0273-2289/89/2203—0311$04.00

Antibodies Immobilized on Inorganic Supports

HOWARD H. WEETALL* AND MICHAEL J. LEE

Ciba-Corning Diagnostics Corp., Medfield, MA 02057

Received December 20, 1988; Accepted March 24, 1989

ABSTRACT

Antibodies and antigens can be covalently coupled to a variety of carriers, both organic and inorganic. The methods for coupling these proteins may be found scattered throughout the technical literature. This report, although it concentrates on inorganic supports, describes several of the more successful methods used in laboratories today. Each of these methods is described in enough detail for the reader to carry out the coupling method of interest in his or her own laboratory. The coupling methods have been divided into two groups, direct and silane. Under each of these general headings are described the specific methodologies.

Index Entries: Antibody; antibodies; protein; coupling; solid-phase; silane; alkylamine; inorganic support; porous glass; magnetic support; immobilized antigen; immobilized antibodies.

INTRODUCTION

There is no universal method for preparing immobilized antibodies. There is no universal carrier and there is no ideal format for their application. Therefore, the support, method of immobilization, and formatting must be chosen by the dictates of the application intended. If the material is designated for an affinity chromatography application or as a particulate in an immunoassay kit, the materials may vary.

*Author to whom all correspondence and reprint requests should be addressed.

This review is not designed to explore all the methods of antibody immobilization, nor is it designed to examine all forms of support materials. We will only consider inorganic supports and covalent attachment.

Inorganic supports can be classified into a few major categories: metals, metal oxides, ceramics, and glasses. Typical examples of supports that fit these categories are:

Bentonite
Colloidal silica
Glass particles
Controlled pore glass
Alumina
Controlled pore titania
Nickel oxide
Iron oxide
Controlled pore zirconia
Charcoal
Hydroxyapatite

Properties of Supports

Porous Glasses

There are compositions of borosilicate glasses that after heat treatment can be leached to form porous glass frameworks (*1*). During heating the glass forms two phases, one is a silica and the other is rich in boric acid and therefore acid soluble. This phase may be leached from the glass leaving behind a porous framework of high silica content. The pore diameters of the glass at this stage are 30 to 60°A and the pore volume is approximately 28% of the total volume of the material.

Larger pores can be formed in the same glass compositions by mild caustic treatment after the acid leach (*2*). This will enlarge the pore diameters. By careful control of the chemical parameters and treatments one can prepare glasses that exhibit narrow pore size distributions and various pore size and pore volume ranges (Table 1).

The durability of controlled pore glass is a function of the temperature, time, solution composition, volume, pH, glass composition, and surface area. Since the glass is 96% silica it exhibits a high degree of durability but its high surface area contributes to a higher than normal solubility.

Generally, one can expect solubility of these materials to increase by a factor of 1.5 for every 10°C increase in temperature. Even so, these materials, when crushed and sieved to the desired particle size range, make an outstanding support for antibody immobilization.

Porous Silica, Titania, Alumina, and Zirconia Bodies

The methods of preparation of these materials are similar and based on methodology devised by R. A. Messing (*3*). The resultant porous cer-

Table 1
Controlled-Pore Glass Physical Properties

Pore diameter, A	Pore volume, SA[a] (M^2/g) at PV=0.70	cc/g, 1.00
75	249	356
125	149	214
175	107	153
240	78	111
370	50	72
700	27	38
1250	15	21
2000	9	13

[a]SA represents the surface area. PV reprecents the Pore volume in cc/g. The data shows the surface area of the particles at 0.70 and 1.00 cc/g.

amic bodies have pore diameters ranging between 100–1000°A with pore sizes +/−10% of the average value.

The process uses a liquid binder with an inorganic oxide to yield a mixture of uniform consistency. The mixture is dried and fired below the sintering temperature of the metal oxide. Typical binders include: water, acetic acid, sodium acetate, formic acid, propionic acid, and magnesium acetate.

Another type of porous ceramic uses hydrogels prepared by acidification of alkaline silica solutions (4). The hydrogel containing ammonia or an amine is autoclaved to a temperature where the media become vaporized and produces pores. The average pore diameters produced by this method range between 300–1200°A with an average pore volume of 1.0 cc/gm.

There are several other methods for the preparation of porous inorganic materials. All of these processes require some form of particle association through a binder followed by firing or precipitation and drying. The resultant particles all have another property in common; they have available oxide or silanol groups on their surfaces. These chemical groups can be used for the covalent attachment of antibodies to the particle surface.

Iron and Nickel Oxides

Iron oxide can be prepared by several methods. One method involves the use of commercially available iron oxide powders. These powders can be silanized directly and used for the attachment of antigens or antibodies. The particles are rather large and have a tendency to clump, particularly after they have been in contact with a magnetic field, since they retain some residual magnetism. Paramagnetic particles are also available commercially. These particles are prepared by a precipitation process that produces material having an average particle size of less than one micron.

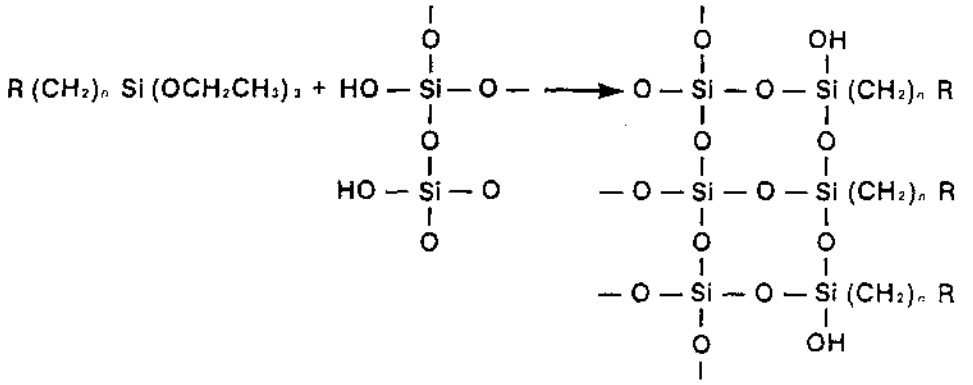

Fig. 1. Schematic representation of silane coupling to an inorganic support. In this example, gamma-aminopropyl-triethoxysilane has been used.

The particles can be purchased already silanized (Advanced Magnetics Corp., Cambridge, MA). These materials have been successfully used for immunoassays.

Latex particles impregnated with iron oxide are also commercially availfrom Seradyn Inc. (Indianapolis, IN). These materials show paramagnetic properties and have also been successfully used for immunoassays.

Nickel oxides produced by precipitation or directly out of the chemical supply house have been used in a manner similar to the iron oxides. As with the iron oxides, they can be silanized directly or entrapped in latex particles to confer magnetic properties to the latex.

Preparation of Silanized Carriers

The inorganic carrier is treated with an organosilane containing an organic functional group at one end and a alkoxysilyl group at the other end of the molecule. Coupling of the silane to the carrier is via the alkoxysilyl group of the coupling agent and the metal oxide or silanol group on the support. The general reaction scheme is shown in Fig. 1.

