A Novel Solvent System for Solid-Phase Synthesis of Protected Peptides: The Disaggregation of Resin-Bound Antiparallel β-Sheet

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Summary: This paper reports the application of the solvent system of lithium bromide in tetrahydrofuran for the disaggregation of protected peptide bound to the Kaiser oxime resin. Fourier-transform infrared spectroscopy is used to characterize the aggregating structure and to follow the disaggregation. The solvation of resin-bound peptide has practical consequences in that the yield of the nucleophilic cleavage of protected peptides from the Kaiser resin is greatly increased.

Solid-phase peptide synthesis, as developed by Merrifield,[1] is a powerful tool for the study of peptide conformation and biological activity. The aggregation of resin-bound peptides has been correlated with low-yield coupling steps in stepwise solid-phase synthesis.[2-4] In the course of our work on the development of a solid-phase fragment condensation methodology,[5] we have found that aggregation of resin-bound peptides can interfere with the nucleophilic cleavage reaction which is necessary to produce protected peptide fragments.[5] We report herein a structural explanation for, and a solution to, this problem using an unusual solvent system which we have also found to be extremely effective for the solubilization of protected peptide fragments.[6]

Aggregation of resin-bound peptides has been observed by solid-state NMR,[2,7] and the structure of the aggregates has been shown by Fourier-transform infrared spectroscopy (FTIR) to be antiparallel β-sheet.[8-11] To minimize this problem, the solvent employed for a solid-phase synthesis must swell the polymer support and solvate the resin-bound peptide. Various solvents have been utilized with these requirements in mind.[2,3,10-13] We report the use of lithium bromide (2 M) in anhydrous tetrahydrofuran (THF),[14] which is extremely effective in disrupting β-sheet structure in resin-bound peptides. This solvent also provides maximal swelling of peptide-resins. For peptide-resins that aggregate in methylene chloride (DCM), nucleophilic cleavage yields in 2 M LiBr/THF are greatly increased relative to yields in DCM.

Table I. Swelling of Peptide-Resins[20]

	solvent			
sample[a]	DCM	DMF	THF	2 M LiBr/THF
Kaiser oxime resin	2.7	2.5	3.3	3.3
peptide A	2.2	2.1	2.3	2.5
peptide B	1.6	1.6	1.8	2.4
peptide C	1.6	1.6	1.7	2.6

[a] A = Boc-DAEFRHDS-resin[21] (0.35 mmol peptide/g resin), B = Boc-AGYGSTQTAGSDSSLT-resin[22] (0.06 mmol/g), C = Boc-LMVGGVVIA-resin[23] (0.40 mmol/g).[15] Volumes are normalized relative to a volume in methanol of 1.0 mL. This volume approximates the dry volume of the resin (1.0 mL is the volume of 0.50 g of oxime resin and of 0.42 g of peptide B).

Table II. Nucleophilic Cleavages of Resin-Bound Peptides[26]

peptide	nucleophile	solvent	% yield	% yield in 2 M LiBr/THF
A	Gly salt[17]	DCM	97	94
B	HOPip[27]	DMF	57	83
C	HOPip[27]	DCM	90	99
D	Ala salt[17]	DCM	35	95

Seebach has reported the powerful solvating effects of anhydrous solutions of lithium halides in tetrahydrofuran.[14] We are able to solubilize the extremely insoluble peptide H_2N-LMVGGVVIA-CO_2H (residues 34-42 of the amyloid-β protein) at high concentration (>100 mg/mL) in 2 M lithium bromide in THF.[6,14] Difficulties in cleaving the resin-bound peptide D (residues 1-41 of the 42-amino acid amyloid-β protein[15,16] of Alzheimer's disease[17]) from the Kaiser oxime resin[5,18] led us to try this solvent system for the solvation and nucleophilic cleavage of resin-bound protected peptides.

