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A RAPID NON-ACIDOLYTIC METHOD FOR DEPROTECTION AND REMOVAL OF PEPTIDES FROM SOLID PHASE RESINS - APPLICATIONS OF AMMONIUM FORMATE CATALYTIC TRANSFER HYDROGENATION

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ABSTRACT: Ammonium formate aided catalytic transfer hydrogenation has been employed in the cleavage, and concommitant deprotection, of the pentapeptide leucine enkephalin from the Merrifield peptide resin under ambient conditions of temperature and pressure in a neutral medium.

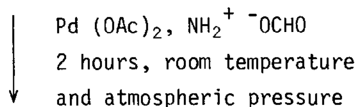
The acidolytic cleavage of protected peptides from solid phase resins often results in unacceptable degradation of the desired product.[1] Alternatives to HF or HBr/TFA treatments using milder reagents would have the advantage of increasing yields of higher quality products and might be exploited in the synthesis of complex sequences not otherwise obtainable by conventional methods. We recently demonstrated the utility and advantages of ammonium formate catalytic transfer hydrogenation (CTH) for the mild, rapid and selective removal of benzyl-based functionalities in solution phase peptide synthesis.[2] We now wish to report our results using a similar CTH system for the cleavage and simultaneous deprotection of peptides anchored to standard Merrifield polymers _via_ a benzyl-ester linkage. This method affords highly pure products in very short reaction times as ascertained by the synthesis of natural and radiolabelled (carbon-14) forms of the opiate-like pentapeptide, leucine-enkephalin. With a [U-^{14}C-glycine] residue at position 2 of leu-enkephalin, the cleavage reaction was found to be more than 70% complete after 10 minutes, and >95% after 120 minutes, as shown in Figure 1.

Using conventional catalytic hydrogenation, Schlatter _et al._ treated a Merrifield peptide resin with hydrogen gas at 60 psi and 40°C for 24 hours to obtain a 71% yield of Boc-protected# [Val^5] enkephalin;[3] Jones under similar conditions obtained a 56% yield of Boc-protected leucine enkephalin.[4] With the use of cyclohexene as the hydrogen transfer agent and at 70°C under ambient pressure, Khan and Sivanandaiah obtained a 20% overall yield of purified biologically active bradykinin.[5] In contrast, the method reported here is carried out at atmospheric pressure and ambient temperature under neutral conditions. Thus, using a Cbz-Tyr-Gly-Gly-Phe-Leu OCH_2-Polymer (Scheme 1), yields as high as 94% of leucine enkephalin were isolated _after_ a single gel filtration. The purity of this once-gelled material was assessed as $\geq$95% by analytical reversed phase HPLC techniques.

The rapidity of the cleavage and deprotection reactions under catalytic hydrogenation conditions is undoubtedly due to a facile hydrogen transfer with formate ion participating in the rate determining step. While formic acid appears to retard the rate of hydrogenolysis

SCHEME 1

Cbz-Tyr-Gly-Phe-Leu-OCH_2-Polymer



NH_2-Tyr-Gly-Gly-Phe-Leu-OH (>95% yield)

in solution phase when 10% palladium-on-carbon is used as the catalyst,[2] the substitution of such counter ions as dicyclohexylammonium or triethylammonium does not affect the reaction course or rate; in fact, the latter salts permit the use of non-polar solvents such as toluene or dichloromethane as alternative solvents for the reaction. As with previously reported hydrogenation techniques, the ammonium formate CTH conditions permit removal of standard hydrogenolyzable protecting groups including carbobenzoxy, benzyl, and nitro (arginine), while leaving untouched such acid labile groups as the Boc and t-butyl ester or ether functions, from the resin bound peptides. The extension of the above method for use with the sulfur containing amino acids methionine and cysteine or for sulfur-based amide bond surrogates[6] is presently under investigation.

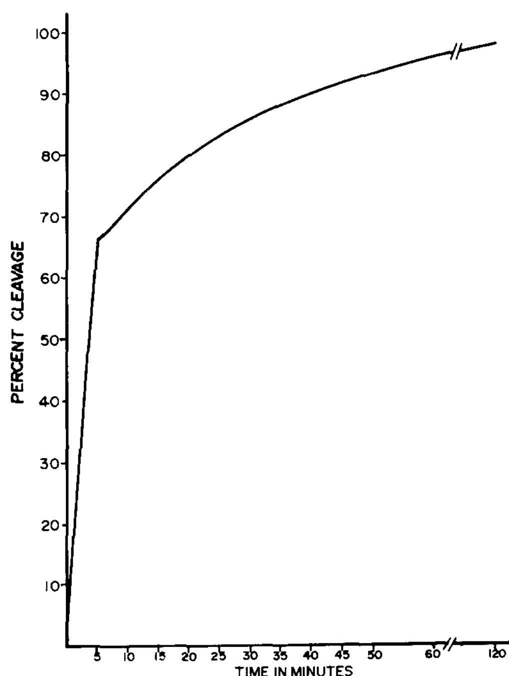


FIGURE 1. CTH removal of Tyr-[U-^{14}C]Gly-Gly-Phe-Leu from a 1% divinylbenzene crosslinked Merrifield polystyrene resin using ammonium formate as hydrogen donor and palladium black, generated *in situ* from palladium (II) acetate, as catalyst.