Typical organosilanes commercially available include epoxy, vinyl, aminoalkyl, sulfhydryl, haloalkyl, and aminoarylsilanes. The silane can be attached to the support in either organic water immiscible solvents (benzene, toluene, xylene) or in aqueous solutions. Silanes can be attached by evaporation of the organic solution leaving the silane behind or by heating, refluxing, or even allowing sufficient time for reaction at room temperature.

There are several general considerations that one must always remember. Since inorganic particles are friable materials, one should never use a stirring bar when working with these materials. Stirring in this manner will "grind" the particles. Particles can be separated for washing or assay by several convenient methods. These include: filtration, centrifugation,

settling, aspiration, or magnetic separation. Several of these methods can be utilized for the further sizing of inorganic particles particularly when clumping has occurred or when one wants to utilize only a specific size range of particles. Particles may be sized on the basis of settling times, centrifugation speed or filter porosity. One must decide on the best method generally by considering what particle range is desired and choosing the method most likely to yield the desired particle range.

Organic Silanization

If it is possible, the support material should be cleaned. This can be accomplished by either heating to a temperature that will burn off any organics adsorbed to the support or it can be carried out by boiling in a 5% nitric acid solution for 45 min, followed by exhaustive washing to remove any residual acid. The product can be dried and carefully stored. The procedure described here for an aminosilane can be used with any methoxy or ethoxy silane. For chlorosilanes, organic silanization procedures must be utilized.

To one gram of clean inorganic support material add 75 mL of 10% gamma-aminopropyltriethoxysilane (or any other desired silane) dissolved in toluene or similar solvent. The preparation is refluxed for 12–24 h, washed with toluene, followed by acetone. The product is air dried. Stability can be improved by heating the dried product to 110°C for 4–8 h.

Aqueous Silanization

This procedure, although it does not produce as many functional groups, does appear to produce a product with more even distribution of silane, which has shown greater stability over long term continuous usage in aqueous environments.

The carrier is first cleaned as previously described. If the support has been burned out, it is necessary to rehydrate the surface. This is accomplished by boiling in distilled water for 30 min to an hour before carrying out the silanization. If the product has been cleaned by the nitric acid wash it is not necessary to dry the material prior to use, it may be silanized directly after a thorough wash. The washed carrier is suspended in a 10% solution of silane dissolved in distilled water. The volume of solution is not important as long as there is sufficient volume to completely cover all the support material. The pH is lowered to pH 4.0 with HCl, and the mixture heated in a water bath at 75°C for 3–4 h. The product is filtered or centrifuged down, and washed with a volume of water 2× that used for silanization. It is dried overnight at 110–115°C. With submicron magnetic particles, the drying step should be avoided. Drying these samples will cause irreversible clumping. When the protein is coupled these materials may need extensive "cure." This is accomplished by placing the coupled support material in a water bath at 56°C for several hours or until no protein can be detected in the supernatant solution.

Silane Evaporative Coupling

The support material previously cleaned as described, and also dried, is added to enough silane dissolved in acetone or other volatile solvent that is nonreactive with the silane, to cover the particles thoroughly. Generally a 1% solution is sufficient for the coupling. The solution is evaporated to dryness leaving behind the silane adsorbed and reacted to the support. The support must be washed with the same solvent to remove unreacted silane and dried. The method is simple and requires no special equipment.

Carrier Activation (Direct, Non-Silane Methods)

Cyanogen Bromide (7)

To one gram of support material suspended in 5–10 mL of water, cooled to 4°C is added 250 mg of solid cyanogen bromide. The solid should be added slowly and the pH of the suspension maintained between pH 10 and 11 with NaOH solution. After the cyanogen bromide has been added the suspension is allowed to stand in the ice bath for an additional 30 min. The product is washed with distilled water until the pH of the water no longer changes and the odor of the cyanogen bromide is undetectable. Be sure to carry out all procedures in a working fume hood, since the cyanogen bromide is a very toxic compound. The washed support is now ready for immediate coupling to protein. The mechanism of action may be similar to that observed with conventional hydroxy-containing compounds that give reactive imidocarbonates with cyanogen bromide. However, in this case the hydroxyl groups are in the form of silanol residues.

Aldehyde Derivative

A method of preparing an active aldehyde has been described by Bursecz (5). The product is prepared by mixing 6 mL of a 2% aqueous solution of epiamine/g of support for 1 h. The epiamine is prepared by heating ethylenediamine to 100–110°C and adding slowly with stirring epichlorohydrin homopolymer of 2500 MW at a rate of 1 mole chloromethyl group to 6 moles ethylenediamine. This is heated for 3 h with stirring. The mixture is neutralized with NaOH. The product is filtered and the excess ethylenediamine removed by vacuum distillation. After reaction of the support with the epiamine, it is filtered and air dried. The product can then be reacted with glutaraldehyde by using a 1% solution for about 1 h. The final activated support can be washed with distilled water, air dried, and stored.

A simpler method is to prepare a solution of polylysine (6) at a ratio of 100 mg/g of support. This is allowed to adsorb at room temperature in a 10 m*M* phosphate buffer at near neutral pH overnight. The product is washed with distilled water. At this point, the support can be dried and stored.

Silane Activation and Coupling

The previous sections of this article have described some of the many types of inorganic support materials available, and the means by which the surfaces of these supports may be derivatized with reactive groups to provide attachment points for antigens or antibodies. For the purposes of this present discussion, we will assume that these latter molecules are all proteins, and, therefore, this section will deal with the principal methods of coupling proteins to the derivatized supports.

Protein molecules contain several types of reactive groups that can be used to form covalent bonds to solid supports. The most frequently used are the amino groups, followed by sulfhydryl, carboxyl, and aromatic groups. In addition, as described above, the functional group on the solid support can be (among others): an aliphatic amine, a sulfhydryl, an aromatic amine, or an epoxy group. Consequently, a considerable variety of methods is available for the coupling of proteins to supports. The methods vary in complexity from a simple one-step process to sequences involving three or more steps.

In one-step methods, the primary derivatization of the solid support is with a group that can react directly with proteins. Examples of such groups are oxiranes (epoxides) and imidocarbonates.

Two-step methods are the most common, and either employ an initial activation of the functional group on the solid support so that it can react with a protein, or they involve the preliminary attachment of a bifunctional "linker" substance to the solid support, followed by coupling of the protein to the linker. The linker may have the same reactive function on each end ("homobifunctional") or the two ends may have different functional groups ("heterobifunctional"). In either case, the group on the end that is not attached to the solid support is capable of reacting directly with a protein. Alternatively, the linker may be attached first to the protein. In this case it must be of the heterobifunctional type, since the free end must not be capable of reacting with a second protein molecule, but only with the solid support.

In three-step methods, the functional group on the solid support is first coupled to a linker molecule that is not in itself capable of reaction with a protein, but that must be further modified. Alternatively, the protein may be modified so that it will react with the linker attached to the solid support (but not with another protein molecule).

For any given functional group on the solid support, the method of choice will depend on the nature of the protein. For example, with some antibodies it may be found that coupling through an amino group on the immunoglobulin molecule decreases the binding affinity, whereas coupling through a sulfhydryl has little effect on the activity.

