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Solid phase peptide synthesis on *J*anda*J*el™ resin

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Abstract—A comparative synthesis of the classic difficult sequence ACP(65–74) on 2% crosslinked *J*anda*J*el and 1% crosslinked polystyrene–divinylbenzene, (PS–DVB, Merrifield resin) by Boc/benzyl chemistry is presented. The *J*anda*J*el batch used was of nearly double the loading of PS–DVB, yet 12.2% less of the *des*-Val[65] contaminant was observed in the *J*anda*J*el synthesis. These results suggest that the increased swelling of the *J*anda*J*el resin relative to traditional Merrifield resin afforded a higher yield of the pure peptide by permitting greater solvent-mediated disruption of secondary structure in the resin-bound peptide.

Since the inception of the solid phase method for the preparation of peptides and proteins,[1] enormous work has focused on addressing the impediments to high-yield, high-purity syntheses. Many coupling/deprotection problems are known to be sequence, not amino acid-dependent;[2] these 'difficult sequences' are generally attributed to the formation of β-sheet structure by the resin-bound peptide.[3] A variety of approaches have been used to address this problem, including the use of increasingly polar solvents (DMSO>DMF/NMP>DCM),[4] in situ addition of base during preactivation,[5] elevated temperature,[6] and more reactive coupling agents.[7–10]

The use of polar solvents, in situ neutralization, and improved coupling agents have been instrumental in the refinement of solid phase peptide synthesis, while other modifications are of limited applicability. For example, elevated temperature, used to denature on-resin secondary structure, can promote racemization, α→β rearrangements at Asp-Gly/Ser sequences, the formation of pyroglutamyl-capped peptides upon Gln deprotection, and dehydrated Asn/Gln side chains. In contrast, new coupling agents, including the HOAt additive,[11] aminium variants HATU[7] and TFFH,[8] and the phosphonium salts PyAoP[9] and PyBroP,[10] generally perform better than the commonly-used HOBt ester, usually with reductions in racemization and more complete acylation. Unfortunately, the higher cost of these coupling agents precludes their wholesale replacement of the standard HBTU, BOP, and carbodiimide/HOBt protocols for routine application.

An alternate route to increasing the yield of stepwise chain assembly focused on improving the polarity of the original PS–DVB resin to afford greater accessibility of the growing peptide chains to acylation and deprotection. Resins with more polar backbone structures, most notably polyacrylamide,[12] polyethylene glycol (PEG)-polystyrene graft,[13] and PEG-crosslinked acrylate/acrylamide resins,[14] have found varying degrees of usage in peptide synthesis. These resins are more compatible than PS–DVB with the relatively polar organic solvents used to disrupt on-resin secondary structure by competition for backbone amide hydrogen bonds. While they have repeatedly shown benefits in syntheses using Fmoc chemistry, most of these resins are unsuitable for Boc chemistry due to varying survival rates of their backbone and/or graft linkages upon repeated N_α-deblocking steps with neat TFA and final handle cleavage with anhydrous HF. Additionally, these resins are often of a 'sticky' consistency, making them difficult to handle, and have poor loadings. Toward the end of introducing a generally applicable improvement in the yields of solid phase peptide synthesis *without the use of additives and/or possibly deleterious reaction conditions*, we have used the recently-developed *J*anda*J*el solid support[15] to prepare

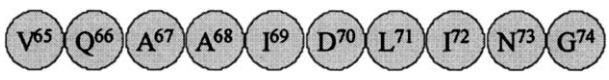

Figure 1. The primary structure of acyl carrier protein (65–74), assembled on *J*anda*J*el and PS–DVB resins.

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the classic difficult sequence, acyl carrier protein (65–74) (Fig. 1).[16]

The increased swelling of *J*anda*J*el relative to PS–DVB in organic solvents such as THF, dioxane, and benzene has been shown in our laboratory to provide improved yields in a variety of solid phase organic synthesis applications. These benefits have been independently confirmed by Shibasaki and co-workers in the development of polymer-supported catalysts.[17] We therefore reasoned that similar improvements might be attained in solid phase peptide synthesis, as the swelling of *J*anda*J*el in DMF is over 2.5 times that of PS–DVB at the same resin crosslinking (14 versus 5.2 mL/g resin). Unlike most of the PEG, acrylamide, and acrylate-based resins designed for use in Fmoc chemistry, *J*anda*J*el is stable to both neat TFA and anhydrous HF, and can be manipulated no differently from standard PS–DVB. This sparked our investigation into the utility of *J*anda*J*el in peptide synthesis using Boc chemistry.

