Immobilization of immunoglobulins on silica surfaces

Stability

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The development of new immunosensors based on surface-concentration-measuring devices requires a stable and reproducible immobilization of antibodies on well-characterized solid surfaces. We here report on the immobilization of immunoglobulin G (IgG) on chemically modified silica surfaces. Such surfaces may be used in various surface-oriented analytical methods. Reactive groups were introduced to the silica surfaces by chemical-vapour deposition of silane. The surfaces were characterized by ellipsometry, contact-angle measurements and scanning electron microscopy. IgG covalently bound by the use of thiol–disulphide exchange reactions, thereby controlling the maximum number of covalent bonds to the surface, was compared with IgG adsorbed on various silica surfaces. This comparison showed that the covalently bound IgG has a superior stability when the pH was lowered or incubation with detergents, urea or ethylene glycol was carried out. The result was evaluated by ellipsometry, an optical technique that renders possible the quantification of amounts of immobilized IgG. The results outline the possibilities of obtaining a controlled covalent binding of biomolecules to solid surfaces with an optimal stability and biological activity of the immobilized molecules.

Covalent immobilization of various biomolecules on solid surfaces has been widely used for different purposes. Among the applications are affinity chromatography (Weetall, 1972), immobilization of enzymes (Mosbach, 1983) and solid-phase analytical techniques (Guesdon & Avrameas, 1981). Biomolecular interactions at solid surfaces are also of fundamental interest in the rapidly growing field of molecular electronic devices (Carter, 1982).

Several measuring techniques based on optical (Giaever, 1976; Nylander *et al.*, 1982–83), electrochemical (Lowe, 1979; Yamamoto *et al.*, 1980; Janata & Huber, 1980) or piezoelectric (Roederer & Bastiaans, 1983) methods have great potential for use as immunosensors. These methods measure directly the change in surface concentration on a solid surface, when for example an antigen interacts with an immobilized antibody. The involve-

ment of specifically labelled molecules is thereby avoided. Because of the possibility of measuring kinetic parameters, such sensors are suited for rapid and automated measuring systems. The possibilities of miniaturization, especially in the case of chemically sensitive field-effect transistors (Janata & Huber, 1980), make these sensors attractive for continuous measurements *in vivo*. However, several fundamental problems remain to be solved before these measuring techniques will be suitable for use as immunosensors.

One problem is to attain a stable attachment of highly specific molecules on the surface of the measuring device. Furthermore, the unspecific adsorption from samples of body fluids such as plasma or serum should be minimized. To obtain appreciable sensitivity for the surface-concentration-measuring devices, the immobilization of, for example, the antibodies to the solid surface should be optimized and each antibody at the surface should be able to interact with an antigen. As reported by Nygren *et al.* (1983), enhanced sensitivity was achieved in an optical measuring tech-

Abbreviation used: SPDP, *N*-succinimidyl 3-(2-pyridyldithio)propionate.

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nique by using affinity-purified antibodies as compared with the immunoglobulin preparation. Highly specific molecules may also be obtained by the use of monoclonal antibodies. However, nothing is known with certainty about the conformation and orientation of antibodies immobilized on a solid phase. Conradie *et al.* (1983) reported an enhanced sensitivity in an enzyme-linked immunosorbent assay when partially denatured antibodies were immobilized on the solid surface. This indicates the possibilities of influencing the orientation and conformation of the antibodies.

Immobilization of proteins on solid surfaces is a complex combination of various interacting forces (Hofstee, 1979; Srinivasan & Ruckenstein, 1980). Differentiation between these forces is difficult. A combination of non-covalent forces may generate stable binding (Hofstee, 1979). However, in this case there is always a risk of molecular re-arrangements, interchange of already bound molecules with others in solution and/or elution of bound molecules in an extreme milieu. The requirement of stability in such a milieu is important when non-disposable sensors are to be used. An immunosensor may be regenerated by breaking the antibody-antigen binding by, for example lowering the pH and/or the surface tension of the medium (van Oss *et al.*, 1979).

For these reasons we have attempted to produce a surface with a chemical composition suitable for covalent binding via thiol-disulphide exchange reactions (Carlsson *et al.*, 1978). This coupling mechanism has the advantage that the number of covalent-bond-forming groups on the protein can be controlled and is possible to quantify. Furthermore, cross-linking and homopolymerization of the protein molecules are avoided.

