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Sonication-Assisted Cleavage of Hydrophobic Peptides. Application in Multipin Peptide Synthesis.

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Abstract: High-power sonication enables hydrophobic peptides, that would otherwise cleave with poor efficiency, to cleave and elute from the solid support in good yield. The method is demonstrated in conjunction with the multipin method of multiple synthesis using a diketopiperazine-forming handle with cleavage at pH 8.3.

By its very nature, simultaneous multiple peptide synthesis by the multipin method[1] necessitates the concurrent handling of peptides that may have significantly different solubility properties. Although rapid screening applications are facilitated by direct, multiple cleavage into physiologically compatible solutions,[2] the attempted cleavage of hydrophobic peptides may result in poor yields, hence unreliable bioassays. A consideration of cleavage conditions expected to enhance peptide yield prompted an investigation of the effect of sonication[3] on the efficiency of cleavage of hydrophobic peptides via the diketopiperazine formation method[4] (Fig. 1).

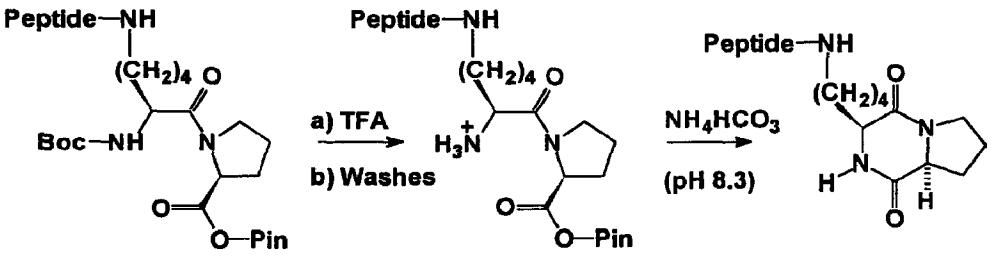

Fig. 1. Peptide Cleavage via Diketopiperazine Formation.
Cleavage into NH_4HCO_3 (pH 8.3), a volatile buffer.

The set of peptides selected for this study (Table 1) encompass a T cell determinant, which has been studied previously in our laboratory. A dinitrophenyl (Dnp) chromophore was incorporated at the N-termini of the peptides to allow cleavage efficiency to be ascertained spectrophotometrically.[5] Inclusion of the Dnp moiety increases the hydrophobicity of an already moderately hydrophobic peptide set. Fauchere and Pliska hydrophobicity parameters[6] for **1-11** are listed in Table 1; values >0.7 generally indicate poor solubility in aqueous solution. The test peptides proved to be highly insoluble in most solvents. For example **7**, the least soluble peptide in the set,

Table 1. Hydrophobic Test Peptides used in this Study.

	Peptide[a] Dnp-[X]-cyclo(KP) -X-	Fauchere & Pliska Hydro -phobicity[b]	Molecular Weight (Dalton) Found[c]	Calcd
1	ßLINSTKIYSYFPß	0.79	1961.2	1961.5
2	ßINSTKIYSYFPSß	0.68	1935.5	1935.4
3	ßNSTKIYSYFPSVß	0.65	1921.1	1921.3
4	ßSTKIYSYFPSVIß	0.79	1920.1	1920.4
5	ßTKIYSYFPSVISß	0.79	1919.8	1920.4
6	ßKIYSYFPSVISKß	0.72	1946.7	1947.5
7	ßIYSYFPSVISKVß	0.85	1917.6	1918.4
8	ßYSYFPSVISKVNß	0.71	1919.1	1919.3
9	ßSYFPSVISKVNQß	0.64	1884.2	1884.1
10	ßYFPSVISKVNQGß	0.64	1854.1	1854.1
11	ßFPSVISKVNQGAß	0.60	1762.1	1762.0

a: Dnp = 2,4-dinitrophenyl, ß = ß-Alanine; b: Ref 6. For this study ß = 0.31, Dnp = 1.99, K{in cyclo(KP)} = 1.70; c: ionspray MS recorded on a Perkin Elmer Sciex API III.

was only sparingly soluble in DMSO or DMF. All peptides, however, were soluble in TFA. Peptides were prepared on polyethylene pins which had been radiation grafted with 2-hydroxyethyl methacrylate (HEMA)[1] (loading = 1.10 μmol/pin) and methacrylic acid/dimethylacrylamide[7] (MA/DMA) (loading = 0.18 μmol/pin).