To adequately compare *J*anda*J*el and PS–DVB, all experiments were performed in parallel using a custom-built double vessel, manual stepwise synthesis protocols described elsewhere,[18] and the same amino acid, reagent, and solvent batches. The *p*-oxymethylphenylacetic acid derivative of Boc-Gly was prepared according to literature procedures[19] and coupled to aminomethyl *J*anda*J*el and PS–DVB to obtain the PAM-derivatized resins. Stepwise elongation of the resin bound-peptide proceeded smoothly with no modifications necessary during the *J*anda*J*el synthesis. At the conclusion of the 10 coupling cycles, peptides were cleaved from the supports using anhydrous HF. ESI-MS and analytical reversed phase HPLC of the unpurified cleavage products indicated that the only substantial contaminant is the truncated *des*-Val[65] nonapeptide (Fig. 2).

The *J*anda*J*el synthesis yielded the desired decapeptide in 86.7% yield, while on PS–DVB the product was obtained in 74.5% yield.[20] Critical in this comparison is that the loadings of the *J*anda*J*el and PS–DVB batches used were 1.50 and 0.78 mmol/g, respectively. Low-loading resins are routinely used to reduce the occurrence of interchain aggregation phenomena.[21,22] It was therefore quite exciting that with nearly twice the loading of PS–DVB, a noticeable improvement was observed through the use of *J*anda*J*el resin. Clearly, aggregation difficulties did not plague the *J*anda*J*el resin as much as PS–DVB. Furthermore, the *J*anda*J*el batch used was 2 mol% crosslinked, while the PS–DVB was of 1% crosslinking. Higher-crosslinked resins typically possess greater mechanical stability; this finding has implications in the synthesis of long peptides and proteins, where repeated handling liberates fine resin particles which can obstruct reaction vessel frits.

