Sensors and Actuators B, 13–14 (1993) 176–179

Immobilization and activity of Concanavalin A on tantalum pentoxide and silicon dioxide surfaces

Felix J. B. Kremer[a], Johan F. J. Engbersen[a], Jans Kruise[b], Piet Bergveld[b], Dick A. J. Starmans[c], Jan Feijen[c] and David N. Reinhoudt[a]

[a]*Department of Chemical Technology,* [b]*Electrical Engineering,* [c]*Biomedical Material Research, MESA Institute of University of Twente, P.O. Box 217, 7500 AE Enschede (Netherlands)*

Abstract

The physical adsorption and chemical immobilization of Concanavalin A (Con A) on semiconductor surfaces SiO_2 and Ta_2O_5 are investigated with the help of radioactive-labelled Con A. It is shown that Con A easily forms multi-layered systems and that SiO_2 has preference for the non-labelled protein. Surface coverage by the two methods is compared with respect to stability to mechanical stress and clearly shows the drawback of physical adsorption. In addition, the complexing abilities of Con A for mannose are investigated showing 50% activity compared to Con A in solution for monolayer systems, increasing to 75% for multi-layered systems.

1. Introduction

The most commonly used method at present to measure the glucose level of diabetic patients is with glucose oxidase-based reagent strips [1]. Since this is not a continuous measurement there is no immediate (and automatic) response possible. Therefore, there is much interest in the development of a glucose sensor which can measure continuously the glucose level *in vivo* and eventually trigger an insulin pump [2]. We are currently investigating the ion sensitive field effect transistor (ISFET) as a possible type of sensor for this purpose. ISFETs have the advantages of miniaturization and mass fabrication by IC technology.

A common detection method for glucose in sensors is with the aid of glucose oxidase which converts glucose into gluconic acid and hydrogen peroxide. The oxidation equivalents necessary can be detected with amperometric sensors [3]. This enzyme system has the drawback that it is dependent on oxygen pressure and that glucose is consumed and hydrogen peroxide is produced. Therefore, we investigated the possibility of glucose detection with selectors which recognize glucose without converting it to other products. Concanavalin A (Con A) was applied as the glucose-recognizing molecule in our study since this protein is well studied [4, 5] and is commercially available.

For our sensor application, Con A is immobilized on the surface of an ISFET. In order to obtain insight in the utility of this method we investigated physical adsorption and chemical immobilization of Con A on silicon dioxide and tantalum pentoxide surfaces with respect to the following points: (i) efficiency of Con A immobilization on the surface by the two methods, (ii) stability of Con A layers obtained by physical adsorption and chemical immobilization, (iii) activity of immobilized Con A in the complexation of saccharides.

2. Experimental

2.1. Materials

Silicon dioxide surfaces were thermally grown on three-inch silicon wafers of 0.3 mm thickness. Silicon wafers sputtered with tantalum were used to grow thermally the tantalum pentoxide surface. These surfaces were prepared by the Department of Electrical Engineering, University of Twente. Before use in the immobilization experiments, the surfaces were cleaned by immersion in a mixture of sulfuric acid and hydrogen peroxide (1:1 vol./vol.).

For the chemical immobilization method the semiconductor surfaces were treated with a 10% γ-aminopropyl-triethoxysilane (Janssen Chemica, Belgium) solution in toluene at 80 °C for 4 h and then rinsed twice with methylethylketone in an ultrasonic bath. In a subsequent reaction step the amino groups were derivatized with a 2.5% glutaric aldehyde solution in water for half an hour.

Radioactive Con A (^{125}I labelled, specific activity 36.3 μCi/μg, concentration 78.5 μCi/ml, NEN America) was

0925-4005/93/$6.00

used as a mixture with non-labelled Con A (Sigma) in a ratio of 1:80 000.

Con A solutions for the immobilization experiments were prepared immediately before use. A typical Con A buffer solution consisted of 0.01 M *N*-ethylmorpholine HCl buffer pH = 7.1, 0.02% $MnCl_2$, 0.02% $CaCl_2$ and the appropriate amount of Con A.

Complexation studies with immobilized Con A were performed with a mixture of radioactive mannose (6 ^{14}C labelled, specific activity 270 Ci/mol, concentration 0.2 mCi/ml, Amersham, England) and non-labelled mannose (Janssen Chemica) in a ratio of 1:50. Mannose used in the experiments was dissolved in the same buffer as was Con A, unless otherwise noted.