EXPERIMENTAL

Peptides were synthesized on a Vega Model 50 automated peptide synthesizer. TLC analyses were carried out on Merck F 254 silica gel plates in solvent systems: A) n-butanol/acetic acid/water (4:1:1); B) ethanol/water (7:3); C) n-butanol/acetic acid/ethyl acetate/water (1:1:1:1); and D) chloroform/methanol/acetic acid/water (15:10:2:3), and the R_f values are expressed as R_fA, R_fB, R_fC, and R_fD, respectively. HPLC analyses were performed with a C-18 reversed-phase Zorbax column (6.2 x 250 mm) using a 0.25 molar ammonium acetate (pH 4.1)/methanol gradient (30-40% over 20 minutes). Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter at the sodium-D line.

Cbz-Tyr-Gly-Gly-Phe-Leu-OCH_2-Polymer

Starting with a chloromethylated polystyrene resin (Sigma: Cl = 1.1 meq/g; crosslinked with 1% divinylbenzene), Boc-Leucine was esterified by Marglin's procedure[7] to yield 0.49 mmol/g Boc-Leu incorporation. The remaining Boc-amino acids were added sequentially using DCC-mediated coupling following our standard procedures,[6] except for the N-terminal tyrosine residue which was coupled as its N-α-carbobenzoxy-p-nitrophenyl ester in the presence of 1-hydroxybenzotriazole.

For the rate study the synthesis was repeated, but a sample of [U-^{14}C] glycine was incorporated at position 2 of leucine-enkephalin as its Boc derivative.

Deprotection and removal of peptide by CTH/ammonium formate; Tyr-Gly-Gly-Phe-Leu·HOAC:

1.0 g of Cbz-Tyr-Gly-Gly-Phe-Leu-OCH_2-Polymer was swelled in 12 ml of DMF containing 1.0 g of palladium acetate for 2 hours. Upon addition of a solution of ammonium formate (1.0 g in 0.5 ml water) a deposition of palladium was observed with rapid evolution of gases after a one minute induction period. After stirring for 2 hours, the resin was filtered and washed with 2 x 30 ml of 50% aqueous acetic acid. The filtrate was taken to dryness *in vacuo* below 40°C and the resulting light-brown residue was applied to a Sephadex G-15 column (96 x 2.8 cm) and eluted with 30% aqueous acetic acid. The column fractions (6.8 ml per tube) in tubes 71 through 85 were pooled and lyophilized to yield 231 mg of leucine-enkephalin acetate (93.9%).

Leucine enkephalin thus obtained was found to be homogeneous on tlc in four different solvent systems (R_fA 0.52, R_fB 0.72, R_fC 0.69, and R_fD 0.43), and at least 95% pure by analytical reversed phase HPLC (k' = 3.46, retention time = 10.1 min), $[\alpha]_D^{22}$ + 25.4° (c 1.1, 95% AcOH); Lit. $[\alpha]_D^{22}$ + 25.4°,[8] $[\alpha]_D^{20}$ + 26.4° (c 1, 95% AcOH).[9] Amino acid analysis: Tyr (0.99), Gly (2.00), Phe (1.08), Leu (0.99). Analysis calculated for $C_{28}H_{37}N_5O_7 \cdot CH_3COOH \cdot 2H_2O$: C, 55.27; H, 6.97; N, 10.75. Found: C, 55.07; H, 6.84; N, 11.29.

Monitoring rate of cleavage from Merrifield resin by CTH/ammonium formate

A 0.9 g sample of Cbz-Tyr-[U-^{14}C]Gly-Gly-Phe-Leu-OCH_2-Polymer in 25 ml DMF was treated as above, but in an open reaction vessel containing a gas dispersion tube that permitted free exchange of solvent medium but not catalyst or resin. At intervals of 0, 2, 5, 10, 15, 20, 30, 45, 60, and 120 minutes, reaction aliquots of 100 μl were withdrawn, diluted with 10 ml of Triton X-100 scintillation cocktail, and ^{14}C readings were taken on a Beckman Model LS 750 scintillation counter and plotted against time (Figure 1). The nearly quantitative removal of peptide from resin was confirmed by 1) isolation of purified ^{14}C-Leu-enkephalin and by

2) the virtual lack of radioactive readings on spent resin (even accounting for an estimated "screening" factor).

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\# Abbreviations used: Boc - t-butoxycarbonyl; Cbz - carbobenzoxy; Polymer - Polystyrene crosslinked with 1% divinylbenzene; HPLC - high performance liquid chromatography; CTH - catalytic transfer hydrogenation; DMF - dimethylformamide; Standard nomenclature for amino acids follows IUPAC-IUB Commission on Biochemical Nomenclature, J. Biol. Chem., 241, 555 (1967); 247, 977 (1972).

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