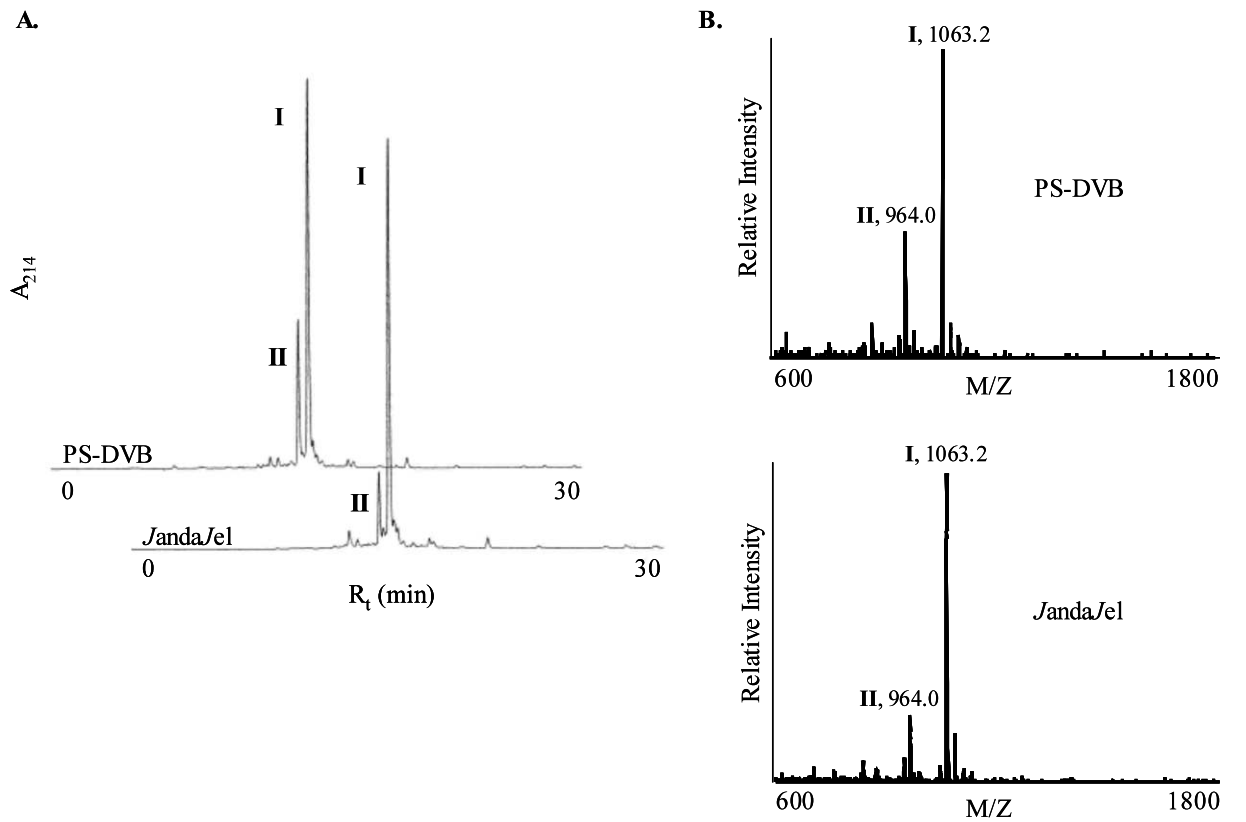

Figure 2. (**A**) Analytical RP-HPLC and (**B**) ESI-MS analysis of unpurified cleavage products. Peaks I and II are the full length and truncated *des*-Val[65] products, respectively, by ESI analysis. For HPLC analysis, exactly 20 μg of material was injected; elution was by a gradient of 0–67%B over 30 min (solvent A, 0.1% TFA in water; solvent B, 0.09% TFA in 90% acetonitrile/10% water). Mass spectra were taken using samples 1 μM in peptide, summed over three scans to approximately 1×10^5 counts.

To investigate the potential of this resin in the chemical synthesis of a protein, a well-characterized 62-amino acid protein, 4-oxalocrotonate tautomerase (4OT),[23] was synthesized in parallel using these two resins under the same rigorously identical conditions, vide supra. Analytical RP-HPLC with on-line ESI-MS detection was used to quantitate the yield of both 4OT syntheses.[24] In this comparison, both syntheses yielded comparable amounts of the full-length protein (8.2 and 8.6% for PS–DVB and *J*anda*J*el, respectively), again an unexpected result considering the loading and crosslinking disparity between these two resins.

The interplay between resin swelling and peptide chain solvation has been the subject of much discussion in the past twenty-odd years. Merrifield has suggested that nonpolar solvents which provide optimal swelling of the microporous PS–DVB resin might lead to collapse of the resin-bound peptide by poor solvation.[1] Consequently, new solvent systems were developed that simultaneously provide improved resin swelling and minimization of peptide chain aggregation phenomena.[25] We have shown that crosslink modification (1,4-bis(4-vinyl)phenoxybutane versus DVB) can yield a resin which provides tangible benefits in the synthesis of a difficult sequence using routine Boc chemistry protocols. Further investigations of the utility of *J*anda*J*el resin in peptide synthesis will be reported in due course.

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18. See Ref. 5. Peptides were synthesized on 0.2 mmol scale, with couplings performed using 2.2 mmol amino acid, 2.0 mmol HBTU, and 5.8 mmol DIEA in 4 mL DMF, preactivated for 1 minute. Deprotections were achieved using 2×1 minute neat TFA treatments.
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20. Full-length and truncated product yields were calculated by integration of respective HPLC peaks as percentages of total peak area.
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24. In both cases, the expected 4OT charge states were observed in a peak in the LC/MS chromatogram of crude material (*J*anda*J*el, 16.999 min peak, $M^{4+}=1699.15$, $M^{5+}=1359.25$, $M^{6+}=1132.95$; PS–DVB, 16.933 min peak, $M^{4+}=1698.75$, $M^{5+}=1359.55$, $M^{6+}=1132.65$; the-

oretical based on average isotopic mass, $M^{4+}=1698.66$, $M^{5+}=1359.13$, $M^{6+}=1132.78$).

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