Examples of several useful coupling procedures are presented below. They are arranged according to the functional group on the solid support, and the functional group on the protein.

General Considerations

The ratio of protein to solid support given in the following descriptions is that generally used in the authors' laboratories for particles of diameter approximately one micron. For larger particles, i.e., those with lower surface area per gram, the ratio may be decreased. After coupling, solid supports are washed several times in protein-containing buffer (usually phosphate buffered saline, with 0.1% bovine serum albumin (BSA) and 0.1% sodium azide), and stored in the same buffer at 4°C.

Alkylamine Solid Support to Protein Amino Groups

Two-step methods using homobifunctional linkers (both ends react with amino groups) are presented first. The third method is a three-step procedure that consists of first introducing carboxyl functions onto the support, then converting these to active esters that can in turn react with amino groups on the protein.

GLUTARALDEHYDE

To 100 mg of solid support material is added 10 mL of 2.5% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.0. The reaction is allowed to continue to 1 h. The product may take on a brown or magenta color, particularly if the glutaraldehyde is technical grade.

The product is washed repeatedly with buffer, until it has no odor of glutaraldehyde, and then used immediately for coupling. Coupling to proteins is effected by incubation of the solid support with a solution of 15 mg of protein in 10 mL of 0.1 *M* phosphate, pH 7.0, with gentle agitation, for 3–15 h at room temperature. This is followed by washing 3 times with phosphate-buffered saline containg 0.1% BSA (PBS/BSA) (Fig. 2).

DISUCCINIMIDYL SUBERATE

One hundred milligrams of solid support is suspended in 10 mL methanol containing 10 mg of disuccinimidyl suberate (Pierce Chemical Co., Rockford, IL). The mixture is shaken for 1 h, and the product is then washed twice with 10 mL of methanol per wash. The particles are suspended in a solution of 15 mg protein in 0.1 *M* phosphate buffer, pH 8.0, and gently agitated for 1–2 h at room temperature. Finally, the particles are washed three times with PBS/BSA and stored in the same buffer (Fig. 3).

This method may be preferable to the glutaraldehyde procedure if a longer "spacer" is required between the solid support and the protein. Both methods are very gentle and rather simple to master.

SUCCINYLATION: ACTIVE ESTER FORMATION

One gram of alkylamine support material is suspended in 100 mL of 0.05 *M* sodium phosphate buffer, pH 6.0, and 0.3 g of succinic anhydride is added. After shaking for 15 h at room temperature, the solid is washed three times with 100 mL of water, twice with 100 mL of methanol, and once with 100 mL of dioxane. The particles are then suspended in a solution of

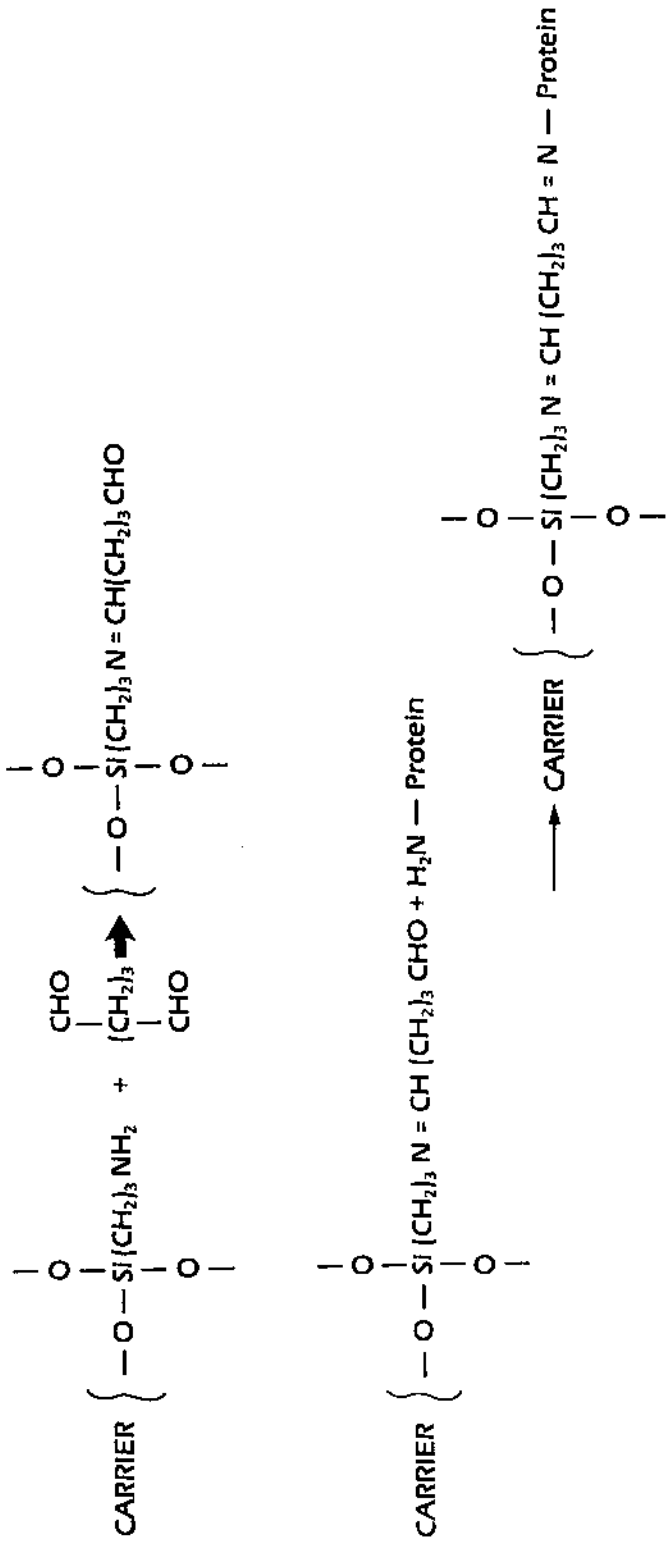

Fig. 2. The coupling reaction between an alkylamine support and glutaraldehyde and coupling to protein.