Fig. 2 summarises 66 cleavage experiments. MA/DMA grafted pins carrying peptides 1-11 were subjected to four cleavage conditions with 0.1M NH_4HCO_3 (pH 8.3): without sonication (15 h) and with sonication (1 h) in both water and 40% MeCN(aq). In the case of the HEMA grafted surface, which is less hydrophilic than the MA/DMA graft surface, only 0.1M NH_4HCO_3 in 40% MeCN(aq) was used in the cleavage studies. Although moderately hydrophobic sequences have been cleaved into aqueous buffer from MA/DMA without sonication assistance[1,2,4], only 11, the least hydrophobic peptide in the study set, cleaved to a satisfactory extent after 15 h in NH_4HCO_3(aq) (Fig. 2A). With 1 h sonication, greatly improved yields were obtained for 9-11. Some improvement was also seen for 3, 6 and 8. Little improvement, however, was observed for the more lipophilic sequences. Even when MeCN was included as a cosolvent in the cleavage solution, the most hydrophobic sequences only cleaved with moderate efficiency under the action of sonication. Following cleavage, 1-8 rapidly precipitated. Similar results were found when these experiments were performed with 0.05M HEPES buffer (pH 7.8) with and without MeCN (results not shown). In the case of the HEMA graft (Fig. 2B), superior cleavage was always obtained with 1 h sonication as opposed to a 15 h passive cleavage. Once again the differences were most pronounced in the case of the more hydrophobic sequences. Sonication more than doubled yields for 2, 4 and 7.

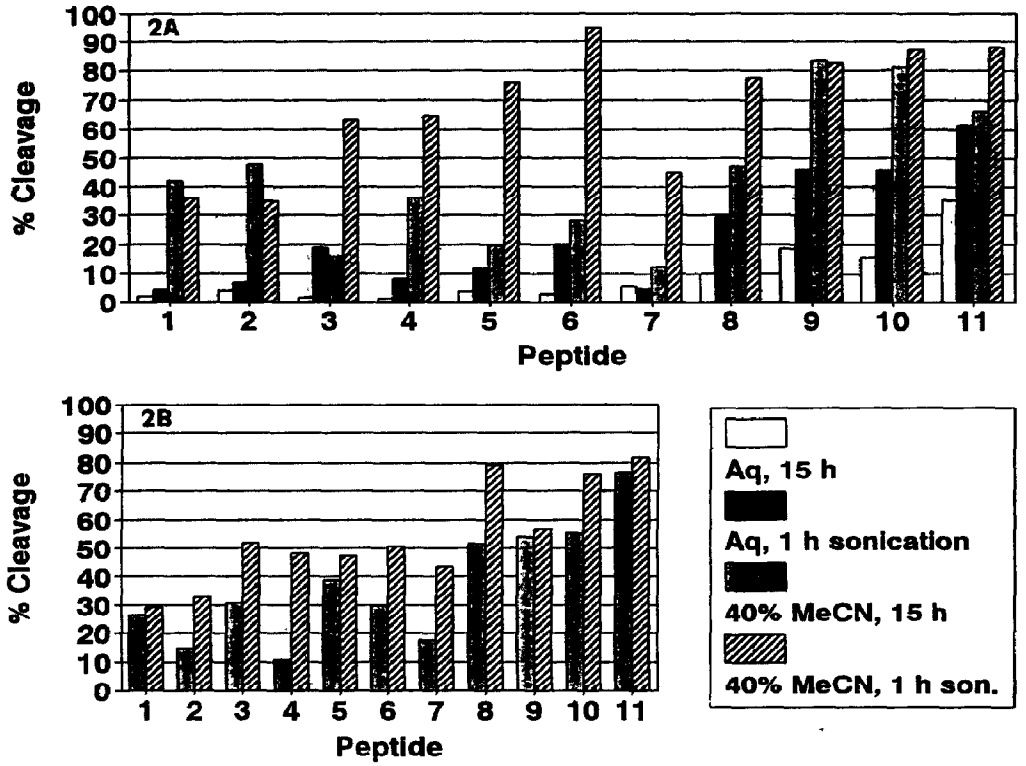

Fig. 2. Cleavage of hydrophobic peptides 1-11 from pins into 0.1M NH_4HCO_3 (pH 8.3). 2A: cleavage from MA/DMA grafted pins; 2B: cleavage from HEMA grafted pins.