In our studies, silicon slides covered by spontaneously formed silica were used as the solid phase. This material is also used in surface-concentration-measuring devices such as ellipsometry (Azzam & Bashara, 1977) and the chemically sensitive field-effect transistor. Silica is used as the solid phase in many h.p.l.c. applications. The slides used in our studies may therefore also serve as a model for the interaction of biomolecules with such silica.

Chemical groups suitable for covalent binding of biomolecules may be introduced on silica surfaces by silanization. A smooth, reproducible and stable silane film of monolayer character is required for the surface-concentration-measuring techniques. These requirements can be fulfilled by chemical-vapour deposition of a suitable silane on the silica surfaces.

Various methods exist suitable for the study of amount, stability and biological activity of immobilized molecules. However, only a few of these measure directly and in an uncomplicated way,

namely without introduction of radioactive (Baszkin & Lyman, 1980) or fluorescent labels (Watkins & Robertson, 1977), the amount of immobilized molecules. Furthermore, labelling has been shown to influence the absorption properties of many proteins (Lensen *et al.*, 1984). Ellipsometry is an optical technique with which it is possible to measure the amount of bound material on a smooth reflecting surface. It is also possible to follow the kinetics during adsorption from solution.

We report here on a stable binding of IgG to chemically modified silica surfaces. The stability of the proposed covalent binding of IgG to these surfaces was compared with the stability of IgG adsorbed on four types of silica surfaces differing in solid surface tension. Before binding and adsorption of IgG, the surfaces were characterized by ellipsometry, contact-angle measurements and scanning electron microscopy. The proteins were characterized by isoelectric focusing.

Materials and methods

Surface pretreatment

All surfaces used were polished single crystalline silicon (1.1.1) phosphorus-doped wafers with a resistivity of 6–8 Ω -cm (Wacker Chemitronic, Burghausen, West Germany). The wafers were cut into 3 mm $\times$ 3 mm slides and washed as described by Jönsson *et al.* (1985a). This treatment rendered the slides hydrophilic, and they are referred to below as 'hydrophilic slides'. Amino groups were introduced at the surface of these slides by chemical-vapour deposition as described by Jönsson *et al.* (1985a). These slides are referred to below as 'amino slides'. After silanization, slides to be further modified were immediately transferred to dichloromethane (Merck, Darmstadt, West Germany) and allowed to react with 10 mM-2-carboxyethyl 2-pyridyl disulphide in the presence of 100 mM-dicyclohexylcarbodi-imide (Aldrich, Beerse, Belgium) and 40 mM-2,6-dimethylpyridine (synthetic grade; Merck) for 2 h in a desiccator with gentle shaking. The 2-carboxyethyl 2-pyridyl disulphide was synthesized from 2,2'-dipyridyl disulphide and 3-mercaptopropionic acid (Fluka, Buchs, Switzerland) by the method of Carlsson *et al.* (1978). After extensive rinsing in dichloromethane, the slides were kept in ethanol until use. These slides are denoted 'pyridyl slides'. Before binding of IgG to these slides they were reduced with 100 mM-dithiothreitol (Fluka) in 10 mM-sodium phosphate buffer, pH 7.5, for 30 min and extensively rinsed in the same buffer supplemented with 1 mM-EDTA (P-EDTA buffer). These slides are denoted 'thiol slides'. The proposed chemical steps involved for pyridyl and thiol slides are shown in outline in Scheme 1.

Scheme 1. Proposed chemical reaction steps involved in connection with pyridyl and thiol slides

(a) Synthesis of pyridyl slides. Amino slides were allowed to react with carboxyethyl 2-pyridyl disulphide in the presence of dicyclohexylcarbodi-imide (DCCD) and 2,6-dimethylpyridine, thereby introducing pyridyl disulphide groups to the slides. (b) Synthesis of thiol slides. Pyridyl slides were reduced with dithiothreitol (DTT), exposing the thiol groups on the surfaces. These groups may then be made to react with SPDP-modified IgG via thiol-disulphide exchange reactions, thereby covalently attaching the protein to the thiol slides by disulphide bonds.

Fig. 1. Proposed chemical structures for hydrophilic, amino, pyridyl, thiol, control and methyl slides

Slides were also prepared that underwent the silanization procedure but without introduction of aminosilane (Jönsson *et al.*, 1985b). These slides are referred to below as 'control slides'.

Methyl groups were introduced on washed slides as described by Jönsson *et al.* (1985a). These slides are denoted 'methyl slides'.