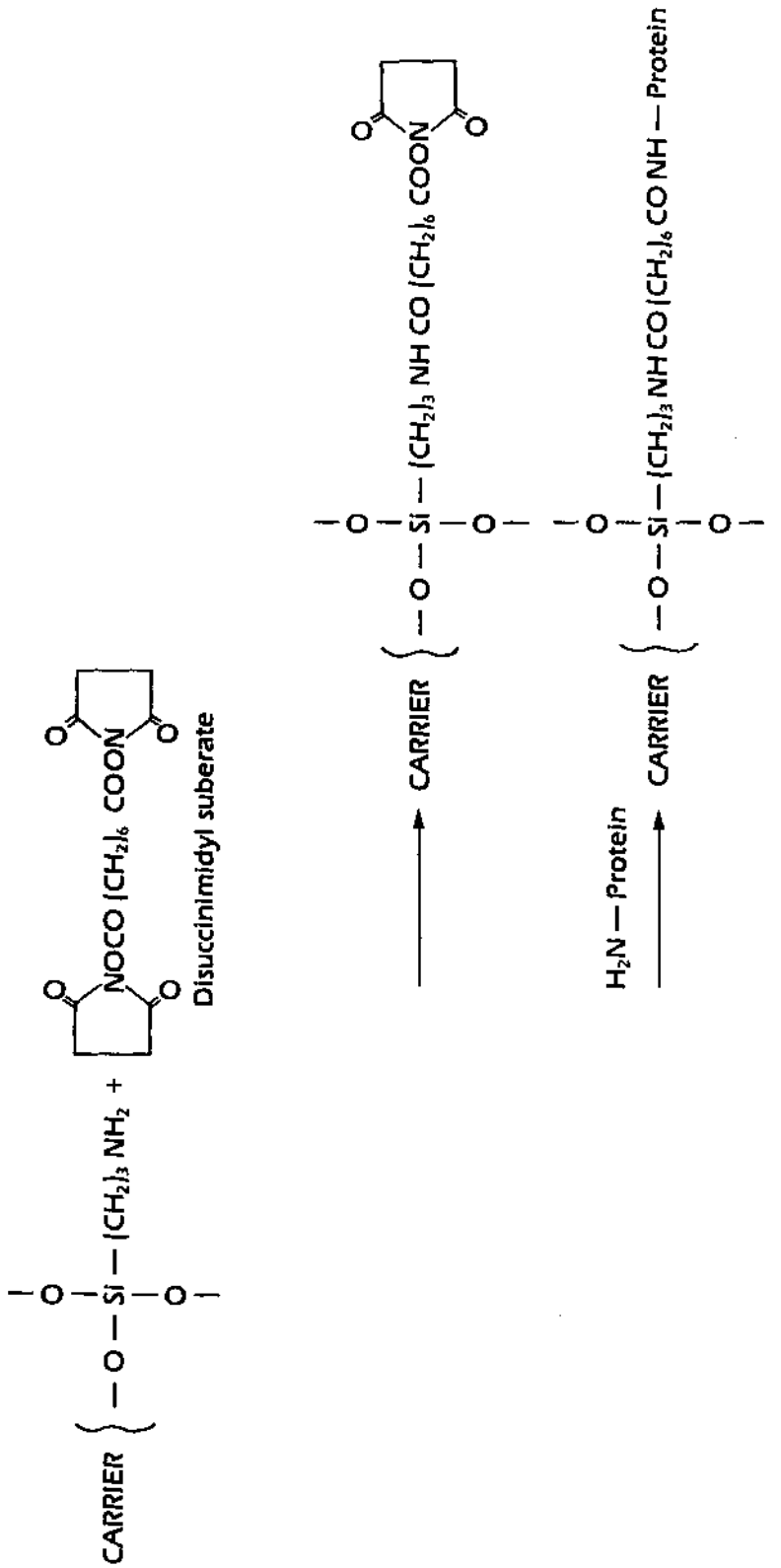

Fig. 3. Coupling of dissuccinimidyl suberate to an amino-carrier and subsequent coupling to protein.

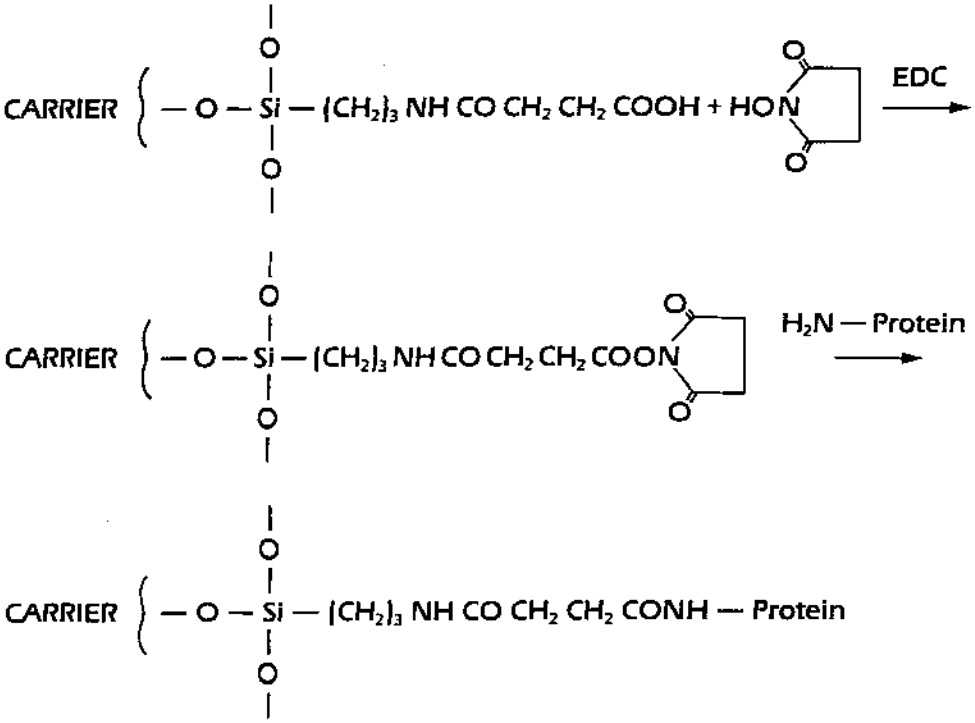

Fig. 4. Coupling of a carboxy carrier to *N*-hydroxysuccinimide using a carbodiimide coupling reagent with subsequent coupling to protein via the active ester.

1.2 g *N*-hydroxy-succinimide (NHS) and 1.9 g 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC) in 100 mL of dioxane and shaken for 90 min at room temperature. The product is washed twice with 100 mL of dioxane, and twice with 100 mL of methanol. The particles may be stored at this stage in 10 mL of dioxane in a tightly capped container at room temperature.

For coupling to protein, 1 mL of the dioxane suspension, containing 100 mg of active ester particles, is added to 15 mg of the protein dissolved in 10 mL 0.1 *M* phosphate buffer, pH 8.0. The mixture is gently agitated for 1–2 h at room temperature. The solid support is then washed 3 times with PBS/BSA, and stored in the same buffer. Any active ester groups not coupled to antibody will be deactivated by coupling to the BSA in the storage buffer (Fig. 4).

This is a method of coupling proteins via their amino groups under mild conditions, using an active intermediate that can be prepared beforehand and stored for a few weeks, provided that it is protected from moisture.

