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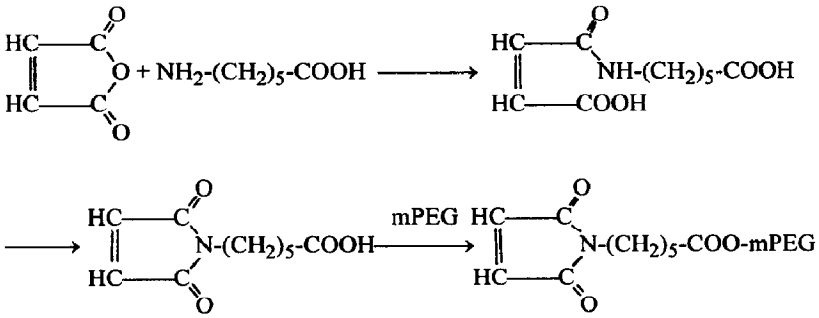
Preparation of a New PEGylation Reagent for Sulfhydryl-containing Polypeptide

Wei tang, Yuan Chang, and Xin-Yuan Liu

Shanghai Institute of Biochemistry, Academia Sinica, China
320 Yue-Yang Road, Shanghai, 200031

Abstract: N-maleimido-6-amino-caproyl ester of PEG was preparated in the presence of DCC etc., as a new sulfhydryl-PEGylation reagent substituded for mal-sac-PEG.

Recently, chemical modification, especially PEGylation of native or recombinant proteins has been widely applied for altering their potency. The bifunctional crosslinking reagent, N-maleimido-6-amido-caproyl ester of 1-hydroxy-2-nitro-4-benzene sulfonic acid (mal-sac-HNSA, (a)) can be used for conjugation of sulfhydryl-containing compound(protein, peptide etc.) to PEG-NH_2(b)[1-2]. However the synthesis of (a) is rather complicated and that of (b) is very difficult[3-7]. Here we describe a procedure without preparation of (a) and (b) to make a new activated PEG ester directly as below, which can be used for sulfhydryl-PEGylation of proteins or peptides.



(c) (d)

At first, maleic anhydride reacted with 6-aminocaproic acid for 4 hours at 25°C , then more acetic acid was added and refluxed under a Dean-Stark water separator over night. The acetic acid was removed in vacuo

and the oily residue containing (c) was dissloved in chloform, filtered and chmomatographed over a silica gel column in chloroform:acetic acid (95:5). Then the pure product (c) was obtained. Finally, the product (d) (mal-sac-PEG) can be made by the reaction of (c) with mPEG (MW5000) in methylene chloride, in the presence of 4-dimethylaminopyridine(DMAP) and N,N-dicylohexylcarbodiimide (DCCI), followed by purification by precipitation with ether, recrystallization from ethanol, and lyophization to give a powder. The total yield was 50-60%.

The data of UV-spectra show that : the yield for the attachment of the N-maleimido-6-amidocapoic acid to the PEG is >90%. The activated PEG ester is readily soluble in water or methylene chloride. It is stable for several months when stored at -20°C .

In a first example,bovine serum albumin(BSA) was modified with activated PEG(d).The BSA was treated with dithiothrcitol(DTT) previously and DTT was removed by a Sephadex G-25 column in 0.05M phosphate buffer (pH6.0), then mal-sac-PEG was added in excess, stirred at 25°C for several hours or over night. Results indicated that one or more PEG molecules could been bound to one BSA molecule.

In another example, the mutants of recombinant γ-interforon (rIFN-γ), i.c., 25Cys-rIFN-γ. were crosslinked to the activated PEG (d) as above. The yield was about 50-80%. The PEG-25Cys-rIFN-γ had full bioactivity relative to the unmodified one. The result will be shown in another paper.

In conclusion, a simple high yielding method for the preparation of a new sulfhydryl-PEGylation reagent is reported. It needs only 3-step reactions for the preparation of sulfhydryl--PEGylation reagent rather than 7-steps that reported in literature, and the difficult steps for making HNSA and PEG-NH2 were averted. Because the yield of the reaction of mal-sac-HNSA to the mPEG is not high, and the separation of the products is not easy, compared with the previous reagent , the purity of the new reagent is much higher and therfore can be directly used more efficiently for sulfhydryl-PEGylation of medically important proteins or peptides at one or more specific sites.

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