PEPTIDYL-tRNA. IX. THE USE OF o-NITROPHENYLSULFENYL GROUP AS N-PROTECTING GROUP IN THE SYNTHESIS OF PEPTIDYL-tRNA*

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SUMMARY

The use of o-nitrophenylsulfenyl group (NPS) as N-blocking group in the synthesis of peptidyl-tRNA is demonstrated by the preparation of val-gly-[^{14}C]phe-tRNA. The synthesis involves a reaction between N-hydroxysuccinimide ester of NPS-val-gly and [^{14}C]phe-tRNA. The NPS group is removed from the N-blocked peptidyl-tRNA by treatment with 0.2 M sodium thiosulfate at 30° C for 2 hrs.

Peptidyl-tRNAs are considered intermediates in protein biosynthesis. The chemical synthesis of peptidyl-tRNA using aminoacyl-tRNA as starting material may be accomplished in three major steps: 1. The preparation of a suitable N-blocked amino acid with an activated carboxyl group; 2. Reacting the N-blocked carboxyl activated amino acid with aminoacyl-tRNA; 3. Removal of the N-blocking group from the peptidyl-tRNA. In several previous publications from this laboratory, the chemical synthesis of different oligopeptidyl-tRNAs has been reported (1-4). The synthesis is based on the reaction between a N-hydroxysuccinimide ester of N-monomethoxytrityl amino acid (or peptide) and aminoacyl-tRNA. The N-blocking group is removed after the acylation reaction by mild acidic treatment (5% dichloroacetic acid at 4° C for 5 min). The acylation reaction

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was found to be specific, and the tRNA was not affected neither by the acylation reaction nor by the acidic treatment used for the removal of the N-blocking group. Although the monomethoxytrityl group was used successfully as a N-blocking group in the preparation of different oligopeptidyl-tRNAs, it was found that in several cases (as in the case of N-hydroxysuccinimide ester of N-blocked phenylalanine or valine) the yield of the acylation was low, probably because of steric hindrance. Similar steric hindrance effects were previously observed when trityl group was used as a N-blocking group in peptide synthesis (5). In order to overcome these difficulties, Zervas, Borovas and Gazis (5) introduced the o-nitrophenylsulfenyl group (NPS) as N-blocking group in peptide synthesis. The NPS was removed from the N-blocked peptide by treatment with two equivalents of hydrogen chloride in ethanol (or nonpolar solvents). Other investigators used sodium thiosulfate (6), thiophenol or mercaptans (7) for removal of the NPS group from the N-blocked peptide.

In this communication we wish to report on the use of NPS as the N-blocking group in the synthesis of val-gly-phe-tRNA. NPS-val-gly was prepared according to Zervas, Borovas and Gazis (5). N-Hydroxysuccinimide ester of NPS-val-gly (III) was prepared by reacting NPS-val-gly (I) (4 mmoles) dissolved in ethylacetate (50 ml) with N-hydroxysuccinimide (II) (4 mmoles) in the presence of dicyclohexylcarbodiimide (4 mmoles). The reaction mixture was kept at room temperature overnight. The dicyclohexyl-urea which precipitated out was removed by filtration and washed with dimethylformamide. The combined filtrate and washing was evaporated to dryness under reduced pressure. The residue was dissolved in acetone (5 ml), then ether (100 ml) was added and the crystals obtained after cooling were collected by filtration (yield 75%, m.p. 155° C). The

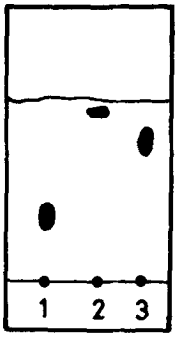


Fig. 1. Thin layer chromatography on microchromatoplates coated with silica gel by dipping. Solvent: ethylacetate (9)-dimethylformamide (1). The spots were made visible by charing with aqueous sulfunic acid 1:1 (v/v) or by iodine vapors. NPS compounds are yellow. 1, NPS-val-gly; 2, N-hydroxysuccinimide ester of NPS-val-gly; 3, N-hydroxysuccinimide.

product moved as a single spot in thin layer chromatography (Fig. 1) and the elemental analysis was: Cal.: N, 13.20%; S, 7.54%. Found: N, 12.94%; S, 7.05%.

The tripeptidyl-tRNA, val-gly[^{14}C]phe-tRNA, was prepared in the following way: [^{14}C]phe-tRNA (IV) (60,000 counts/min specific activity 470 mC/mmole) dissolved in 0.5 ml of 0.2 M veronal buffer pH 8.0 was added to a solution of N-hydroxysuccinimide ester of NPS-val-gly (III) (10 mg) in freshly distilled dimethylsulfoxide (2 ml). The reaction mixture was shaken in a Vortex test tube mixer for several min, then kept at 30° C for 2 hrs. Cold 10% dichloroacetic acid (2.5 ml) was added, and after 10 min at 0° C cold dimethylformamide (2 ml) was added. The tRNA was isolated by centrifugation (15,000xg for 20 min at 4° C). The precipitate was washed with cold ethanol (3 ml), dried in a vacuum desiccator and dissolved in 0.1 M acetate buffer pH 5.0 (0.06 ml). In order to remove the N-blocking group, a solution of 0.4 M sodium thiosulfate (0.06 ml) was added and the reaction mixture was kept for 2 hrs at 30° C. Then 10% dichloroacetic acid

(1 ml) and dry ethanol (3 ml) were added. The tRNA was isolated by centrifugation, the precipitate washed with 3 ml ethanol (or acetone-ether 1:1), dried in a vacuum desiccator, and finally dissolved in 0.1 M acetate buffer pH 5.0. An aliquot was treated with 0.5 M NaOH for 60 min at 30° C and the alkaline hydrolyzate was analyzed by paper electrophoresis. More than 98% of the radioactivity moved as val-gly-phe and none as phe (Fig. 2). When a sample of the peptidyl-tRNA which had not been treated with alkali was analyzed by paper electrophoresis all the radioactivity remained at the origin.