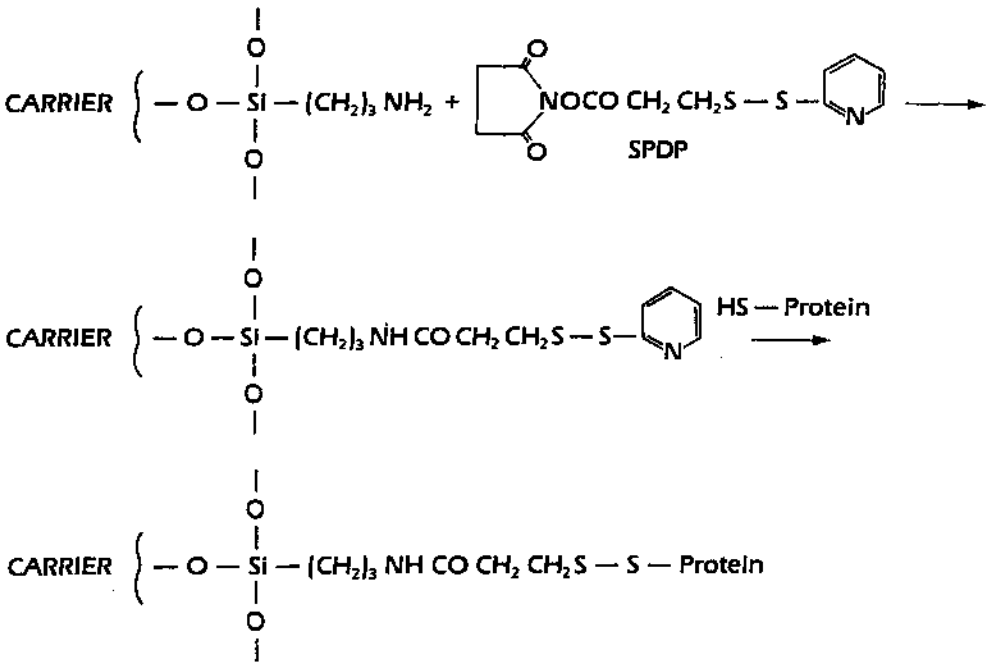

Fig. 5. Coupling to an amino support via activation with SPDP.

Alkylamine Solid Support to Protein Sulfhydryl Groups

These methods use heterobifunctional linkers. (One end reacts with amino groups on the solid support and the other with sulfhydryl groups on the protein.)

SUCCINIMIDYL 3-(2-PYRIDYLDITHIO)PROPIONATE (SPDP)

The solid support (100 mg) is suspended in 10 mL of 0.1 M phosphate buffer, pH 8.0, to which is added dropwise 10 mg of SPDP in 1 mL of methanol. The suspension is agitated at room temperature for 1 h. The particles are then washed three times with 10 mL of 0.1 *M* phosphate, pH 7.4, containing 1 m*M* disodium EDTA. The final wash is decanted, and the sulfhydryl-containing protein (15 mg) is added in 10 mL of the phosphate-EDTA buffer, and the mixture is gently agitated for one hour. The particles are then washed and stored in PBS/BSA (Fig. 5).

SUCCINIMIDYL 4-(*N*-MALEIMIDOMETHYL)
CYCLOHEXANE-1-CARBOXYLATE (SMCC)

The solid support (100 mg) is suspended in 10 mL of 0.1 *M* phosphate buffer, pH 7.0, and the mixture warmed to 30°C. To the stirred suspension is slowly added 10 mg SMCC in 1 mL of dioxane. Stirring is continued for 2 h, then the particles are washed three times with 10 mL of phosphate buffer, pH 7.4, containing 1 m*M* disodium EDTA. The final

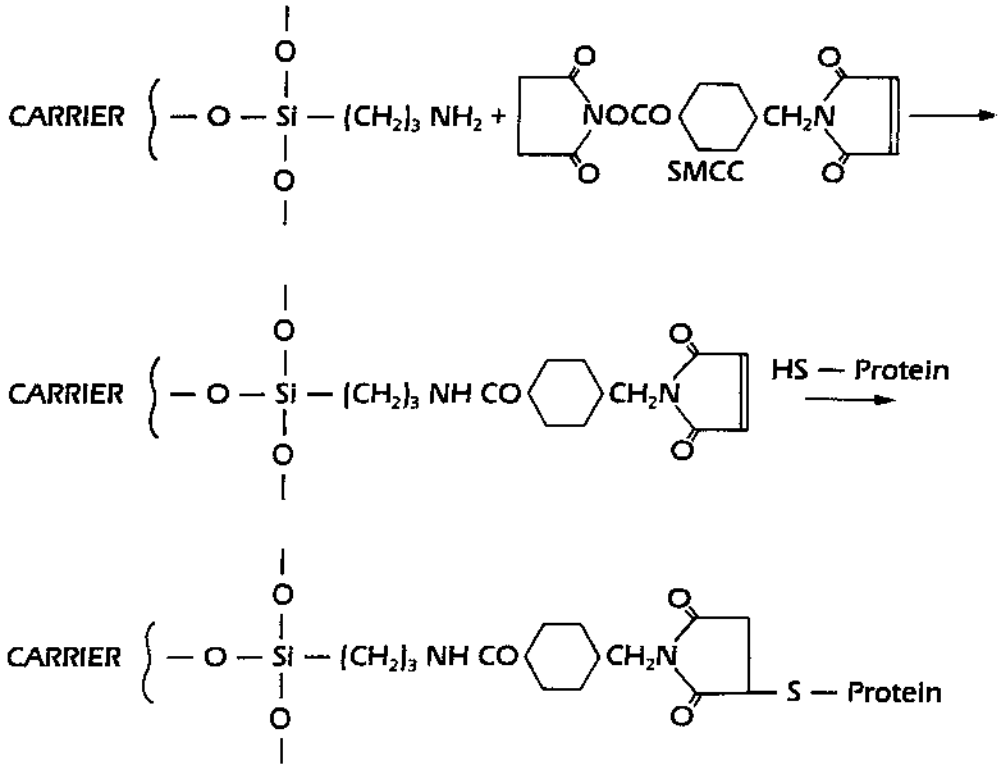

Fig. 6. Coupling to an amino support by initial activation with SMCC.

wash is decanted and the sulhydryl-containing protein (15 mg) is added in 10 mL of the same buffer and the mixture gently agitated for 1 h. The particles are washed and stored in PBS/BSA (Fig. 6).

The advantage of this method over the one using SPDP (above) is that in this case, the coupling to the protein is via a thioether bond. This is more stable than the disulfide bond, which is susceptible to reductive cleavage by active thiols. However, the maleimido intermediate, prior to coupling, is less stable than the pyridyldithio intermediate.

Arylamine Solid Support to Various Protein Functional Groups

DIAZOTIZATION

This is a method that couples principally to tyrosine residues in the protein, but histidine, lysine, cysteine, tryptophan, and arginine also react to a lesser extent. It is a somewhat complicated procedure (4 steps) but may yield an active product in cases where the glutaraldehyde method does not work.