It is most likely that sonication improves the rate of elution of cleaved peptides from the support, rather than increasing the rate of cyclization. Nevertheless, sonication enhances diketopiperazine cleavage efficiency and can greatly reduce cleavage times required for hydrophobic sequences. We have also found sonication to be useful in eluting peptides cleaved with ammonia vapour from the support[8], and in accelerating base cleavage. Where very hydrophobic peptides are encountered, addition of MeCN, which is relatively non-toxic to cells[9], aids the cleavage process. Even in cases where an organic cosolvent could not be tolerated, sonication greatly enhances cleavage efficiency, unless the peptides are very hydrophobic. As NH_4HCO_3 is a volatile buffer, the cleaved peptide solutions (or suspensions) can be freeze dried and reconstituted in DMSO (for example) for subsequent bio-testing. The technique is easily applied to the multipin system. Mounted pins are immersed into racked polypropylene tubes, which contain the cleavage buffer. These are, in turn, placed into the sonication bath, ensuring a good liquid junction.

Peptide Synthesis. Peptides were prepared on radiation grafted[1,7] polyethylene detachable crowns, and functionalised with a preformed diketopiperazine-forming handle[1,2]. The crowns were fitted to polypropylene support pins, and mounted on a plastic holder designed to hold 96 individual pins. Peptide synthesis was performed using Fmoc protected amino acids; those requiring side-chain

protection were as follows: Arg(Pmc), Asn(Trt), Asp(OtBu), Gln(Trt), Glu(OtBu), His(Boc), Lys(Boc), Ser(tBu), Thr(tBu), Trp(Boc) and Tyr(tBu). Coupling reactions were performed in polypropylene microtitre trays using 150 μL of activated amino acid solution (Fmoc-Axx-OH/BOP/N-methylmorpholine/HOBt, 100 mM: 100 mM: 150 mM: 100 mM) in DMF. Couplings were performed for either 2 h or 16 h at 25°C. Fmoc deprotection was performed by treating pins with 20% piperidine in DMF (50 mL/96 pins) for 20 min. Deprotection was followed by a DMF wash, 3 MeOH washes and air drying. Dnp-ßAla-OH[4] was coupled using the conditions listed above. Peptides were side-chain deprotected by treatment with TFA/anisole/ethanedithiol (95:2.5:2.5, v/v/v) (50 mL/96 pins) for 2.5 h. The pins were then soaked in MeOH (10 min), 1% AcOH in MeOH/H_2O (1:1, v/v) (1.5 h) and H_2O (10 min) and shaken dry.

Peptide Cleavage. Peptides were cleaved into 0.1M NH_4HCO_3 in either H_2O or MeCN/H_2O (2:3, v/v). (MA/DMA pins: 800 μL; HEMA pins: 3.0 mL). Cleavage from MA/DMA pins was performed in racked 1 mL BioRad tubes using pins fitted to the holder used throughout synthesis. Tubes were held in a Micronic rack and supported on a wire frame during sonication. Cleavage from HEMA pins was performed in 5 mL polypropylene tubes. Sonication was performed in a 1000 W ultrasonic bath for 1 h at <30°C. When cleavage was performed without sonication, pins were left soaking for 15 h at 20°C. Cleavage efficiency was established by cleaving residual peptide from the pins with 2M NaOH in EtOH/H_2O (1:1, v/v)(800 μL/pin or 3.0 mL/pin) with 30 min sonication.

Peptide Analysis. The poor solubility properties of the test peptides precluded analysis by reverse phase HPLC and TLC. All peptides were examined by ionspray MS (Table 1). Peptide **11** was subjected to amino acid analysis.[10] Peptide concentrations, hence cleavage efficiencies, were determined by freeze drying peptide solutions or suspensions, reconstituting the peptides in TFA and determining absorbances of standard solutions at 381 nm.[5]

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