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METHOD OF BINDING, BY COVALENT BONDS, PROTEINS AND POLYPEPTIDES TO POLYMERS USING CYANATES

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ABSTRACT OF THE DISCLOSURE

Organic cyanate compounds are employed for binding organic compounds containing a primary or secondary amino group to polymers containing one or more hydroxyl and/or primary and/or secondary amino groups. The invention is useful, among other things, for binding water soluble enzymes to water insoluble polymers while preserving the activity of the enzyme.

This is a continuation-in-part of Ser. No. 783,761, filed Dec. 13, 1968, and now abandoned.

The present invention is concerned with a method for binding, by covalent bonds, a polymer containing at least one group of the formula —XH, wherein —XH represents a member selected from the group consisting of hydroxyl groups, primary and secondary amino groups, and an organic compound containing at least one group of the formula —YH, wherein —YH represents a member selected from the group consisting of primary and secondary amino groups in which the polymer is reacted with a compound capable of forming a reactive derivative thereof, whereupon the derivative as formed is reacted with the organic compound.

Such a process is previously known. Organic compounds containing one or more groups of the formula —YH, that it is of special interest to bind to polymers, are particularly proteins, polypeptides, peptides, and amino acids and derivatives thereof. Examples of such compounds are enzymes, antibodies, protein and/or peptide hormones, antigenic proteins, allergens or haptens. An important example is antibodies which, for analytical determination, can be bound specifically to polymers by such a process. Other examples of such organic compounds are derivatives of carbohydrates, for example, polysaccharides containing an amino group of the formula —YH. Examples of polymers containing one or more groups of the formula —XH are polysaccharides such as dextran, cellulose, starch, dextrin and agar-agar (Sepharose®), copolymers of dextran with epichlorohydrin or derivatives of these compounds such as hydroxyethyl cellulose and 2-hydroxy-3-(4-amino-phenoxy)-propyl-substituted copolymers of dextran with epichlorohydrin. The polymer may also be polyaminostyrene.

Examples of compounds capable of forming a reactive derivative of the polymer as set forth above according to the prior are are agents for introducing azide groups, isocyanate groups, and diazonium groups. The preparation of the reactive derivative is often complicated and

the results are difficult to reproduce. It is also known to introduce reactive groups in a polymer containing one or more groups of the formula —XH by treating said polymer with cyanogen bromide. The latter compound is, however, very toxic, which represents a disadvantage when working on a technical scale.

Although the cyanogen bromide yields a reactive derivative, which presents excellent properties from several viewpoints, it must be held a disadvantage that it is difficult to vary the reactivity of the derivative by this method in view of what is desired in each special case.

It has now been found possible to avoid or essentially reduce the disadvantages above referred to. To this effect there is used an organic cyanate compound containing one or more cyanate groups as a compound capable of forming a reactive derivative of the polymer.

Preferred compounds are those of the formula

$$R(OCN)_x$$

in which R is an organic residue, for example, a halogenated alkyl group, a substituted aromatic group, a heterocyclic ring system or a cycloaliphatic group and x is the integer 1 or 2, preferably 1. Substituents in the aromatic groups are, for example, nitro, halogen such as chloro, alkyl such as methyl and tert.butyl, alkoxy such as methoxy.

The compound used according to the invention, which contains at least one cyanate group, can be of very different nature. Thus, it may be an aliphatic, alicyclic-aromatic or heterocyclic cyanate compound, substituted or unsubstituted. Examples of such compounds are β,β,β-trichloroethylcyanate, 1-adamantylcyanate, phenylcyanate, o-nitrophenylcyanate, p-nitrophenylcyanate, m-chlorophenylcyanate, p - methoxyphenylcyanate, o-tert. butylphenylcyanate, 2,2-dimethylphenylcyanate, 2,4,6-tritert.butylphenylcyanate, 2 - naphthylcyanate, 1,4-dicyanatobenzene.

As mentioned above the coupling process thus comprises two distinct steps. The first one of these, the preparation of the reactive derivative, is usually carried out in such a manner that the polymer containing one or more groups of the formula —XH is contacted with the organic cyanate compound in question. The reaction is carried out in an alkaline medium, for example in an aqueous alkaline medium. Most suitable pH-values are primarily those which lie between 7 and 13. The reaction can also be carried out in other solvents than water, such as those which are miscible with water, e.g. acetone and dioxane. Compounds which may suitable be used to adjust the desired pH-value or pH-interval may be sodium hydroxide, calcium hydroxide, sodium hydrogen carbonate, sodium carbonate, potassium carbonate, triethylamine.

It is relatively simple for a person skilled in the art to find the suitable pH-value or pH-interval because the pH is selected inter alia according to reaction temperature and reaction time. The reaction can thus be carried out at different temperatures such as in the range of from 0 to 50° C. and the reaction time can vary within broad limits, from a few minutes to hours. The formed reactive derivative of the polymer is suitably subjected to purification before being bound to the organic compound, for example by washing with suitable solvents, such as water and acetone. The second step of the invention, i.e. binding the reactive derivative to the organic