The aminoaryl support material is prepared by the following two-step procedure: To 5.0 g of carrier is added 50 mL of chloroform and triethyla-

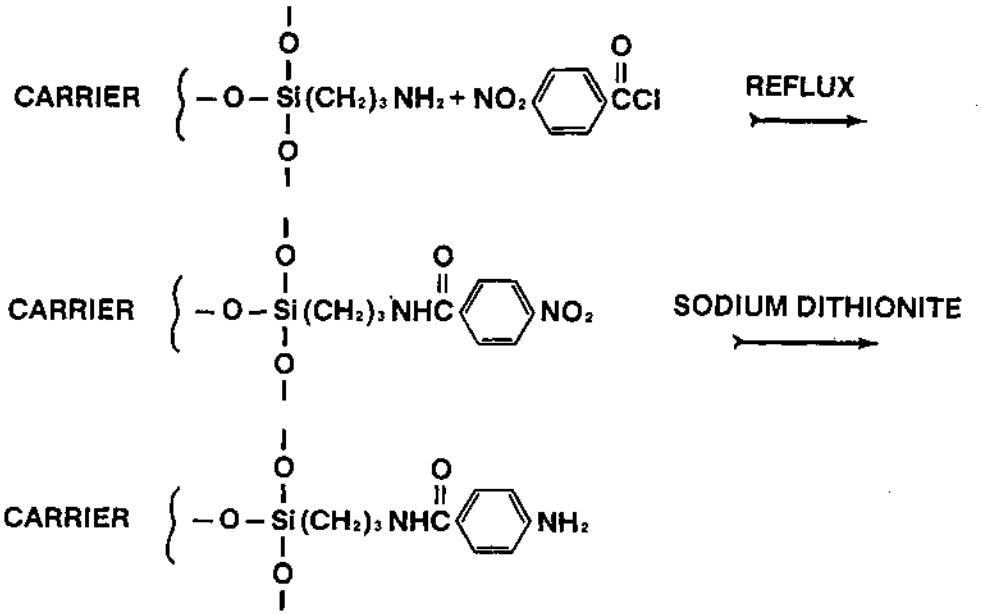

Fig. 7. Preparation of an aminoaryl support material by coupling the amino group to *p*-nitrobenzoylchloride followed by reduction.

mine (10% v/v). To the suspension is next added 0.5 g of *p*-nitrobenzoylchloride. The reactants are refluxed overnight, then the solid is washed with chloroform and allowed to air dry. Reduction is accomplished by boiling the carrier in a 10% solution of sodium dithionite in enough water to well cover the product. After 30 min of boiling, the support is separated while hot, and then exhaustively washed with 0.01 *M* HCL until the odor of the dithionite can no longer be detected. The product is finally washed with distilled water until the pH is near neutral. The support can be stored at this point either in distilled water or dry (Fig. 7).

Diazotization is carried out as follows: One hundred milligrams of aminoaryl solid support is suspended in 10 mL of 2 *N* HCL, and cooled to 4°C in an ice bath. The mixture is then gently shaken, and 1 mL of 1% sodium nitrite is added. The presence of free nitrous acid is verified by the use of pH paper and starch-iodide paper. After 30 min, excess HNO_2 is destroyed by adding solid urea or sulfamic acid until the starch-iodide paper no longer shows a purple color when a drop of the reaction medium is placed on the paper. The particles are then washed with ice-cold distilled water until the pH is close to neutral. Successful activation of the support can be verified at this stage by adding a small sample to a saturated solution of beta-naphthol in 0.1 *M* borate buffer, pH 9.0. The solid support material should immediately become bright orange in color.

The activated particles are added to an ice-cold solution of 15 mg of the protein in 10 mL of 0.1 *M* borate buffer, pH 8.0, and gently agitated

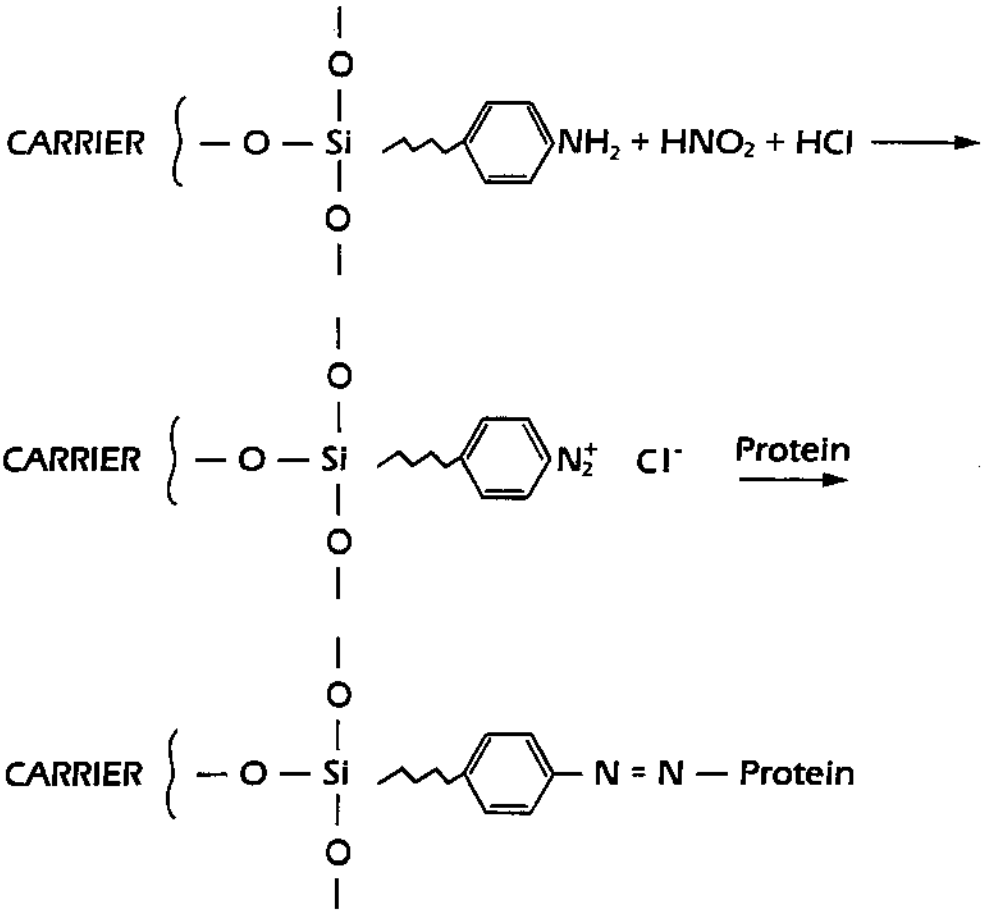

Fig. 8. Activation of an arylamine to form the diazonium group followed by coupling to protein.

for 15 h at 4°C. After this, the protein solution is replaced by a solution of 1 g glycine in 10 mL borate buffer, and incubation continued for 1 h at room temperature to inactivate any remaining diazonium groups. The solid is washed three times with PBS/BSA and stored in the same buffer (Fig. 8).

Sulfhydryl Solid Support to Protein Sulfhydryl

The first method described below is a two step method using a homobifunctional linker. The method presented after that consists of a preliminary activation of the solid support sulfhydryl, so that it can react with the protein sulfhydryl.

