Wolfbeis 1991; Göpel et al. 1992; Göpel and Ziegler 1992; Karube and Yokoyama 1993; Göpel 1994; Urban 1999).

Apart from traditional syntheses in large-scale application, cellulose chemistry has also developed a number of cellulose derivatives used in biotechnology or in practical biochemistry as an insoluble support matrix for biomolecules (see e.g. Chibatu 1978; Ohba et al. 1979; Gemeiner and Breier 1982; Ugarova et al. 1983; Simionescu et al. 1985; Woodward 1985; Hayashi and Shimizu 1988; Braun and Meuret 1989; Comfort et al. 1989a,b; Göpel et al. 1992; Hermanson et al. 1992; Pyun et al. 1996).

Special fields of application are affinity chromatography and, above all, the immobilization of enzymes and immunoproteins. In this connection, all the essential immobilization categories are found, such as carrier entrapment, ionic and covalent binding as well as a large diversity of variants of the biocompounds applied. Table 1 shows some examples of enzyme immobilization on structurally modified celluloses.

The examples in Table 1 could be extended both with respect to further cellulose derivatives such as cellulose carbonates, chloroacetyl cellulose, bromoacetyl cellulose, etc., and with regard to further biocompounds such as other enzyme species, NAD and pyridoxal phosphate coenzyme, vitamin B_{12}, immunoproteins, etc. Interest was focused on preparative or analytical (e.g. chromatographic) applications where high loading densities of the carrier compounds with biocompounds were of importance.

In (bio)chemical sensor technology – especially in enzyme and immunosensors – cellulose derivatives have been applied as well (see e.g. Tsuchida and Yoda 1983; Nilsson and Mandenius 1994; Palmisano et al. 1997). The development here proceeded from enzyme electrodes to electron (e)-mediator-functionalized support matrices, e.g. ferrocene derivatives and redox-mediator-functionalized polymers to reduce the e-transfer barrier between enzyme structure and measuring electrode as well as improve signal transfer (see e.g. Frew and Hill 1987a,b; Fultz and Durst 1982).

Applications in fiberoptic sensors were, among others, the immobilization of pH indicators via divinylsulfone at cellulose (Weigl et al. 1993) or the immobilization of enzymes, e.g. urease and glucose oxidase (GOD), at sodium-periodate-activated cellulose-acetate membranes in combination with pH indicators (Sansurbino et al. 1994).

However, cellulose-acetate membranes have not become established as a support matrix in practical biosensor application. These membranes lack decisive structural prerequisites – such as reactive groups and the possibility of selective structural design for biosensor application.

The most important building block of a biosensor is an analyte-sensitive structural unit – the so-called transducer solid phase. It generally consists of a polymeric support matrix into which a biocompound for analyte recognition according to the biomolecular complementarity principle is incorporated. For example, the enzyme–substrate complex is a typical analyte recognition principle in biosensor technology. The analyte takes on the role of the enzyme substrate, and substrate conversion serves as a ‘chemical signal’ which, for example, is transformed into an electrical or optical signal via e-mediator or chromogen structures also present in the transducer solid phase. The latter signal is then transmitted to the measuring principle surface, e.g. an electrode, a field-effect transistor or fibre optics (see e.g. Frew and Hill 1987a; Wolfbeis 1991; Göpel and Ziegler 1994).

The main deficiencies in biosensor application are unstable transducer solid phases with unstable biomolecules, uncontrolled signal losses and transverse sensitivities due to substances other than the analyte. The structure of the support matrix plays a decisive role in solving these problems.

Among the support materials used for biosensors (see e.g. Ulman 1991; Wolfbeis 1991; Göpel et al. 1992; Göpel 1994), polysaccharide structures, e.g. cellulose structures, are likely to have biomolecule-compatible properties and a wide range of structural modification possibilities, not least due to their natural occurrence in communities with proteins (Pazur et al. 1970; Wykes et al. 1971; Vegarnd and Christensen 1975; Daele et al. 1992; Mannhalter 1993).

Among the cellulose derivatives, in particular, NH₂-functionalized cellulose derivatives (NH₂-celluloses) give rise to great expectations concerning their suitability as support matrices for biocompounds.