2.2. Methods

Adsorption and chemical immobilization experiments were performed using polypropylene/polyethylene disposable 50 ml syringes (Aldrich Chemie, Belgium). The syringe was closed with a pressure cap allowing the thermostation of the syringe without leaching out of the radioactive material. Six semiconductor substrates were placed in a disposable Teflon sample holder, which was placed in the syringe (Fig. 1). Both the syringe and sample holder were only used once.

At the start of each experiment the sample part of the syringe in which the semiconductor plates were placed was filled with 20 ml of buffer solution without protein in such a way that no air/water interface was present in the sample compartment. The adsorption of Con A was started by addition of the appropriate amount ($\approx$1 ml) of the buffered Con A solution (concentration 2×10^{-5}–5.2×10^{-2} g/ml) to the buffer solution in such a way that no air/water interface was formed, followed by gentle mixing. The amount of Con A in the solution was determined with a gamma counter (1282 Compugamma, LKB, Wallac, Turku, Finland). After the immobilization period, the remaining protein solution in the sample compartment of the syringe was removed and the semiconductor surfaces in the syringe were rinsed five times with buffer solution. Subsequently the semiconductor surfaces were put in 6 ml vials and counted in the gamma counter. The protein immobilized on the surface was calculated from the detected amount of ^{125}I and the known ratio of labelled and non-labelled protein.

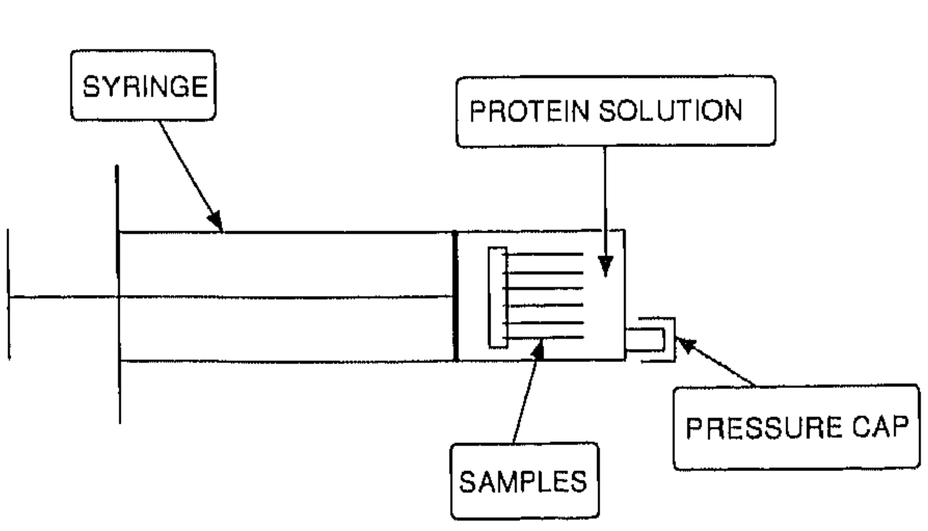

Fig. 1. Schematic drawing of the syringe with Teflon holder for protein adsorption experiments.

3. Results and discussion

The aim of this study was to investigate the efficiency of immobilization of Concanavalin A on semiconductor surfaces and the determination of the binding activity of the immobilized protein. The semiconductor surfaces used were Ta_2O_5 and SiO_2 as these materials are the basic supply of the ISFET sensor. The concept for sensor application is to immobilize the protein chemically, but in order to gain insight into the efficiency of this method, it was compared with a method in which the protein was only physically adsorbed onto the surface. Using the diameter of Concanavalin A (80 Å) [6] it is calculated that the surface can be covered with about 3.8 $\mu g/cm^2$ Con A if a monolayered structure is formed. By studying the dynamics of surface coverage in addition to the quantity of Con A immobilization, information can also be obtained about the type of immobilization. Fast physical adsorption of the protein on the surface is observed during the first hours and the total amount of protein adsorbed during this period coincides with the amount calculated for the formation of a monolayer. In the subsequent hours, the rate of adsorption is considerably lower, pointing to the formation of a multi-layered protein system (Fig. 2).

