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Activation of Sepharose with *N,N′*-Disuccinimidyl Carbonate

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ABSTRACT

A method is described for the activation of Sepharose with *N,N′*-disuccimidyl carbonate. The activated carbonate reacts smoothly with amine-containing ligands yielding stable carbamate derivatives.

Index Entries Activation, of Sepharose with *N,N′*-disuccimidyl carbonate; Sepharose, activation with *N,N′*-disuccinimidyl carbonate; *N,N′*-disuccinimidyl carbonate, activation of Sepharose with.

INTRODUCTION

We have recently described the activation of polysaccharides with *p*-nitrophenyl chloroformate and hydroxysuccinimide chloroformate (*1,2*). The activated polymers reacted with proteins and amino-containing ligands yielding stable and uncharged carbomate derivatives. Of the two activated polymers used, the hydroxysuccinimide-containing moiety was found to be more convenient and more efficient, since shorter reaction times were needed for coupling with amino groups and higher coupling yields were obtained (*1–3*). Another advantage over the *p*-nitrophenyl derivative is that no yellow color appears during the coupling reaction.

The major disadvantage is using the hydroxysuccinimide chloroformate is that it is unstable under normal storage conditions and is therefore not commercially available. Consequently, any laboratory that considers using these compounds for the activation of polysaccharide resins would have to prepare the hydroxysuccinimide chloroformate

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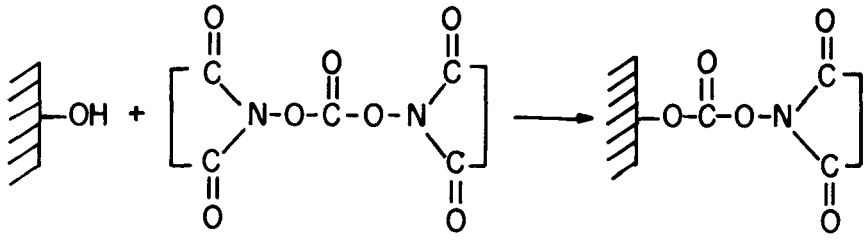
from phosgene and hydroxysuccinimide. Alternatively, a ready-made activated column containing the hydroxysuccinimide carbonate can be purchased commercially (Sigma Chem. Co., St. Louis, MO). In this case, however, the user is limited to a fixed amount of activation that is predetermined by the commercial source.

Because of the excellent performance of the hydroxysuccinimide carbonate columns for coupling amino-containing ligands, we look for an alternative method of their preparation.

In the following we describe the activation of hydroxyl-containing polymers (polysaccharides and others) with *N,N'*-disuccinimidyl carbonate (DSC).

DSC is a stable colorless compound commercially available (Polysciences, Inc.) and can be stored for years in a refrigerator (4). The reaction of DSC with hydroxyl containing polymers is shown in the following scheme:



EXPERIMENTAL DETAILS

In a typical procedure, Cl-Sepharose (10 g) is dehydrated (washed) successively with 100 mL portions of graded acetone–water mixtures (1:3, 1:1, and 3:1) culminating with dry acetone. If filtered, about 7.0 g of Sepharose treated under these conditions are equivalent to 10 g of wet (H_2O-swollen) gel. The filtered gel is resuspended in 10 mL dry acetone containing the required amount of DSC at 4°C. In this particular procedure, 800 mg DSC (representing about 300 μmol/g wet wt gel) were used. Under continuous stirring, a solution containing between 1.5 and 2 molar ratio (with respect to the desired amount of DSC) of either triethylamine (in this case, 0.75 mL triethylamine in 10 mL pyridine) or dimethylaminopyridine (650 mg in 10 mL acetone) is added slowly. The suspension is stirred for 1 h at 4°C. The reaction mixture is filtered on a sintered glass funnel and washed successively with solutions of cold acetone, 5.0% acetic acid in dioxane, methanol, and isopropanol. The methanol washings are checked for the presence of free reagents by diluting an aliquot with 0.25*M* NH_4OH and reading at 261 nm (E^{M}_{cm} = 10.000) (5). The activated gel (5.0 g filtered from isopropanol is equivalent to 10 g wet gel) is stable for several months at 4°C when stored in isopropanol.

Using this procedure and these quantities of reagents, gels containing between 25 and 35 μmol/g gel (10–15% yield of active hydroxy-

succinimide carbonates) are obtained (*5*). The amount of reactive groups can be further increased using more DSC, but the above-described levels of active groups are sufficient for most practical purposes.

By comparison, Sepharose was activated under the same conditions with *p*-nitrophenyl chloroformate and hydroxysuccinimide chloroformate. The resulting gels contained about 45 μmol/g gel.

Amino-containing ligands and proteins were coupled to the activated resins by reaction in 0.1–0.2*M* phosphate buffer (pH 7.5) or fresh solutions of 0.1–0.2*M* $NaHCO_3$ at 4°C for 4–16 h. Using a column containing 17 μmol/g wet gel of hydroxysuccinimide, 12 out of 15 mg trypsin added were coupled as determined by UV measurements of the unbound protein. Coupling of other ligands was performed in a manner similar to that described earlier (*1,2*).

CONCLUSIONS

The activation techniques described here can be used for both protein immobilization and affinity chromatography since stable and uncharged carbamates are the products. Among the other advantages of this method is the regeneration of the original hydroxyl groups from active moieties that have failed to couple the desired ligand.

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