The proposed chemical structures of the different surfaces are shown in Fig. 1.

Ellipsometry

Ellipsometry measures the change in polarization of a light-beam on reflection from a surface (Fig. 2). The technique has been used by a number of workers to study the adsorption of proteins on solid surfaces (e.g. see Vroman & Adams, 1969; Cuypers *et al.*, 1983).

A complete description of the ellipsometric set-up together with a calibration of the technique with radiolabelled IgG are presented elsewhere (Jönsson *et al.*, 1985a).

Surface characterization

Three different runs of washing and chemical modifications were surface-characterized. From each run at least three slides for each type of surface were measured.

The thicknesses of the silane and silica films on the silicon for the different slides were determined by ellipsometry (Jönsson *et al.*, 1985a).

The surface energies of the slides were evaluated by contact-angle measurements. The measurements were performed in the following way. A number of non-wetting liquids, over a range of surface tensions, were placed as small droplets on the slide, and the contact-angle, θ , for each was evaluated. A so-called Zisman plot was then constructed, with $\cos \theta$ as the ordinate and the surface tensions of the liquids as the abscissa. The interception of the resulting straight line with $\cos \theta = 1$ gave the critical surface tension of the surface, γ_c . The γ_c values derived from such plots correlate well with the chemical nature of the solid surface (Zisman, 1964).

The contact angle is also a function of the liquid surface tension and the solid surface tension. Both surface tensions may have polar and dispersive contributions. The polar contribution represents

Fig. 2. Measurement principle for the ellipsometer

Monochromatic light from the laser was converted into elliptically polarized light by the polarizer and quarter-of-wavelength plates. With proper settings of the first polarizer plate this light, after reflection on the silicon slide, was converted into linearly polarized light. The light could be extinguished by the setting of the second polarizer placed after the silicon slide, and the minimum light transmission was detected. In the case of silicon the angular setting of the first polarizer, Δ , is proportional to the amount of immobilized IgG on the surface. To ascertain an impurity-free air/liquid interface a continuous rinsing system was used. The slides could in this way be taken in and out of the cuvette without introducing any change in the immobilized protein film.

electrostatic and metallic interactions, hydrogen-bonding and dipole interactions. The dispersive contribution represents the London interaction between non-polar molecules. The derivation of the dispersive and polar contributions of the solid surface tension, γ_s^d and γ_s^p , from the contact-angle data is in general complex. In the present work, the solid surface tension (γ_s), γ_s^d and γ_s^p have been calculated by using the geometric-mean approximation method (Kaelble, 1971). In these calculations a computer program was developed to calculate best-fit values of γ_s^d and γ_s^p by least squares. The contact-angle measurements were performed as described by Jönsson *et al.* (1982).

The measurements were carried out in air and on slides dried in N_2 gas. However, thiol slides were not characterized. This was due to difficulties in obtaining a reproducible drying process for these slides after the reduction with dithiothreitol in an aqueous solution (Scheme 1).

Slides were examined in a scanning electron microscope as described by Jönsson *et al.* (1985b).

IgG modification and binding

Rabbit IgG was prepared and characterized as described by Jönsson *et al.* (1985a).

The IgG was treated with SPDP (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by Carlsson *et al.* (1978) to a degree of modification of 6–8 SPDP groups per protein molecule, a value based on $A_{280}^{1\%} = 13.5$ for IgG. Before incubations with the slides, the IgG was transferred to P-EDTA buffer by gel filtration on Sephadex G-25 (Pharmacia Fine Chemicals). The SPDP-modified IgG was compared with IgG in isoelectric focusing on LKB Pagplate, pH 3.5–9.6 (LKB, Bromma, Sweden). Two different batches of IgG were used in this investigation. These batches showed essentially identical isoelectric-focusing patterns.

One crucial point when handling the slides is the transfer of the slides through the air/liquid interface and the accompanying risk of transfer of impurities to the slides. The reproducibility of the experiments was markedly enhanced by using glass filter funnels with a continuous back flow of buffer during all handling steps of the slides (Fig. 2b). The continuous flow of buffer ensured that a fresh liquid surface was created.