The rate of adsorption is lower in this second stage due to the weaker protein–protein interaction than the surface–protein interaction. Protein–protein interaction is also observed upon changes in concentration of the protein in the solution. In the range of lower protein concentrations, the rate of adsorption to the surface is almost linear with concentration. In the higher con-

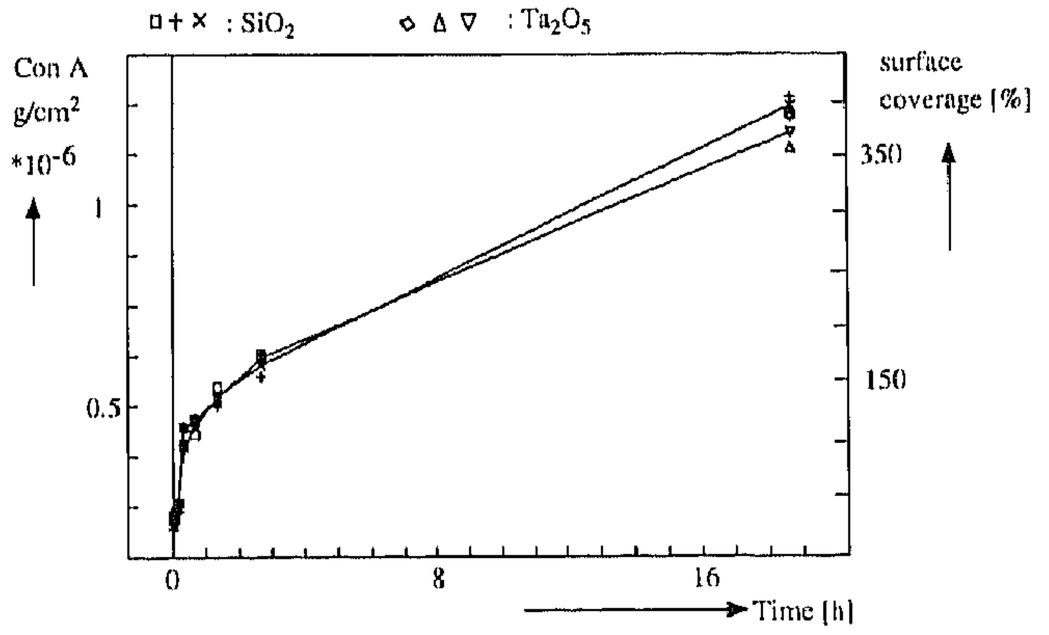

Fig. 2. Time dependence of physical adsorption on tantalum pentoxide and silicon dioxide surfaces in a solution containing a Con A concentration of 5.05×10^{-2} mg/ml.

centration range the adsorption rate levels off due to protein–protein interactions in the solution (Fig. 3).

Since in these experiments a mixture of radioactive protein and non-labelled protein is used we checked the possibility of differences in adsorption behaviour to the surface for these two types of Con A. As the labelled protein is modified by iodination it may be expected that the hydrophobic properties of the protein are changed and, in the case of silicon dioxide surfaces, differences in adsorption between labelled and non-labelled protein are indeed found (Fig. 4). The surface shows a preference for adsorption of the unlabelled protein. The tantalum pentoxide surface shows almost no discrimination. In the lower concentration range of the radioactive protein the same adsorption behaviour is found for both surfaces.

For the chemical immobilization, the rate of adsorption is completely different. Rapid reaction takes place on the chemically modified surfaces and surface coverage is complete within 15 min. After this period a slower physical adsorption occurs in which multilayers of protein are formed (Fig. 5).

Comparison of the stability of chemically immobilized layers and physically adsorbed layers shows that the physically adsorbed layers are removed almost completely after frequent washing steps. The chemically treated surface loses only the physically adsorbed multilayer structure, but the covalently immobilized monolayer remains on the surface (Fig. 6). Therefore, for sensor application it is necessary to immobilize the protein chemically.

