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IMPROVED SOLID PHASE PEPTIDE SYNTHESIS ON NON-CROSSLINKED RESINS

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Most solid phase peptide synthesis today employs the Merrifield type crosslinked resins, although the use of these resins presents problems of steric hindrance, diffusion and slow reaction rates (1). When crosslinked resins are used, only a small fraction of the peptide chains are confined to the surface of the resin beads and most of the synthesis occurs within the matrix of the resin (2). Recent publications (3-5) have described the use of a 25% chloromethylcopolystyrene as a backbone for solution phase synthesis of peptides. The authors feel that this method has promise, but not enough data is available. In order to improve reaction conditions for solid phase peptide synthesis, we have employed a non-crosslinked chloromethylcopolystyrene resin. In this communication we present data comparing the rates of peptide synthesis in solution, on a crosslinked resin and on our non-crosslinked resin employing both the p-nitrophenyl active ester and dicyclohexylcarbodiimide (DCC) methods.

Non-crosslinked chloromethylcopolystyrene was prepared (6) from linear polystyrene (7), and is soluble in benzene. This indicates the absence of a significant degree of crosslinking (6). A chlorine analysis of 14.45% indicated that 62.2% of the phenyl groups were chloromethylated. Non-crosslinked t-BOC-glycyl-chloromethylcopolystyrene was prepared by reacting 2.94 gm (containing 12 m moles of Cl) of non-crosslinked chloromethylcopolystyrene, 1.75 gm (10 m moles) of t-BOC-glycine and 1.4 ml (10 m moles) Et_3N in 20 ml of EtOH at 60°C for 48 hours. This resin was treated in the manner described by Merrifield (8) yielding 2.71 g of non-crosslinked glycyl-chloromethycopolystyrene after removal of the t-BOC protective group (8). Amino acid analysis revealed 1.49 m moles of glycine per gm of resin. This resin is insoluble in DMF and $CHCl_3$.

p-Nitrophenyl Active Ester Method (9) 133 mg (containing 0.20 m moles of glycine) of the above resin ester was preswelled in 10 ml of $CHCl_3$ and then reacted with 77.2 mg (0.20 m moles) of p-nitrophenylcarbobenzoxy (Cbz)-L-leucinate in a total of 20 ml of $CHCl_3$ at 25°C for 24 hrs. The kinetics of this reaction were studied by following the rate of formation of p-nitrophenol

at 315 mμ, on the Cary 14 recording spectrophotometer. A plot of ($\frac{x}{a(a-x)}$ vs time), where a is the original concentration of the active ester and x is the concentration of p-nitrophenol, gave a straight line indicative of second order kinetics. A rate constant of 1 x 10^{-3} liter $mole^{-1}$ $min.^{-1}$ was obtained which compares favorably to that constant (3.44 x 10^{-3} liter $mole^{-1}$ $min.^{-1}$) obtained for the reaction of the active ester with ethylglycinate in solution under the same conditions. U.V. data indicates that both reactions went to 80% completion in 24 hours. Amino acid analysis of the dipeptide resin gave the following ratios: Leu 0.81, and Gly 1.00. When the commercially available (10) crosslinked glycine resin ester was studied under the same conditions the rate was approximately 100 times slower.

Dicyclohexylcarbodiimide (DCC) Method (11) 133 mg (containing 0.20 m moles of glycine) of non-crosslinked resin ester was preswelled in 10 ml of DMF and reacted with 53 mg (0.2 m mole) of Cbz-L-leucine and 41.2 mg (0.2 m mole) of DCC in a total volume of 20 ml of DMF at 25°C. The reaction was followed by measuring the concomitant decrease in DCC (λ 263 mμ) and Cbz-L-leu. (λ 265 mμ) at 264.5 mμ. A second order plot gave a straight line with a rate constant of 2.90 liter $mole^{-1}$ $min.^{-1}$. The resin was treated with 30 ml EtOH, filtered and washed 3 x with 20 ml portions of each of the following solvents: DMF, EtOH, and AcOH. It was then deblocked by stirring for 4-1/2 hrs in 10 ml of 30% HBr in AcOH at 25°C. Amino acid analysis at 25% reaction and at completion gave the following ratios: Gly 1.00, Leu 0.256 and Gly 0.99, Leu 1.00. This indicates that the rate of decrease in the DCC concentration as measured by u.v. analysis, is a direct measure of peptide synthesis. Under the same conditions a rate constant of 1.06 x 10^{-2} liter $mole^{-1}$ $min.^{-1}$ was obtained for the crosslinked resin.

These studies demonstrate a distinct rate enhancement in solid phase peptide synthesis. Further studies involving the use of this resin are in progress.

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