Underivatized sulfhydryl solid supports are susceptible to oxidation on storage, and need to be reduced before carrying out the reactions de-

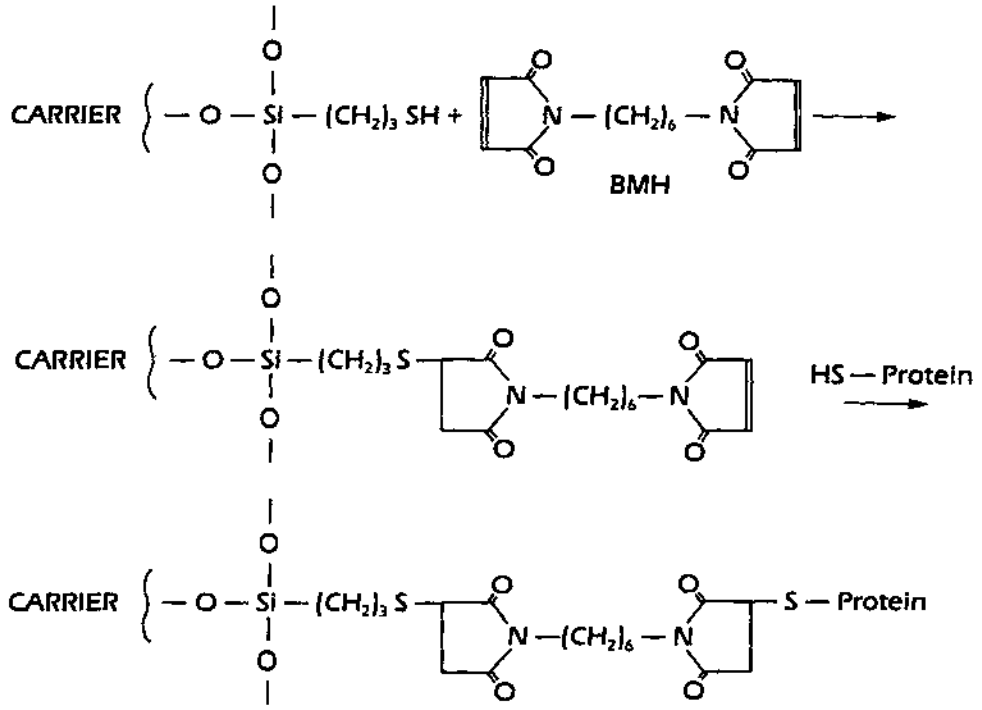

Fig. 9. Coupling of a reduced sulfhydryl support to protein through activation with BMH.

scribed below. In order to reduce the material, it is suspended in a solution of dithiothreitol (DTT), (0.03 *M*) in 0.1 *M* Tris/HCL, pH 8.0, containing 1 m*M* EDTA, and shaken for 30 min. It is then extensively washed with the same buffer (without DTT) until all the DTT has been removed. This may be ascertained by the lack of a reaction of the wash solution with 2,2′-dipyridyl disulfide (DPDS). If DTT is present, the addition of 1 mL of 1.5 m*M* DPDS to 2 mL of the wash will give an extinction reading at 343 nm, the characteristic absorbance wavelength of 2-thiopyridone.

1,6-Bismaleimidohexane (BMH)

One hundred milligrams of fully reduced sulfhydryl solid support is added to 10 mL of 0.1M phosphate, pH 7.0, containing 1 m*M* disodium EDTA. Ten milligrams of BMH (Pierce Chemical Co., Rockford, IL) dissolved in 1 mL of acetone is added, and the suspension shaken for 1 h. The product is washed three times with 10 mL of the phosphate-EDTA buffer, and then suspended in a solution of 15 mg protein in the same buffer. Reaction is allowed to proceed at room temperature for 16 h. The solid is then washed with PBS/BSA and stored in the same buffer (Fig. 9).

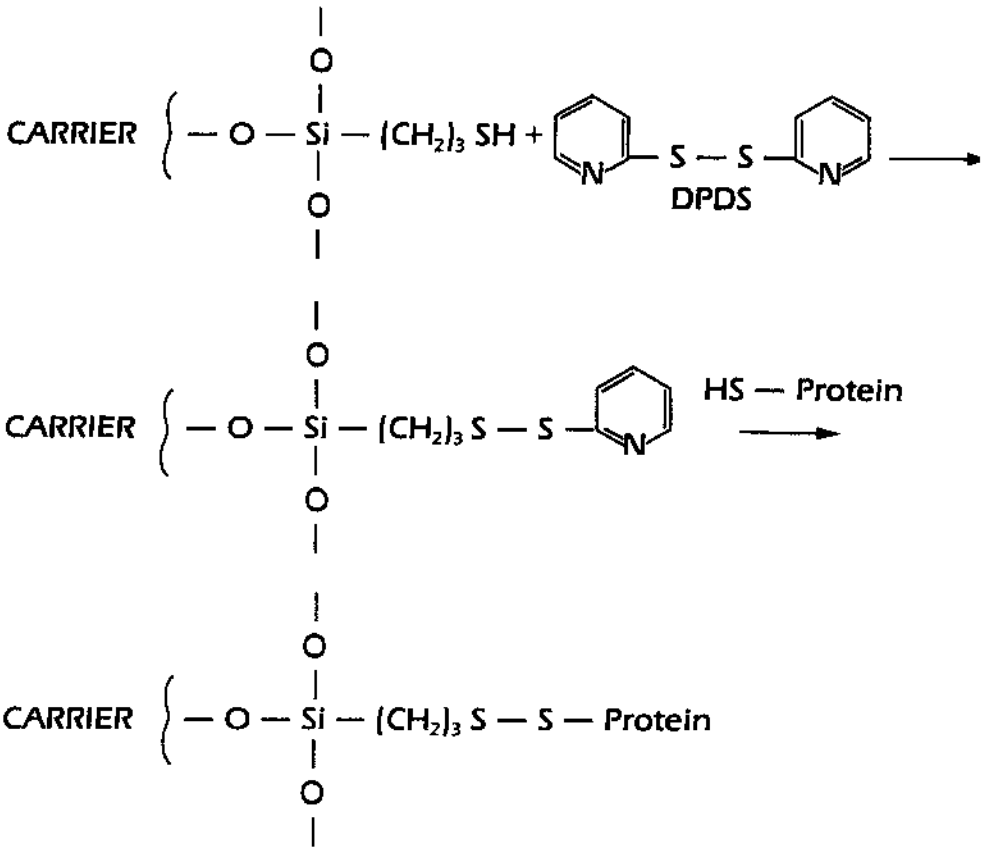

Fig. 10. Coupling of a fully reduced sulfhydryl support to protein through activation with DPDS.

ACTIVATION WITH 2,2'-DIPYRIDYLDISULFIDE (DPDS)

The fully reduced solid support (100 mg) is suspended in 10 mL of 0.1 *M* Tris/HCL buffer, pH 8.0, containing 1 m*M* EDTA and 1.5 m*M* DPDS. The reactants are shaken for 30 min then washed repeatedly with Tris/HCL, pH 8.0, 1 m*M* EDTA, until no DPDS can be detected by measuring extinction at 281 nm.

Next, the protein solution (15 mg in 10 mL of Tris/HCL/EDTA buffer) is added, and the mixture gently agitated for 1 h. Finally, the product is washed with PBS/BSA until no 2-thiopyriodone is detected by measuring extinction at 343 nm (Fig. 10).

Sulfhydryl Solid Support to Protein Amino

This method is for proteins with no sulfhydryl groups, and uses a heterobifunctional linker.

