Internal Aggregation during Solid Phase Peptide Synthesis. Dimethyl Sulfoxide as a Powerful Dissociating Solvent

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Internal aggregation within the peptide-resin matrix during solid phase peptide synthesis may be overcome by the use of dimethyl sulfoxide as solvent for both acylation and deprotection reactions.

The occurrence of so-called 'difficult sequences' which undergo strong association within the peptide-resin matrix is a major problem in solid phase peptide synthesis.[1] Onset of internal association is a consequence of inadequate solvation of the protected peptide chains and is characterised by substantial and often critical reduction in the rates of both acylation and deprotection reactions. For expanded gel resin supports, there is also a sharp reduction in resin volume. Although information about peptide sequences likely to give rise to these probelms is accruing,[2] it has not so far been possible to predict the occurrence of 'difficult sequences' reliably. We have, therefore, devised an experimental test for the propensity of individual residues towards aggregation, based upon the effect of insertion of guest residues into a strongly aggregating host sequence.[3,4] This has enabled study of the effects of variation in amino acid and side chain protecting group structures. It has now been further applied in a wide ranging solvent and solvent–additive study with important results for the synthesis of aggregating sequences.

Oligo(alanyl)-valine sequences undergo strong internal association when synthesised on both polystyrene[5] and polydimethylacrylamide[6] supports. The onset of internal association is a function of chain length, and in this sequence it commences particularly early at the deprotected hexapeptide stage. In continuous flow synthesis[7] it is easily detected spectroscopically by the slow release of chromophoric fluorene derivatives into the flowing reagent stream during deprotection reactions. This results in a characteristic flattening and broadening of the deprotection elution profile (Fig. 1). It is also readily detected during acylation steps.[6] Insertion of one or two guest residues into the oligo(alanyl)-valine sequence as shown in **1** delays the onset of aggregation depending on their structures. Data for a large number of inserted residues with varying side chain protecting groups have been reported and discussed.[3,4]

Fmoc-Ala$_n$-X-X-Ala$_3$-Val-polydimethylacrylamide **1**

H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH **2**

For the solvent study we selected the sequence as shown in **1** [X = Glu(OBu^t)]. On synthesis using pentafluorophenyl ester derivatives in dimethylformamide (DMF), it undergoes internal association after a total of eight amino acid residues have been added. Solvents can be judged better or worse than DMF in promoting aggregation by carrying out resin-equilibration and deprotection steps in this solvent and detecting the length

Table 1 Deprotection peak heights obtained during the synthesis of **1** [X=Glu(OBu^t)] by the Fmoc-polyamide procedure[7] expressed relative to residue 6 = 100%[a]

	Solvent	Conc. % in DMF	Ala-11	Ala-10	Ala-9	Ala-8	Ala-7	Glu-6
1	*N,N*-DMF	100			32.4	37.6	82.3	100
2	*N,N*-Dimethylacetamide					30.9	78.6	100
3	*N,N*-Diisobutylformamide						9.8	100
4	*N*-Methylpyrrolidone				26.1	55.2	76.5	100
5	*N*-Methylformamide						45.9	100
6	Formamide						22.6	100
7	Dichloromethane	50					19.0	100
8	*N*-Methylurea	20		28.8	27.2	35.3	98.6	100
9	*N,N'*-Dimethylurea	20	34.8	33.1	31.0	34.2	104.6	100
10	*N,N,N',N'*-Tetramethylurea						28.7	100
11	1,3-Dimethylethylene urea					33.9	78.9	100
12	1,3-Dimethylpropylene urea						7.5	100
13	Urea	10				46.0	109.0	100
14	Guanidine HCl	20				24.5	96.9	100
15	Lithium bromide	5	35.0	40.3	58.2	93.0	119.9	100
16	Lithium bromide	10	54.4	66.0	91.7	103.5	131.2	100
17	Lithium perchlorate	10				33.0	109.2	100
18	Potassium thiocyanate	10	53.3	58.9	54.5	74.1	122.1	100
19	Ethylene carbonate	20				11.6	103.4	100
20	DMSO		49.4	90.5	110.4	111.6	109.3	100
21	DMSO	50		44.5	63.8	95.6	100.3	100
22	DMSO	20	46.0	43.1	40.0	53.0	104.9	100
23	Sulfolane	20	35.9	34.9	30.6	30.8	100.9	100

[a] Deprotection was carried out using piperidine (20% in the appropriate solvent or solvent-additive mixture). Where the concentration is not given, the solvent was used neat. In most cases, additive concentration was limited by solubility. All acylation reactions were in DMF. Synthesis was carried out on a LKB Biolynx continuous flow synthesiser. Peak heights were obtained spectrometrically at 275 nm.