Another important feature which has been studied is the remaining affinity of immobilized Con A towards saccharides. Con A is a tetramer with four saccharide binding sites and it may be anticipated that upon immobilization the activity is reduced due to the blocking of part of these sites. We have measured the affinity of Con A towards mannose since this monosaccharide is known to have one of the highest binding affinities towards free Con A [7]. A chemically immobilized monolayer of Con A still shows an activity of about

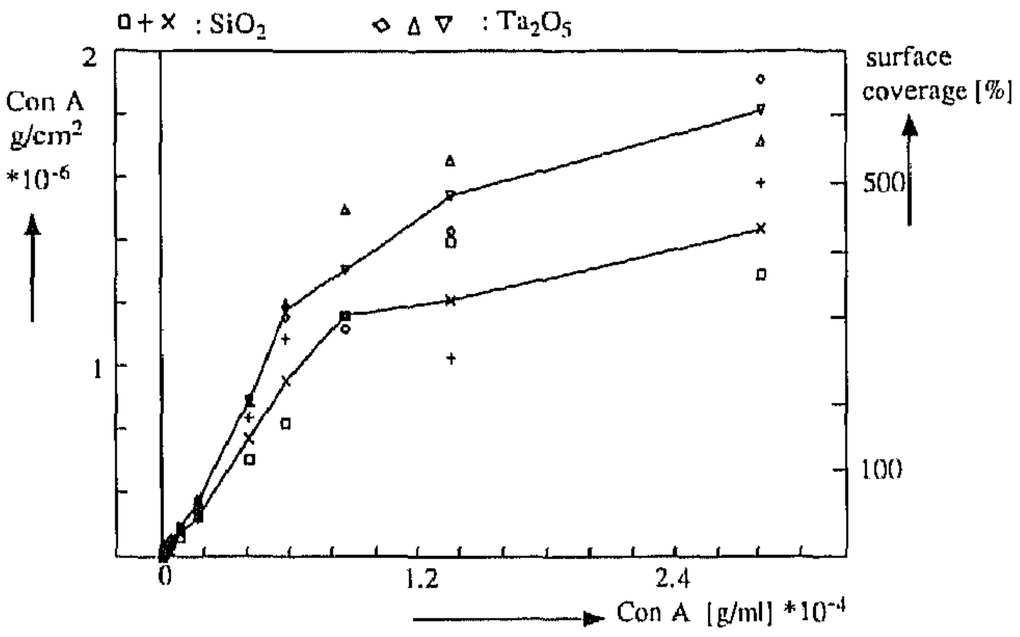

Fig. 3. Dependence of the physical adsorption process on the concentration of Con A in the buffer solution. Samples are measured after 24 h of equilibration in the Con A solution. The horizontal axis gives the concentration of Con A buffer used.

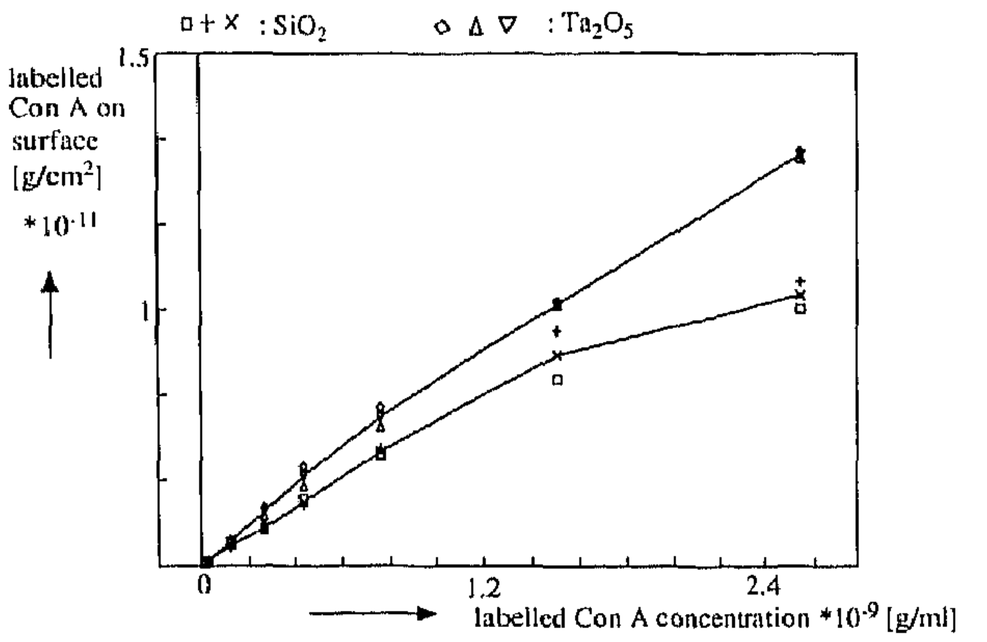

Fig. 4. Differences in adsorption of labelled and non-labelled Con A on tantalum pentoxide and silicon dioxide surfaces. The horizontal axis gives the concentration of the labelled Con A in the buffer solution. The total concentration of Con A is constant at 1.0×10^{-2} mg/ml.