Binding and adsorption of IgG and SPDP-modified IgG on control, amino, thiol and methyl slides took place during incubation of the slides in polystyrene Petri dishes. The dishes were first placed in

the glass filter funnel in a continuous flow of P-EDTA buffer. Thereafter the slides were placed in the dishes and the dishes were lifted out of the funnel. IgG was then added to the dishes to a final concentration of approx. 100 µg/ml. The dishes were incubated in a humidified atmosphere for 18–24 h. Extensive rinsing in P-EDTA buffer was carried out by immersing the dishes together with the slides in the funnel followed by the removal of the dish. The slides were now measured in the ellipsometer and again transferred to the Petri dishes via the funnel. In parallel experiments it was ascertained that the slides, as measured by ellipsometry, were not influenced by P-EDTA buffer incubation without any IgG added. The adsorbed amount of IgG was therefore calculated as the differences in ellipsometric parameters for such slides and slides incubated with IgG.

The stability of the IgG on the various surfaces was tested by replacement of the P-EDTA buffer in the slide-containing dishes with the media A–E (Table 1). The slides were again incubated for 16–18 h, rinsed in P-EDTA buffer, and measured in the ellipsometer as described above.

To make certain that these incubation media had no effects on the unexposed surfaces, e.g. oxidation or removal of silane layers, slides without surface attached IgG were also incubated in media A–E. No effects of the various media on the surface structure of the different slides could be detected by the ellipsometer.

The detergent used was Triton X-100 (Sigma

Chemical Co., St. Louis, MO, U.S.A.). All chemicals were of analytical grade and all reactions were carried out at $22 \pm 1^\circ\text{C}$ unless otherwise specified. The water used was double-distilled. All glassware and dishes used for the incubations were washed overnight in Hellmanex (Hellma, Müllheim, East Germany) before use. This wash was followed by rinsing in tap water and distilled water.

Results

Table 2 shows the film thicknesses and contact-angle data for the various surfaces used in this investigation. The film thickness for the hydrophilic slides represents the spontaneously formed silica on the silicon surface. The values for layer thicknesses for the other surfaces express the total thickness including the spontaneously formed silica. As is evident from a comparison of control slides and hydrophilic slides in Table 2, the silica layer does not considerably increase its thickness at the temperatures used for the silanization. The further increase in layer thickness for amino slides could thus be attributed to the aminosilane.

A series of control experiments was carried out. They include a systematic omission of reaction components involved in the amidization reaction (Scheme 1). Thus 2-carboxyethyl 2-pyridyl disulphide alone, dicyclohexylcarbodi-imide alone, 2-carboxyethyl 2-pyridyl disulphide + dicyclohexylcarbodi-imide, dicyclohexylcarbodi-imide + 2,6-dimethylpyridine, 2-carboxyethyl 2-pyridyl disulphide + 2,6-dimethylpyridine and finally 2,6-dimethylpyridine alone were omitted; other steps in the overall coupling procedure remained the same. In all these cases, except for the omission of 2,6-dimethylpyridine, no change in layer thickness could be detected. In the case of omitting 2,6-dimethylpyridine the layer thickness shown in Table 2 for pyridyl slides was diminished by roughly 10%.

Scanning electron microscopy of the slides revealed smooth surfaces. Silanized slides were indistinguishable from the control and hydrophilic

Table 1. Incubation media used in the stability test of immobilized IgG

Medium	Composition
A	P-EDTA buffer, pH 7.5
B	0.1 M-Glycine, pH 2.5
C	0.05% (v/v) Triton X-100/0.1 NaCl/0.1 M-sodium phosphate buffer, pH 7.5
D	0.05% (v/v) Triton X-100/4 M-urea, pH 2.0
E	50% (v/v) Ethylene glycol/1 M-NaCl

Table 2. Surface characterization, ellipsometric layer thickness and contact-angle data are given.

The numbers each indicate mean $\pm$ maximum deviation for at least three different slides, from three separate runs of washing and chemical modifications. Abbreviation: N.D., not done.

	Layer thickness (nm)	γ_c (mN/m)	γ_s^p (mN/m)	γ_s^d (mN/m)	γ_s (mN/m)
Hydrophilic slides	1.40 ± 0.05	40 ± 2	52 ± 4	17 ± 3	69 ± 7
Control slides	1.50 ± 0.05	25 ± 1	14 ± 2	23 ± 2	37 ± 4
Amino slides	2.0 ± 0.1	37 ± 3	22 ± 2	24 ± 2	46 ± 4
Pyridyl slides	2.4 ± 0.1	32 ± 3	17 ± 2	26 ± 2	43 ± 4
Thiol slides	2.2 ± 0.3	N.D.	N.D.	N.D.	N.D.
Methyl slides	1.55 ± 0.05	22 ± 1	1 ± 1	24 ± 1	25 ± 2

slides, indicating the absence of any particle larger than 20–30 nm.