Merrifield has demonstrated that the swelling of peptide-resins is dependent on both solvent-polystyrene and solvent-peptide interactions.[19] The solvent-dependent

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(11) Narita, M.; Isokawa, S.; Honda, S.; Umeyama, H.; Kakei, H.; Obana, S. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 773. We have tested 10% hexafluoro-2-propanol in DCM and found that it partially disrupts β-structure in our peptides and swells the peptide resins almost as well as 2 M LiBr/THF. However, cleavage yields in this solvent were not improved relative to DCM.
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(15) The sequence of the amyloid-β protein is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA. Peptide D is the product of a fragment condensation synthesis and is a mixture of (1-41) (0.02 mmol peptide/g resin), (26-41) (0.06 mmol/g), and (34-41) (0.20 mmol/g). Details of this synthesis will be reported elsewhere.
(16) The following side-chain protecting groups were used: D(Bzl), E(Bzl), H(Bom), R(Mts), S(Bzl), T(Bzl), Y(Cl_2-Bzl). In peptide A, the aspartate protecting groups are D1(tBu), D7(cHex).
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(18) Lansbury, P. T.; Hendrix, J. C.; Coffman, A. I. *Tetrahedron Lett.* **1989**, *30*, 4915. Salts must be anhydrous for use in 2 M LiBr/THF. Salts were prepared in methanol instead of 1:1 methanol-water. After drying, the salt was dissolved in benzene and dried to remove residual water.
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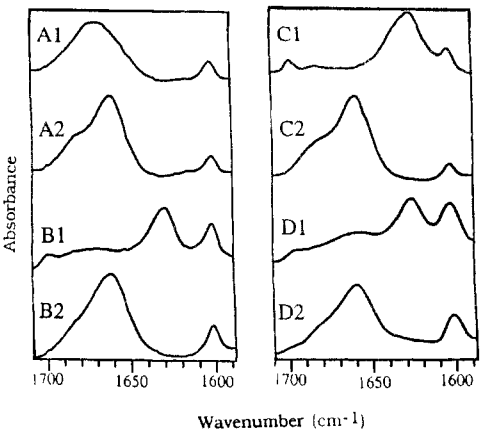

Figure 1. FTIR of resin-bound peptides in DCM and 2 M LiBr/THF. A1: Peptide A in DCM. A2: Peptide A in 2 M LiBr/THF. B1: Peptide B in DCM. B2: Peptide B in 2 M LiBr/THF. C1: Peptide C in DCM. C2: Peptide C in 2 M LiBr/THF. D1: Peptide D in DCM. D2: Peptide D in 2 M LiBr/THF. Peptide D: Boc-(amyloid β1-41)-resin, fully protected.[14,15] The band at 1601 cm^{-1} is due to polystyrene.

swelling of the Kaiser oxime resin was very different from that of the three peptide-resins tested (Table I).[20] THF was very effective in swelling the oxime resin but was a poor solvent for swelling peptide-resins. The peptide-resins differed from each other in their swelling behavior. Peptide A[21] swelled effectively in all of the solvents tested; this behavior was typical of most of the peptide-resins we have analyzed. In contrast, peptides B[22] and C[23] were slightly swollen in DCM and DMF but had a much larger volume in 2 M LiBr/THF. In all cases, peptide-resins reached their greatest volume in 2 M LiBr/THF.

Resin-bound peptides were suspended in solvent and analyzed by FTIR[24] to determine the structural basis for the swelling of peptide-resins (Figure 1). A monomeric amide in DCM absorbs at ca. 1680 cm^{-1}. The presence of a band at 1630 cm^{-1} is indicative of strongly hydrogen bonded β-sheet structure; an additional weak band at ca. 1695 cm^{-1} is indicative of antiparallel β-sheet.[25] Peptide A did not assume β-sheet structure in DCM or in 2 M LiBr/THF (Figure 1; A1, A2). Peptides B, C, and D were aggregated antiparallel β-sheets in DCM (Figure 1; B1, C1, D1). Aggregation was disrupted in 2 M LiBr/THF, as evidenced by the disappearance of the band at 1630 cm^{-1} (Figure 1; B2, C2, D2). The observed amide I band in 2 M LiBr/THF (1660 cm^{-1}) probably represents a lithium-amide complex.[6]