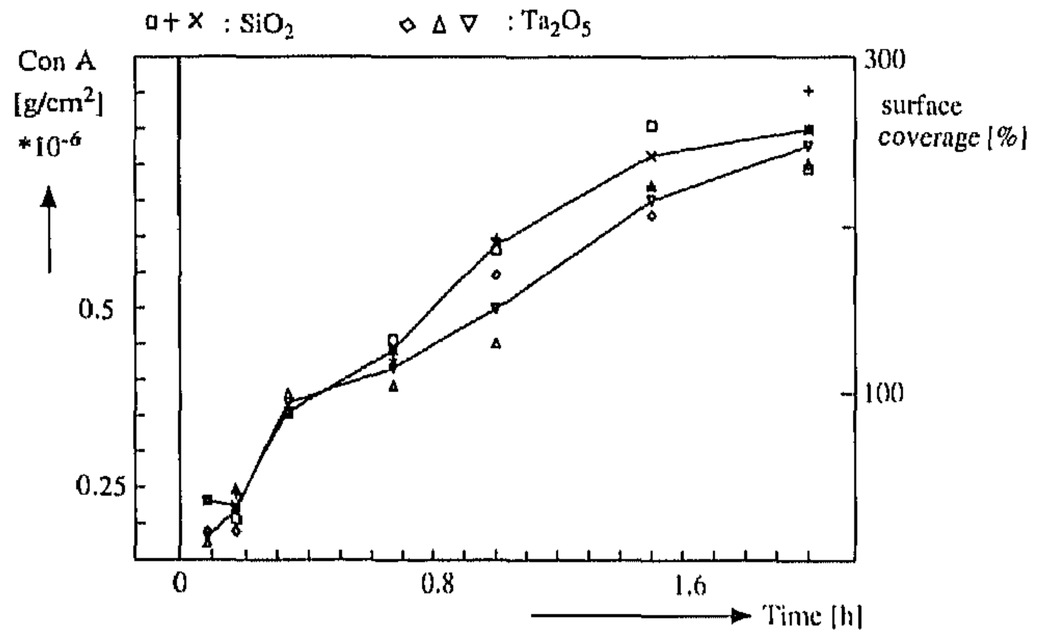

Fig. 5. Time dependence of the chemical immobilization of Con A on tantalum pentoxide and silicon dioxide surfaces pretreated with γ-aminopropyl-trimethylsilane and glutaric aldehyde. Con A concentration in the buffer is 2.32×10^{-2} mg/ml.