In Fig. 3 the isoelectric patterns for IgG and SPDP-modified IgG are analysed.

The parameter tested for differentiation between adsorption and covalent binding to IgG to the surfaces is the stability of the IgG film in an extreme milieu. Fig. 4(a) shows the result of the stability test for IgG adsorbed on control, methyl, thiol and amino slides. In Fig. 4(b) the same test was made for SPDP-modified IgG. Four separate experimental runs with two different batches of IgG were performed. Minor differences, $\pm 15\%$, exist in the adsorbed or bound amount of IgG between separate runs. The qualitative behaviour, however, was the same in all experiments. The bars in Fig. 4 represent the mean values $\pm$ maximum deviations from four different slides of each type of surface modification in one experimental run.

When requirements for covalent binding of IgG are fulfilled (stippled bars in Fig. 4b) the stability

of the bound IgG is superior to all other combinations of surface types and IgG. Notable is the appreciable difference in behaviour for IgG and SPDP-modified IgG on thiol slides.

Both types of IgG adsorbed on methyl slides could be eluted by detergent-containing media, whereas IgG adsorbed on the other surface types was not markedly influenced by this treatment. The lowering of surface tension by ethylene glycol in combination with increased ionic strength (medium E; Table 1) eluted significant amounts of IgG from the amino and control slides. Furthermore, lowering the pH (media B and D, Table 1) elutes some IgG from amino and thiol slides.

Discussion

The purpose of this investigation was to find a stable and reproducible technique for immobilization of immunoglobulins on surfaces suitable for use in surface-concentration-measuring devices. The results are, however, of a fundamental character pertaining to protein–solid-surface interactions in general. To obtain reliable results concerning these interactions it is of extreme importance to characterize both the surface and the protein used for the studies (Greig & Brooks, 1981).

Surface pretreatment and surface characterization

Chemical modification by silanization is usually performed in a liquid phase (Weetall, 1976). This process has been used successfully with some chlorosilanes, whereas alkoxysilanes, such as the aminosilane used in this investigation, have been reported to give rise to multiple silane layers, along with attached polymeric silane particles (Haller, 1978). By using strict anhydrous conditions this can be avoided and smooth layers of monolayer character obtained (Untereker *et al.*, 1977). To obtain aqueous stable aminosilane monolayers we have used a chemical-vapour deposition technique for silanization of the silica surfaces. Although we have no direct evidence for covalent binding of the aminosilane surface, no change in silane layer thickness could be detected after 18 h of incubation in the various media A–E (Table 1). This indicates a stable attachment of aminosilane to the silica surface.

The small variation (± 0.1 nm) in the silane layer thickness as measured by ellipsometry together with the electron-microscopic observations indicate a smooth coverage by the silanes.

The results from the systematic omission of reaction components involved in the amidization reaction suggest that the reaction depicted in Scheme 1 occurs. It is therefore suggested that the increased thickness as compared with the amino slides is due to reaction with 2-carboxyethyl 2-pyridyl disul-

Fig. 3. Isoelectric focusing of IgG and SPDP-modified IgG. Lanes A and F, calibration-kit proteins: amyloglucosidase (pI 3.50), soya-bean trypsin inhibitor, β -lactoglobulin A, bovine carbonic anhydrase B, human carbonic anhydrase B, horse myoglobin (pI 7.35), lentil lectin (pI 8.15), lentil lectin (pI 8.45), lentil lectin (pI 8.65) and trypsinogen. Lanes B and D, IgG, 10 μ g. Lanes C and E, SPDP-modified IgG, 10 μ g. The isoelectric points of the standard proteins are indicated.

Fig. 4. Stability test for IgG

The stability of immobilized IgG (a) and immobilized SPDP-modified IgG (b) was tested by incubation for 16–18 h of slides with immobilized IgG in media A–E (Table 1). The IgG was immobilized to (from the left) control, methyl, thiol and amino slides respectively. The bars indicate the remaining amount of IgG and SPDP-modified IgG after this incubation. The bars representing the incubation in medium A may be used as a reference since this incubation did not influence the amount of immobilized IgG and SPDP-modified IgG on any surface. A simplified view of the solid-surface chemistry is also indicated.

phide. This was also supported by the significant decrease in polar character for pyridyl slides as compared with amino slides evident from the contact-angle measurements. The decreased γ_p^s for pyridyl slides is suggested to be due to reaction of the polar amino groups on the amino slides with 2-carboxyethyl 2-pyridyl disulphide.

