Internal Association in Solid Phase Peptide Synthesis. Synthesis of Cytochrome C Residues 66—104 on Polyamide Supports

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Summary Solvation of peptide–resins in solid phase synthesis may be increased by appropriate choice of side-chain protecting groups, with enhanced efficiency in amino-acid incorporation

SOLVATION of reacting species is usually of crucial importance in solution organic synthesis In its absence, aggregation may occur with precipitation or micelle formation In either case access to reactive groups may be restricted We now report observations which suggest that these phenomena are not limited to the free solution state, but are also operative in modified form in solid (gel) phase synthesis

The C-terminal fragment of cytochrome C comprising residues 66—104 (**1**) is of importance because of its use in partial syntheses[1–3] of analogues of this protein At the suggestion of Professor H A Harbury, we have carried out several assemblies of the 39-residue equine sequence (**1**) by the polyamide solid phase method[4] using *N*-α-fluorenylmethoxycarbonyl[5] (Fmoc) amino-acids Eight of the 39 amino-acids in (**1**) are lysine residues Initially, these were side-chain-protected as t-butoxycarbonyl (Boc) derivatives Aspartic acid (1), glutamic acid (5), threonine (3), and tyrosine (3) residues were protected as t-butyl esters or ethers, and the single arginine as its *p*-methoxybenzenesulphonyl derivative [6] With this combination of protecting groups, a resin loading of *ca* 0 2 mequiv /g, and dimethylacetamide as solvent, a marked and progressive decline in amino-acid incorporation was observed after the 12th residue (t-butyl aspartate) At the same time, the initially highly swollen (*ca* 20 ml/g) polydimethylacrylamide support decreased sharply in volume, becoming more dense and granular in character A similar decrease in amino-acid incorporation was observed using the ω,ω-bisadamantyloxycarbonyl[7] derivative of arginine in place of the *p*-methoxybenzenesulphonyl derivative (Mbs) † We interpret

† This exchange was made to improve the ease of final deprotection of the completed peptide and was unconnected with the foregoing observations

these observations as due to internal aggregation of the peptide chains within the initially mobile resin matrix. This aggregation would result essentially in additional cross-linking of the polymer support, with immobilisation of the polymer strands and loss of its gel-like character. The effect is apparently associated with the long sequence of amino-acids (**2**) containing a preponderance of highly hydrophobic hydrocarbon side chains and t-butyl-based protecting groups, including the single aspartate residue at which resin collapse begins. In agreement with this interpretation, changes in the side-chain protecting group strategy so as to increase slightly the polarity at the start of this sequence, together with a reduction in concentration on the resin to 0·13 mequiv./g, gave much better results. No sudden changes in resin structure were observed and a satisfactory preparation of the 39-residue peptide was achieved.

Glu.Tyr. Leu.Glu.Asn.Pro.Lys.Lys.Tyr.Ile.Pro.Gly.Thr.
66
Lys.Met.Ile.Phe.Ala.Gly.Ile.Lys.Lys.Lys.Thr.Glu.Arg.
Glu.Asp.Leu.Ile.Ala.Tyr.Leu.Lys.Lys.Ala.Thr.Asn.Glu
104

(**1**)

-Ile.Lys(Boc).Lys(Boc).Lys(Boc).Thr(Bu^t).Glu(OBu^t).
85
Arg(Mbs).Glu(OBu^t).Asp(OBu^t).Leu.Ile.Ala.Tyr(Bu^t).Leu.
Lys(Boc).Lys(Boc)-
100

← Direction of synthesis

(**2**)

p-$HOCH_2 \cdot C_6H_4 \cdot OCH_2 \cdot CO \cdot O \cdot C_6H_2Cl_3$-2,4,5

(**3**)

In the final synthesis, all eight lysine residues were introduced as ε-trifluoroacetyl derivatives, hydroxy- and acidic amino-acids were protected as t-butyl ethers or esters as before, arginine as the bisadamantyloxycarbonyl derivative, and methionine as its sulphoxide. Assembly was commenced by attachment of an internal reference-spacer amino-acid norleucine to the amino-resin,[4] followed by the acid-labile linkage agent (**3**). The first residue of the sequence proper (t-butyl glutamate) was introduced as its *N*-biphenylisopropoxycarbonyl derivative using the symmetrical anhydride activating procedure in the presence of *p*-dimethylaminopyridine. This avoided possible problems associated with base-catalysed ester bond formation using base-labile *N*-protecting groups. The following 37 residues were introduced sequentially using exclusively *N*-fluorenylmethoxycarbonyl derivatives following generally the symmetrical anhydride or *p*-nitrophenyl ester (asparagine) coupling and piperidine (20% in dimethylacetamide) deprotection procedures already described.[5] The synthesis was conveniently terminated using Boc.Glu(OBu^t).OH.

All the t-butyl and adamantyloxycarbonyl protecting groups were cleaved and the peptide detached (93%) from the resin by a single treatment with 90% trifluoroacetic acid for 2 h. The crude octa-trifluoroacetyl derivative was chromatographed on diethylaminoethyl cellulose (DE52) using a linear gradient of 0·01—1M ammonium hydrogen carbonate, pH 7·8 at 4 °C. Partial loss of trifluoroacetyl groups occurred during this purification,‡ resulting in heterogeneity in the main peak (43%). This material was collected, all the trifluoroacetyl groups cleaved with 1M aqueous piperidine at 0 °C for 2½ h, and the piperidine removed by gel filtration (Sephadex G25). Chromatography on carboxymethyl cellulose (CM52) using a linear gradient of 0·015—0·25M ammonium acetate, pH 6·0, eluted 53% of the material applied as a single symmetrical peak which was collected and the sulphoxide reduced with excess of thioglycolic acid. After further chromatography on Sephadex G25, the product [Found: Asp, 2·92 (3); Thr, 2·90 (3); Glu, 5·17 (5); Pro, 1·98 (2); Gly, 2·00 (2); Ala, 3·00 (3); Met, 0·97 (1); Ile, 3·42 (4); Leu, 2·96 (3); Tyr, 2·88 (3); Phe, 0·91 (1); Lys, 7·33 (8); and Arg, 0·83 (1)] gave a single spot on t.l.c. (butan-1-ol–pyridine–acetic acid–water, 90:18:60:72, R_f 0·62) and was eluted on analytical h.p.l.c. from both reversed-phase (Microbondapak C_{18}) and ion exchange (Whatman SCX) columns in a position identical to that of authentic cytochrome C residues 66—104 derived from the natural protein. A trace of unreduced sulphoxide was present. The yield from peptide–resin was 14·5%.§

The problems encountered in this synthesis underline the need for careful planning and increased understanding of the solid phase system. Appropriate choice of protecting groups allows modification of the solvation properties of the peptide–resin system and may be of critical importance in the synthesis of difficult sequences containing clusters of hydrophobic residues. Use of a less polar solid phase system as in the conventional polystyrene–methylene chloride combination will not necessarily be advantageous, as attempted solid-phase assembly of the same cytochrome fragment elsewhere has shown.[1]

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‡ Lys(Tfa) was cleaved to the extent of 20—25% when kept in contact with the final buffer for 24 h at room temp.

§ Combination of this product with natural cytochrome C 1—65 fragment is being studied by Prof. Harbury and his colleagues at the Dartmouth Medical School, Hanover, New Hampshire.

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