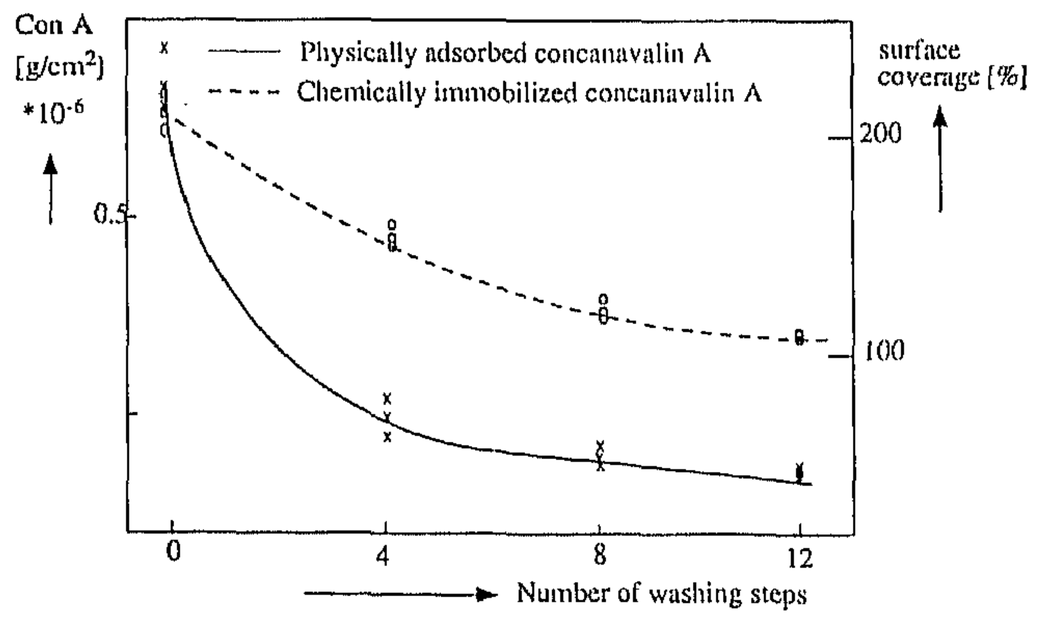

Fig. 6. Stability of chemically and physically immobilized Con A layers after several washing steps by vigorous shaking and rinsing with buffer solution for periods of 20 min.

50% of the value in solution, indicating that half the amount of binding sites are still attainable for the sugar. For the chemically immobilized multi-layered system, the total complexation activity is about 75% of the value of free Con A, which implies that the physically adsorbed layers above the first layer are more accessible for the monosaccharide. The same affinity behaviour is observed for the physically adsorbed layers of Con A. That specific complexation to the binding site rather than aspecific adsorption is the mode of binding of the saccharide is confirmed in an experiment in which demetallized Con A [8] is used instead of Con A in its native form. Demetallized Con A has no sugar binding properties and only a minor amount of non-selective adsorption is observed. Therefore, we can conclude that chemical immobilization of Concanavalin A to the semiconductor surface reduces the selective binding properties of Con A only moderately, which makes this protein suitable as a selective receptor on a sensor surface.

4. Conclusions

During physical adsorption on tantalum pentoxide and silicon dioxide surfaces, Concanavalin A easily forms a multi-layered structure, except at very low protein concentrations. Due to protein–protein interactions the growth of these physically adsorbed layers does not level off.

Labelled Con A, which is derivatized with iodine, shows less adsorption at silicon dioxide surfaces than non-labelled Con A; this is a phenomenon that one has to take into account in labelling experiments.

There is no large difference towards complexation of mannose between chemically immobilized and physically adsorbed layers.

For sensor application, however, chemically immobilized Concanavalin A is preferred above physically adsorbed Concanavalin A due to the mechanical stability of the layers formed.

References

1 R. B. Tattersall, Home blood glucose monitoring, *Diabetologia, 16* (1979) 71.

2 P. D. Home and K. G. M. M. Alberti, in A. P. F. Turner, I. Karube and G. S. Wilson (eds.), *Biosensors: Fundamentals and Applications*, Oxford University Press, London, 1987, Ch. 36, p. 723.

3 W. J. Albery and D. H. Craston, in A. P. F. Turner, I. Karube and G. S. Wilson (eds.), *Biosensors: Fundamentals and Applications*, Oxford University Press, London, 1987, Ch. 12, p. 180.

4 B. B. L. Agrawal and I. J. Goldstein, Protein–carbohydrate interaction. VII. Physical and chemical studies on Concanavalin A, the haemaglutinin of the Jack Bean, *Arch. Biochem. Biophys., 124* (1968) 218.

5 G. H. McKenzie, W. H. Sawyer and L. W. Nichol, The molecular weight and stability of Concanavalin A, *Biochim. Biophys. Acta, 263* (1972) 283.

6 G. M. Edelman, B. A. Cunningham, G. N. Reeke, Jr., J. W. Becker, M. J. Waxdal and J. L. Wang, The covalent and three-dimensional structure of Concanavalin A, *Proc. Natl. Acad. Sci. USA, 69* (1972) 2580.

7 R. D. Poretz and I. J. Goldstein, An examination of the topography of the saccharide binding sites of Concanavalin A and of the forces involved in complexation, *Biochemistry, 9* (1970) 2890.

8 A. J. Kalb and A. Levitzky, Metal-binding sites of Concanavalin A and their role in the binding of α-methyl D-glucopyranoside, *Biochem. J.*, 109 (1968) 669.