The absolute value of the calculated layer thickness should be interpreted with care and not directly translated into molecular dimensions. In the thickness calculations the layer was assumed to be homogeneous. This may, however, not be entirely true for these thin silane layers. Furthermore, it has been shown (Moses *et al.*, 1978; Boerio *et al.*,

1978; Chiang *et al.*, 1982) that the aminosilane molecules may exist in several forms and orientations at a surface. The calculated values of the layer thickness therefore only indicate that no major build-up of multilayers on the surfaces occurs at the various chemical modification steps.

The contact-angle data are further discussed in the following paper (Jönsson *et al.*, 1985c).

The surface characterization should in no way be taken as evidence for the chemical surface structures proposed in Fig. 1. The structures should rather be considered as a simplified view of the surfaces. Especially in the case of the aminosilane attachment to a surface, several structural forms have been suggested. The internal cyclization structure for the aminosilane adopted here was suggested by Moses *et al.* (1978).

IgG binding and stability

Purified serum IgG was used in this study although it is a mixture of active and inactive antibodies. The IgG was prepared from commercially available rabbit IgG and further purified by affinity chromatography. The preparation was free from IgM and IgG aggregates by gel filtration. Analytical isoelectric focusing showed a mixture of proteins with a spectrum of isoelectric points in the pH range 5.5–7 for IgG and 5–6 for SPDP-modified IgG. As could be expected the SPDP modifications, as a result of the reaction of SPDP with amino groups on the IgG, induces an anodic shift in the range of isoelectric points for this IgG. The broad range of isoelectric points is typical for a polyclonal IgG preparation.

To study the type of IgG–solid-surface interactions involved, the stability of adsorbed and bound IgG was tested. Adsorbed protein molecules may show a virtually irreversible adsorption as utilized in adsorptive protein immobilization (Hofstee, 1976). Our results show that the adsorbed IgG is not stable towards changes in the surrounding medium such as lowering the pH or surface tension, incorporation of detergents and/or urea and increase in ionic strength.

Unspecific adsorption on a solid phase may in many analytical applications be minimized by the incorporation of a detergent in the buffer solutions. The detergent should ideally have no influence on the stability and activity of the immobilized molecules. As shown in the present study treatment with a detergent had a remarkable effect on IgG adsorbed on methyl slides. Since solid-phase assays are commonly based on immobilization by adsorption, followed by incorporation of detergent in subsequent incubation steps, this clearly shows the importance of choosing a proper surface as the solid phase. In enzyme-linked immunosorbent assay the solid phase usually consists of polystyrene. This

polymer has been reported to have a critical surface tension in the range 33–43 mN/m (Brash, 1977). This is considerably higher than the value for methyl slides. The amino and pyridyl slides, however, have their critical surface tension within the range reported for polystyrene. The influence of detergent on stability of adsorbed IgG on these slides was also much less pronounced as compared with the methyl ones.

The elution of proteins from alkylamino silica-gel surfaces, similar in structure to the amino slides used by us, has been extensively studied in hydrophobic-interaction chromatography (Hofstee, 1973, 1979; Srinivasan & Ruckenstein, 1980). Elution of IgG from alkylamino surfaces was performed by the combination of lowering the surface tension and increasing the ionic strength. We also tested this combination (medium E) and found substantial elution of both IgG and SPDP-modified IgG adsorbed on amino slides.

The significant difference in stability of SPDP-modified IgG immobilized on thiol slides as compared with the other combinations of IgG and surfaces strongly suggests the covalent binding of IgG via disulphide bonds to thiol slides (Scheme 2). The non-covalent IgG–surface interactions are, for the sake of simplicity, omitted from this Scheme. It may be noted that the degree of SPDP substitution

Scheme 2. Proposed covalent binding of SPDP-modified IgG to thiol slides

can be controlled and therefore also the maximum number of covalent bonds to the solid surface. Evidently it is possible to optimize the degree of SPDP substitution, allowing the best combination of stability and preserved biological activity.

The present paper outlines the possibilities of controlled and reproducible immobilization of IgG on surfaces for use in new immunosensor applications.

Factors involved in determining the amount of IgG immobilized to the various surface types together with the kinetics of the immobilization are presented in the following paper (Jönsson *et al.*, 1985c).

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