AN APPROACH TO THE SEMISYNTHESIS

OF

ACYL CARRIER PROTEIN

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ABSTRACT

The purpose of this study was to prepare several semisynthetic analogues of the acyl carrier protein of E. coli, using the Merrifield solid-phase method for the preparation of the synthetic 1-6 hexapeptide in the protected form. It was hoped that the synthesis of these analogues, and their evaluation by the $^{14}CO_2$ assay and by other methods, might contribute to the study of protein structure and function in general, and those of ACP in particular.

The strategy chosen for the synthesis of the protected 1-6 hexapeptide was to employ acid-stable protecting groups for glutamic, aspartic, and arginine residues, and to cleave the completed peptide from the resin in a protected form using HBr in acetic acid. The p-nitrobenzyl group was chosen for the protection of acidic amino acid side-chains, as this group has often been stated to be stable to HBr in acetic acid.

In the course of this work, however, it became obvious that the stability of the p-nitrobenzyl group to HBr in acetic acid, while greater than that of other benzyl esters, was not sufficient to allow the convenient preparation of peptides protected with this blocking group. Cleavage of the protecting group occurred to an appreciable extent, and thus the product was contaminated with a mixture of deprotected peptides.

Other disadvantages of the p-nitrobenzyl group were also encountered during this study. In particular, problems

of instability were encountered in the preparation of protected amino acid intermediates for peptide synthesis. The p-nitrobenzyl group was also found to give low coupling yields during synthesis, and poor solubility during purification, to amino acids and peptides protected with it. It is clear that the problems of instability and insolubility associated with p-nitrobenzyl ester protection were aggravated by the fact that many of the target peptides bore two p-nitrobenzyl groups per molecule.

The coupling of a crude hexapeptide to the native 7-77 peptide yielded a product which gave some activity in the $^{14}CO_2$ assay after extensive purification of the semisynthetic protein. This result, together with amino acid analysis of the crude peptide, implies that the desired protected hexapeptide composed a significant proportion of the peptide after cleavage from the resin. The insolubility conferred by the p-nitrobenzyl protecting group, however, presumably caused the target peptide to be selectively lost from the mixture during the purification procedures, in favour of deprotected peptides and deletion peptides having greater solubility.

For this reason, the major products which were purified from the crude cleaved peptides were pentapeptides lacking one glutamic acid residue bearing the p-nitrobenzyl protecting group.

These pentapeptides, when coupled to the native 7-77 peptide, gave products which were inactive in the $^{14}CO_2$ assay. This result is interesting, in that it suggests that both Glu^4 and Glu^5 are essential to the interaction between

the 1-6 and 7-77 peptides which maintains the active conformation of ACP. However, these data must be treated as tentative until an active semisynthetic ACP satisfying analytical criteria is prepared by this method. The results obtained by assaying the purified semisynthetic ACP prepared from a crude hexapeptide suggest that a fully-active semisynthetic ACP could be prepared by this method, but they cannot be regarded as conclusive in the absence of supporting analytical data.

The approach to semisynthesis chosen for this study appears to be basically sound, but new acid-stable protecting groups for carboxyl functions are clearly required if the method is to become generally useful.

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