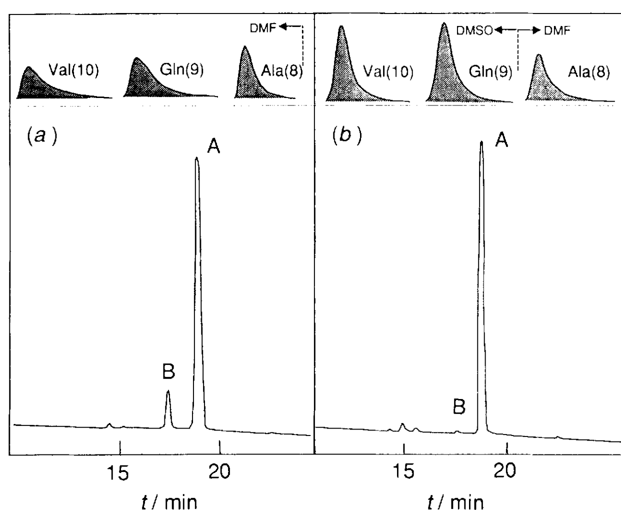

Fig. 1 Deprotection and HPLC elution profiles measured at 215 and 275 nm, respectively, for the Fmoc-polyamide continuous flow synthesis[7] of acyl carrier protein residues 65–74 decapeptide **2** (*a*) in DMF and (*b*) with the last two residues added and deprotected in DMSO. Peak A corresponds to the completed decapeptide **2**, and peak B to the des-valine nonapeptide.

of peptide chain at which aggregation occurs. Deprotection peak height data are collected in Table 1 for residues from Glu-6 onwards in the named solvent or solvent–additive mixture. All syntheses were checked by peptide-resin amino acid analysis.

We draw attention to the following points. Of the simple dipolar aprotic solvents commonly used in peptide synthesis, DMF, dimethylacetamide and *N*-methylpyrrolidone were approximately equal in the test. Diisobutylformamide, *N*-methylformamide and formamide itself were all worse, as was dichloromethane–DMF (1:1). None of the urea type solvents[8] or additives investigated (experiments 8–13) were effective in delaying aggregation. Of the inorganic salts examined (experiments 15–18), added lithium bromide[8,9] was apparently effective though at 10% concentration (experiment 16), amino acid analysis indicated incomplete amino acid incorporation. Potassium thiocyanate was less effective and lithium perchlorate ineffective. Stewart and Klis have reported[9] that added perchlorate or thiocyanate ions were both superior to bromide in facilitating synthesis of a different test peptide. 10% Ethylene carbonate[10] in DMF was worse than DMF itself in this test.

Outstanding amongst the data in Table 1 is that for dimethyl sulfoxide (DMSO) (experiments 20–22).† The length of the peptide sequence [**1**, X = Glu(OBu^t)]) increased from eight residues in DMF to eleven residues in DMSO before aggregation occured, an effect comparable to that produced by insertion of structure-disrupting secondary amino acid residues into the oligoalanyl-valine sequence.[5,6] Lesser but significant effects were observed with DMSO–DMF mixtures. To validate this result we have used DMSO as solvent for the deprotection *and acylation* reactions in the well known 'difficult sequence', residues 65–74 **2** of the acyl carrier protein. This decapeptide undergoes strong internal association during deprotection of the 9th and 10th residues. Addition of the final valine is very strongly hindered. Under our standard conditions completely adequate for the earlier residues (pentafluorophenyl ester–hydroxybenzotriazole coupling, 45 min in DMF), this last acylation is invariably 10–15% incomplete. The results for syntheses of **2**, (*i*) under standard conditions in DMF and (*ii*) with DMSO used for the addition and deprotection of the last two residues are shown in Fig. 1. The final crude product is contaminated with *ca.* 12% des-valine nonapeptide in (*i*) and the expected broadening of the deprotection profiles is observed. There is no evidence for aggregation during the synthesis (*ii*) and a much purer crude product nearly free of nonapeptide (<0.5%) is obtained.

When the same synthesis is repeated using DMSO as solvent for the deprotection steps alone, some 12% of nonapeptide failure sequence is obtained as usual. Reassocia-

† Fluka puriss grade DMSO [absolute, over molecular sieves (<0.01% H_2O), >99.5% (GC)] was used without further purification.

tion within the peptide-resin matrix therefore occurs quite rapidly on reverting from DMSO to DMF as the permeating medium.

We conclude that dimethyl sulfoxide is likely to prove a superior reaction medium for solid phase peptide synthesis in aggregating systems and may provide a solution to the 'difficult sequence' problem. It has been mentioned from time to time in the past,[11] sometimes as an additive to DMF or *N*-methylpyrrolidone, but has found little application. It has good solvation properties for protected peptide sequences and delays or eliminates the onset of internal association. It also appears to be a kinetically superior reaction medium, giving faster activated ester acylation[12] and possibly deprotection reaction rates (Fig. 1) then DMF in non-aggregating sequences. Caution should be taken with peptide sequences containing methionine, cysteine, or other oxidisable residues.

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