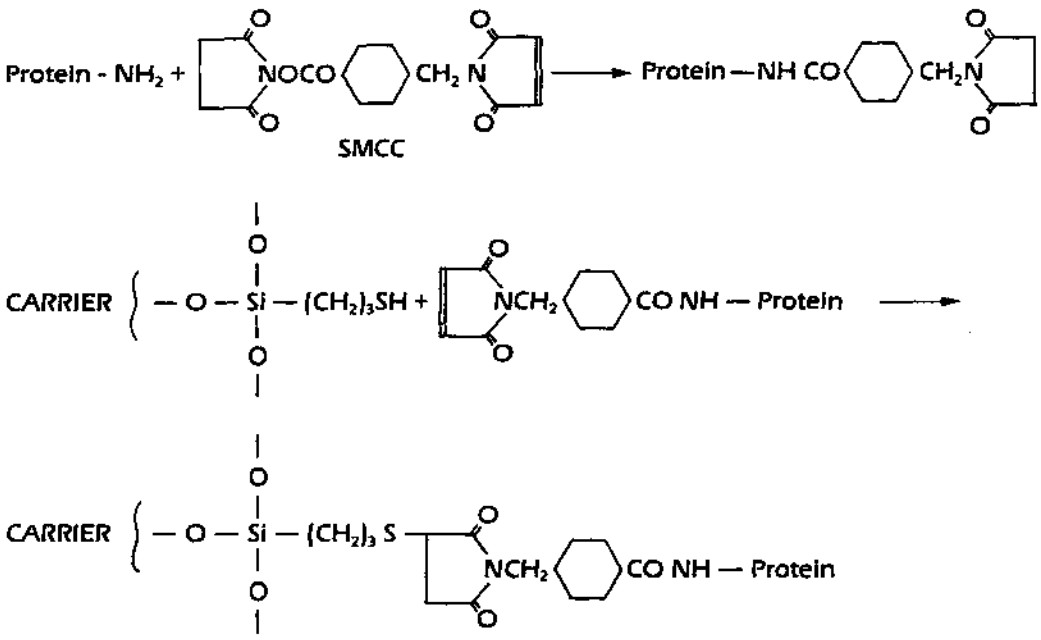

Fig. 11. Coupling of a protein to a sulfhydryl carrier via reaction with SMCC.

SMCC

Fifteen milligrams of the protein to be coupled is dissolved in 1.5 mL of 0.1 *M* phosphate, pH 7.0, and heated to 30°C. To this is added, dropwise, 2.5 mg SMCC in 0.15 mL of dioxane, with stirring. After 2 h, the solution is chromatographed on Sephadex G-25, using 0.1 M phosphate, pH 7.0, containing 1 m*M* EDTA. The protein containing fractions are combined, and the volume made up to 10 mL with the same buffer. To this is added 100 mg of reduced sulfhydryl solid support (*see above for details on preparing the reduced solid support*), and the mixture is gently agitated for 16 h. The product is washed with PBS/BSA and stored in the same buffer (Fig. 11).

Oxirane Solid Support to Protein Amino

The oxirane group itself can react directly with amino groups on proteins, in a one-step procedure.

One hundred milligrams of oxirane derivative is added to a solution of 15 mg of the protein to be coupled in 0.2 *M* carbonate/bicarbonate buffer, pH 9.0. The mixture is gently agitated for 48 h at room temperature. The solid is then washed in PBS/BSA (Fig. 12).

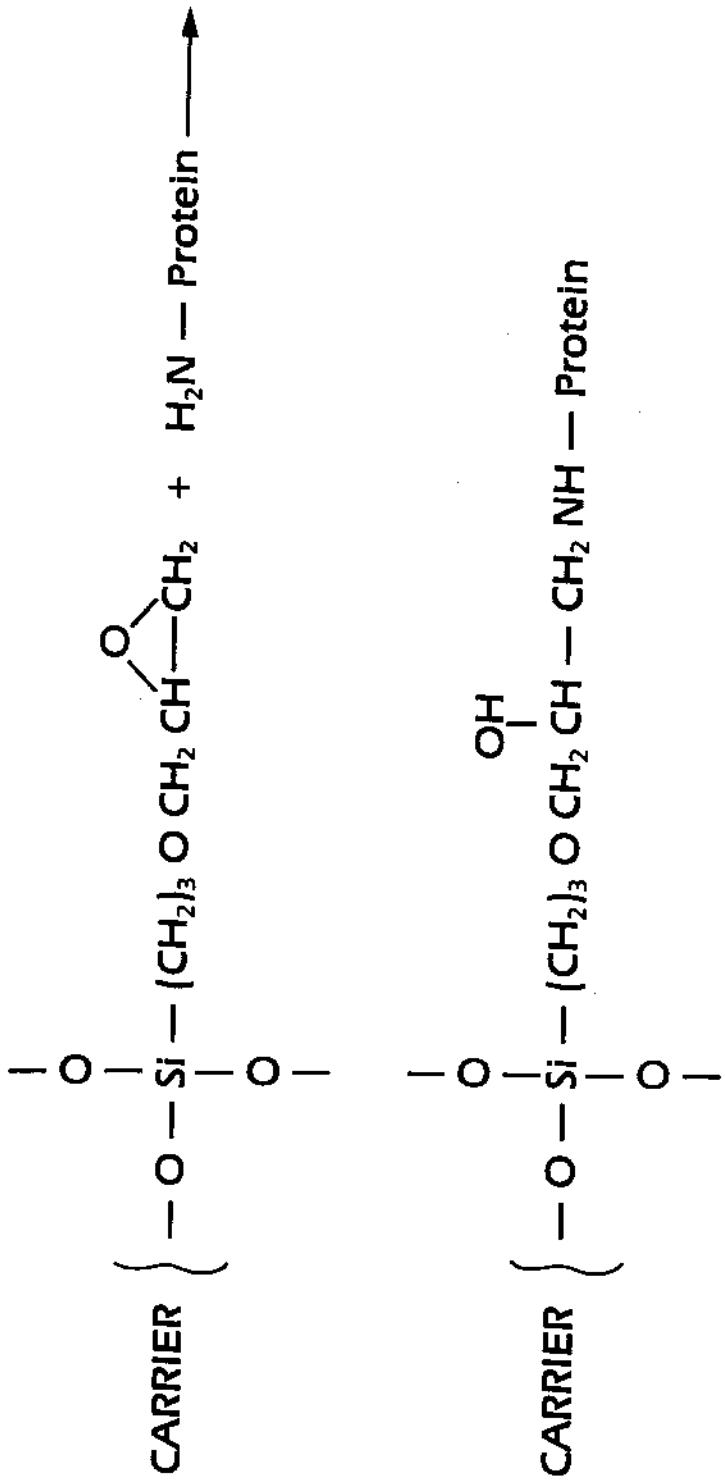

Fig. 12. Coupling of protein to an oxirane (epoxide) activated support material.

REFERENCES

1. Nordberg, M. E. (1944), *J. Amer. Ceram. Soc.* **27,** 299.
2. Hood, H. P. and Nordberg, M. E. (1940), US Patent No. 2,221,709.
3. Messing, R. A. (1975), US Patent No. 3,892,580.
4. Le Page, M. (1973), GR Patent No. 1,334,606.
5. Bursecz, C. F. (1983), US Patent No. 4,415,664.
6. Royer, G. P. and Green, G. M. (1975), *Biochim. Biophys. Res. Commun.* **44,** 426.
7. Weetall, H. H. and Detar, C. C. (1975), *Biotech. Bioeng.* **17,** 295.