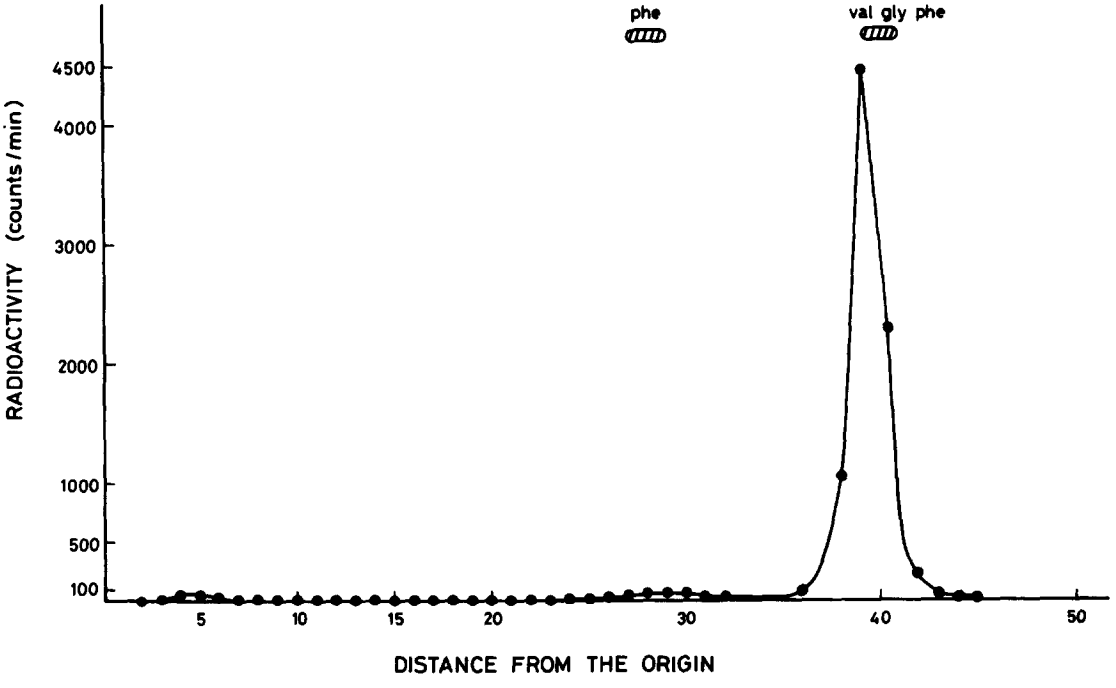
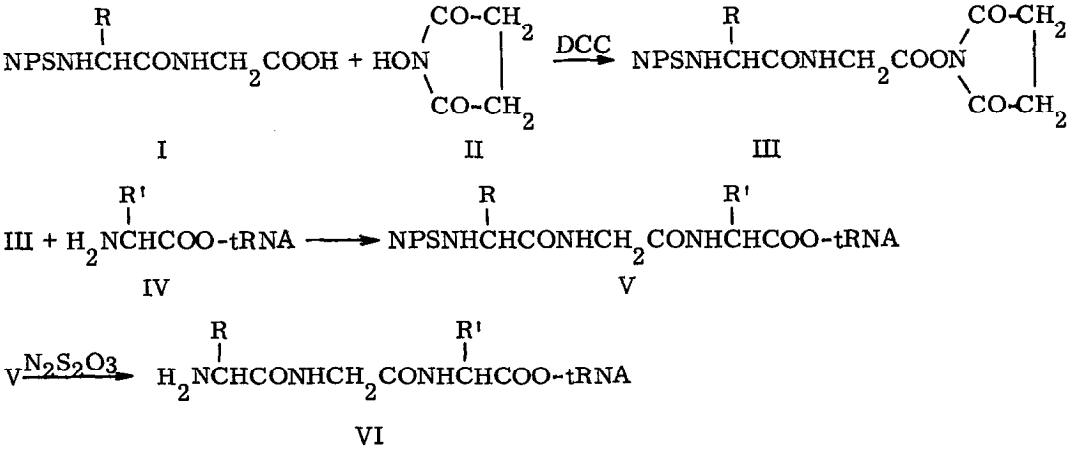


Fig. 2. Paper electrophoresis of the alkaline hydrolyzate of val-gly-[^{14}C]phe-tRNA. The electrophoresis was run on Whatman No. 3 MM paper for 4 hrs at 45 V/cm. The solvent used was 1 M acetic acid, pH 2.5.

A similar procedure was used for the preparation of different oligopeptidyl-tRNAs other than val-gly-phe-tRNA.

The synthesis of val-gly-phe-tRNA using NPS as N-blocking group can be summarized in the following scheme:



NPS = o-nitrophenylsulphenyl; DCC = dicyclohexylcarbodiimide.

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