Most peptides can be cleaved from the Kaiser oxime resin in >90% yield[26] using *N*-hydroxypiperidine[27] (HO-Pip) or amino acid tetra-*n*-butylammonium salts.[18] Peptide A, which does not aggregate, was cleaved in high yield in both DCM[18] and in 2 M LiBr/THF (Table II). However, cleavages of peptides B and D, which form β-sheet aggregates in DCM, proceeded in very low yields. Cleavage yields improved dramatically when 2 M LiBr/THF was used as the solvent. The yield for cleavage of peptide C was high in DCM, but was improved in 2 M LiBr/THF.

The principles illustrated here should be applicable to other reactions in solid-phase peptide synthesis. New solvent systems can be evaluated using FTIR and simple swelling measurements in order to minimize aggregation and increase chemical yields. Our results indicate that 2 M LiBr/THF is a powerful solvent for resin-bound peptides. While couplings to unaggregated resin-bound peptides seem to be slower in this solvent system than in dimethyl formamide (DMF),[28] it may serve as a last resort for coupling to certain resin-bound peptides which aggregate strongly in DMF. For our fragment-coupling strategy, this solvent system may prove invaluable for the solvation of protected fragments which are sparingly soluble in organic solvents.[29]

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(20) Swelling measurements in Table I were made by washing a sample of resin (ca. 0.5 g) with the solvent and suspending the resin in the solvent in a glass column (i.d. 1.0 cm) above a glass frit. The resin was allowed to settle until all excess liquid had drained through the frit. The height of the resin was measured, and the procedure was repeated twice. The same sample of resin was used for all solvents.

(21) The sequence of the protected resin-bound peptide A is derived from the N-terminal region (residues 1-9) of the amyloid-β protein.

(22) The sequence of the protected resin-bound peptide B is derived from the consensus 16 amino acid repeating sequence of two bacterial ice nucleation proteins. (Warren, G.; Corotto, L.; Wolber, P. *Nucl. Acids Res.* **1986**, *14*, 8047.)

(23) The sequence of the protected resin-bound peptide C is derived from the C-terminal region (residues 34-42) of the amyloid-β protein.

(24) FTIR measurements were made using a Mattson Cygnus 100V spectrometer. Peptide-resins were swollen and suspended in solvent. A drop of this suspension was placed between the windows of a solution cell (AgCl windows for DCM; CaF_2 windows for 2 M LiBr/THF.).

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(26) Cleavage yields were determined by amino acid analysis (Waters Picotag) of the resin before and after cleavage. Cleavage products of peptides A, B, and C have been purified and fully characterized.

(27) Nakagawa, S. H.; Kaiser, E. T. *J. Org. Chem.* **1983**, *48*, 678.

(28) The tetrapeptide LMVG was prepared on the Kaiser oxime resin. Boc-amino acids were activated as the preformed symmetric anhydride in THF. Couplings were carried out in 2 M LiBr/THF (1 h) with yields of 60-80%, compared with 95-100% for couplings in DCM. Current efforts include optimization of coupling time to allow quantitative coupling.

(29) We have had some success using 1 M LiBr/THF as solvent for gel permeation (Waters Ultrastyragel 1000-Å column) purification of protected peptides which, due to their insolubility, cannot be easily purified by existing methods.

Alkyne Insertion Reactions of Metal-Carbenes Derived from Enynyl α-Diazo Ketones [$R'CN_2COCR_2CH_2C{\equiv}C(CH_2)_{n-2}CH{=}CH_2$]

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Summary: The title substrates react with a variety of metal catalysts to give metal-dependent product arrays (including tricyclic cyclopropanes and others formally arising from the vinylogous α-keto carbene **3** shown in Scheme I) of both synthetic and mechanistic interest.

Stoichiometric quantities of Fischer carbene complexes [e.g., $(CO)_5Cr{=}C(R)(OMe)$] react both inter- and intra-

