

PREPARATION OF BOLTON AND HUNTER'S REAGENT FOR LABELLING PROTEINS WITH IODINE-125

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The labelling of proteins with iodine-125 by conjugation method has been successfully applied by Bolton and Hunter /1/. They used in conjugation reaction the reactive ester - N-succinimidyl 3-/4-hydroxyphenyl/ propionate - previously labelled with iodine-125 in the benzene ring /2/.

Bearing in mind the increasing interest in this labelling method for radioimmunological and radioreceptor studies, work has been undertaken in our Laboratory on the method of production of Bolton and Hunter's reagent and on the technique of labelling proteins by conjugation on a commercial scale.

The synthesis of inactive N-succinimidyl hydroxyphenyl-propionate was carried out according to a general method of preparation of reactive aminoacid esters described by Anderson /3/. We found this method to be more suitable for preparation of crystalline product than that of Rudinger /4/ used originally by Bolton and Hunter.

The labelling of the ester with iodine-125 was carried out by means of the chloramine-T method. The yield of iodination was more reproducible, if the amounts of chloramine-T, sodium metabisulphite and potassium iodide were twofold greater than those used by Bolton and Hunter. The labelling time was 15 s instead of few seconds used by Bolton and Hunter. This did not decrease the labelling efficiency and permitted to perform the whole labelling procedure without undue hurry.

EXPERIMENTAL

Preparation of N-succinimidyl 3-/4-hydroxyphenyl/propionate.

3-/4-hydroxyphenyl/propionic acid /5 mM/ and N-hydroxy-succinimide /5 mM/ in tetrahydrofuran /5 cm³/ have been treated portionwise at -18 to -20° C with dicyclohexylcarbodiimide /5.5 mM/. The mixture was stirred magnetically at -18 to -20° C for 2 hours and then kept in a refrigerator for several hours. The precipitate of the urea derivative was filtered off and washed with ethyl acetate. The filtrate was dried over anhydrous MgSO₄ and concentrated to a volume of 3 cm³. n-Hexane was added until turbidity of the solvent was observed. After several hours the colourless precipitate was filtered off, washed with a mixture of ethyl acetate and n-hexane and dried in a vacuum desiccator over P₂O₅. About 3 mM of the ester /m.p. 116-126° C/ were obtained. The ester was stable for more than 6 months, when stored under water-free conditions.

Labelling of N-succinimidyl 3-/4-hydroxyphenyl/propionate with iodine-125.

The labelling was carried out in glass vessels 0.7-0.8 cm

high and 1 cm diameter. Per 0.5 μ g of the ester approx. 4 mCi Na^{125}I /20 μ l/, 200 μ g of chloramine-T in 40 μ l of 0.2 M phosphate buffer, pH 7.5, 600 μ g of sodium metabisulphite in 40 μ l of 0.05 M phosphate buffer, pH 7.5 and 800 μ g of potassium iodide in 40 μ l of 0.05 M phosphate buffer were used. The solution was stirred with magnetic stirrer. The metabisulphite was added 15 seconds after chloramine-T. After addition of KI the reaction mixture was immediately transferred to a multidose vial with benzene /2 cm³/ containing 0.05% of dimethylformamide and shaken. The benzene layer was dried over anhydrous MgSO_4 /0.25 g/ and dispensed in portions /2-3/ to multidose vials.

The radiochemical yield was 60-80% based on 12 experiments. The obtained ^{125}I - labelled ester showed the specific activity higher than 4 mCi/ μ g, i.e. 1500 mCi/ μ mole. The radiochemical purity of labelled ester exceeded 70%. The preparation was stored at 4° C as solution in benzene or as solid and was used for labelling proteins during 2 weeks following its production.

REFERENCES

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