Aldehyde-functionalized Ethoxysilanes as New Enzyme Immobilization Reagents

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One-step immobilisation of biomolecules on silica surfaces using aldehyde-functionalised silanes is observed for glucose oxidase fixation.

The fixation of biomolecules on solid surfaces is of importance in various fields of research. In biotechnological processes the immobilisation of enzymes on solid substrates offers an easy and effective separation of the 'catalyst' from dissolved product mixtures and permits the reuse of the enzyme. Development of sensitive biosensors also requires the reproducible and stable immobilization of the biomolecule, for example enzymes or antibodies.

Functionalised ethoxysilanes such as 3-aminopropyltriethoxysilane[1,2] (APTES) or 3-sulfanylpropyltrimethoxysilane[3,4] (SPTMS) have been used as connecting links. Immobilization processes using these reagents require a second activation reagent which is able to couple with the reactive group of the silane and free amino groups of the biomolecule. Therefore, bifunctional crosslinking agents such as glutaraldehyde (GA) and *N*-(γ-maleimidobutyryloxy)succinimide (GMBS) must be used, in addition to APTES or SPTMS, to functionalise the surface prior to the immobilization step. As many immobilization problems stem from the use of crosslinking agents (*e.g.* glutaraldehyde tends to form undesirable polymers[5]), the development of an immobilization technique which does not use a bifunctional crosslinking reagent promises to simplify and improve the attachment of biomolecules. Here we present a new method for the immobilisation of biomolecules using aldehyde-functionalized silanes.[6] The aldehyde group can be used to attach biomolecules directly to the surface, with no bifunctional crosslinking species required. In this case, only one step is necessary for the activation of the silica surface. This is a significant improvement compared to literature methods.

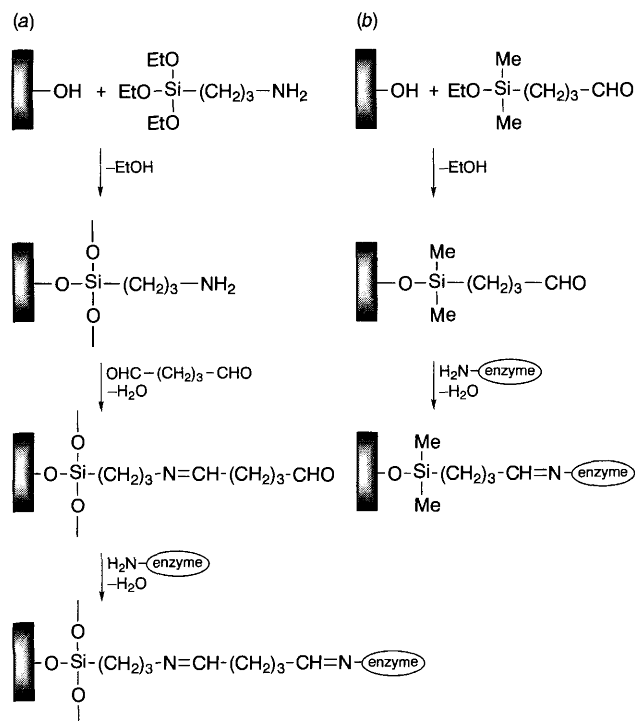

Fig. 1 Immobilization of an enzyme on a silica surface using (*a*) a traditional method (APTES/GA), (*b*) an aldehyde-functionalized silane [$(EtO)Me_2Si(CH_2)_3CHO$]

Fig. 1 schematically shows the immobilization of glucose oxidase (GOD) *via* (*a*) a traditional method using 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GA), and (*b*) our process using monoethoxydimethylsilylbutanal.

The use of monoethoxy- instead of triethoxy-silanes provides another improvement for special applications. Hydrolysis and condensation of the triethoxysilanes can lead to the formation of polymer networks on the surface, which may impede the transformation of a signal between enzyme and transducer in a biosensor or block the functional groups needed for the immobilisation step.

To examine the effectiveness of the new aldehyde-functionalised silanes as immobilisation reagents we used monoethoxydimethylsilylbutanal [$(EtO)Me_2Si(CH_2)_3CHO$] for the fixation of GOD on a substrate of controlled-pore glass (CPG). GOD is a catalyst for the specific oxidation of β-D-glucose with oxygen resulting in gluconic acid and hydrogen peroxide. Therefore, this reaction can be used as an assay for enzyme activity after immobilization. β-D-glucose is added to the immobilized enzyme in oxygenated solution and the production of hydrogen peroxide can be monitored *via* a second enzyme reaction.

Hydrogen peroxide oxidises *o*-phenylendiamine (OPD) to 2,3-diaminophenazine, an orange dye, in the presence of horseradish peroxidase (POD). Production of the dye can be quantified photometrically at λ = 490 nm after adding sulfuric acid.[5]

GOD was immobilized on CPG by the present method and compared to the traditional technique of APTES/GA and SPTMS/GMBS immobilization. The activity of GOD immobilized with these three techniques was compared to enzyme adsorption on native, underivatised CPG. Table 1 shows the extinction caused by GOD immobilized with different techniques directly after fixation and after 3 weeks.

Three samples were prepared identically using each technique. Immobilized GOD activity was very reproducible among these samples. With all methods a relatively high activity was observed. Enzyme immobilized *via* SPTMS/GMBS shows the lowest initial activity. The best results were obtained with the aldehyde-functionalized ethoxysilane. Here, the initial activity of the attached GOD is 20–50% higher than the samples prepared by literature techniques. The last row shows the activity of GOD attached to untreated CPG, in which the GOD

Table 1 Comparison of the activity of glucose oxidase (GOD) immobilised with different techniques. Average of three samples each.

Immobilization method	Initial activity/au	Activity after 3 weeks/au
APTES/GA	0.38	0.64
MPTMS/GMBS	0.22	0.93
Aldehyde silane	0.49	0.77
Native CPG	0.36	0.06

was only immobilized by adsorption. The physisorbed enzyme showed a relatively high initial activity, but this activity decreased close to zero within three weeks. This may be caused by desorption of the GOD from the unmodified CPG. In contrast, the activity of the chemically immobilized enzyme increased over the following weeks as can be seen in the second column. This may be a result of improved access of substrate to enzyme active sites, due to reorientation of the immobilized enzyme during this period. This effect of increasing enzyme activity after the immobilization has not been previously described. However, the immobilized GOD shows this effect very reproducibly. Modification of CPG with each of the three different techniques provides a stable immobilization.

The technique described here has been shown to immobilize glucose oxidase very effectively. Aldehyde-functionalized silanes provide a much simpler, one-step method of immobilising biomolecules on surfaces, and offer enhanced or comparable enzyme activity, relative to literature techniques.

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