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Resin comparison and fast automated stepwise conventional synthesis of human SDF-1α

HIRENDRA PATEL, CHRISTINA A. CHANTELL,* GERMAN FUENTES, MAHENDRA MENAKURU and JAE H. PARK

Department of Chemistry, Protein Technologies Inc., 4675, South Coach Drive, Tucson, Arizona 85714, USA

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Abstract: Human SDF-1α contains 68 amino acids and is a member of the chemokine family of peptides. This long peptide was synthesized stepwise using classical conditions in 101 h. The reaction times were then reduced to deprotection times of 2×2 min and coupling times of 2×2.5 min, resulting in a total synthesis time of 22 h. The effect of different resins, resin substitutions and deprotection reagents on the crude peptide purities was compared. A small portion of crude peptide was purified using an RP-HPLC column and the mass of the final product was confirmed with MALDI-TOF mass spectrometry. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: resin comparison; fast Fmoc SPPS; SDF-1α

INTRODUCTION

Stromal cell-derived factor 1α (SDF-1α, H_2N-KPVSLS YRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNN-NRQVCIDPKLKWIQEYLEKALNK-OH) or CXC chemokine ligand 12α (CXCL12α) is a member of the chemokine family of peptides, which is involved in basal leukocyte trafficking and homing, as well as development [1]. Many scientists are interested in SDF-1α because the interaction of SDF-1α with its receptor, CXCR4, is involved in HIV pathogenesis [2] and cancer metastasis such as in breast cancer [3] and lung cancer [4].

SDF-1α has been produced and isolated from *Escherichia coli* through recombinant protein expression. This process is not feasible with respect to labeling, purity, reproducibility, scale up, good manufacturing practice (GMP) and selective modifications [5].

To the best of our knowledge, there are no publications on the stepwise conventional Fmoc solid-phase peptide synthesis of SDF-1α. However, there is one article on a general synthetic approach for chemokines (the 'resin swap' method) that contain SDF-1α. This resin swap method is also feasible for chemokines that have *C*-terminal proline residues such as eotaxin [5].

In 1997, Ueda *et al.* [6] reported the synthesis of an SDF-1α analog by chemical ligation. The fragments of this analog were synthesized using an *in situ* neutralization Boc chemistry protocol, then assembled using chemical ligation.

In this article, we demonstrate the first conventional stepwise Fmoc solid-phase peptide synthesis of SDF-1α. We also reduce the deprotection and coupling times without any special techniques and compare the effect of different resins, resin substitutions and deprotection reagents on the crude peptide purities.

MATERIALS AND METHODS

Stepwise Peptide Synthesis

Peptides were synthesized on Protein Technologies, Inc. Symphony, Prelude or Tribute peptide synthesizers at the 20-μmol scale (25 μmol on the Symphony) using a 10-fold excess of Fmoc-amino acids (200 mM) relative to the resin. Fmoc-Lys(Boc)-Wang-PS-low-loaded (LL) resin (0.26 mmol/g) and Fmoc-Lys(Boc)-Wang-PS resin (0.55 mmol/g) were purchased from Novabiochem. Fmoc-Lys(Boc)-CLEAR resin (0.45 mmol/g) was purchased from Peptides International and Fmoc-Lys(Boc)-HMPB-ChemMatrix resin was purchased from Matrix Innovation. Deprotection was performed using either 20% piperidine/DMF or 2% DBU/20% piperidine in DMF. Coupling was performed using a 1 : 1 : 2 mixture of amino acid/HCTU/NMM in DMF. Fmoc-amino acids, HCTU, 400 mM NMM in DMF and 20% piperidine in DMF were supplied by Protein Technologies, Inc. DBU was purchased from Sigma-Aldrich. The side-chain-protecting groups for the amino acids were Trt for cysteine, histidine, asparagine and glutamine, (^{t}Bu) for aspartic acid, glutamic acid, tyrosine, serine and threonine, pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for arginine and ^{t}Boc for lysine and tryptophan. Cleavage reactions were carried out in a 95 : 2 : 2 : 1 mixture of TFA/water/anisole/ethanedithiol (EDT) for 2 h.

Peptide Analysis

Crude peptides were precipitated with ether and dissolved in HPLC grade water. Peptides were then analyzed on a Varian microsorb-MW 300 Å, 5 μm, C-18 HPLC column,

*Correspondence to: Christina A. Chantell, Department of Chemistry, Protein Technologies Inc., 4675, South Coach Drive, Tucson, Arizona 85714, USA;
e-mail: info@peptideinstruments.com

250 mm × 4.6 mm over 60 min using a gradient of 5–95% aqueous MeCN with 0.1% TFA at 1 ml/min. Detection was at 214 nm. The peptides masses were confirmed by MALDI-TOF mass spectrometry at the University of Arizona Mass Spectrometry Facility.

RESULTS AND DISCUSSION

In our 2008 study, we reported that HCTU is a very reactive and relatively inexpensive coupling reagent, which can be used as a substitute for the more expensive coupling reagent HATU. As such, we were able to use HCTU to perform fast Fmoc solid-phase peptide synthesis with coupling times of 5 min or less [7]. From our published [8] and unpublished studies, we found that lower substituted resins improved the crude purity of long or difficult peptides compared to higher substituted resins.

Therefore, we decided to synthesize SDF-1α on Fmoc-Lys(Boc)-Wang-PS-LL resin (0.26 mmol/g), which has the lowest resin loading in our stock. We initially synthesized the peptide with a classical SPPS protocol. The Fmoc-protecting group was removed using 20% piperidine in DMF for 3 min followed by 17 min and the resulting free amine group on the resin was coupled 2 × 30 min with Fmoc-protected amino acids

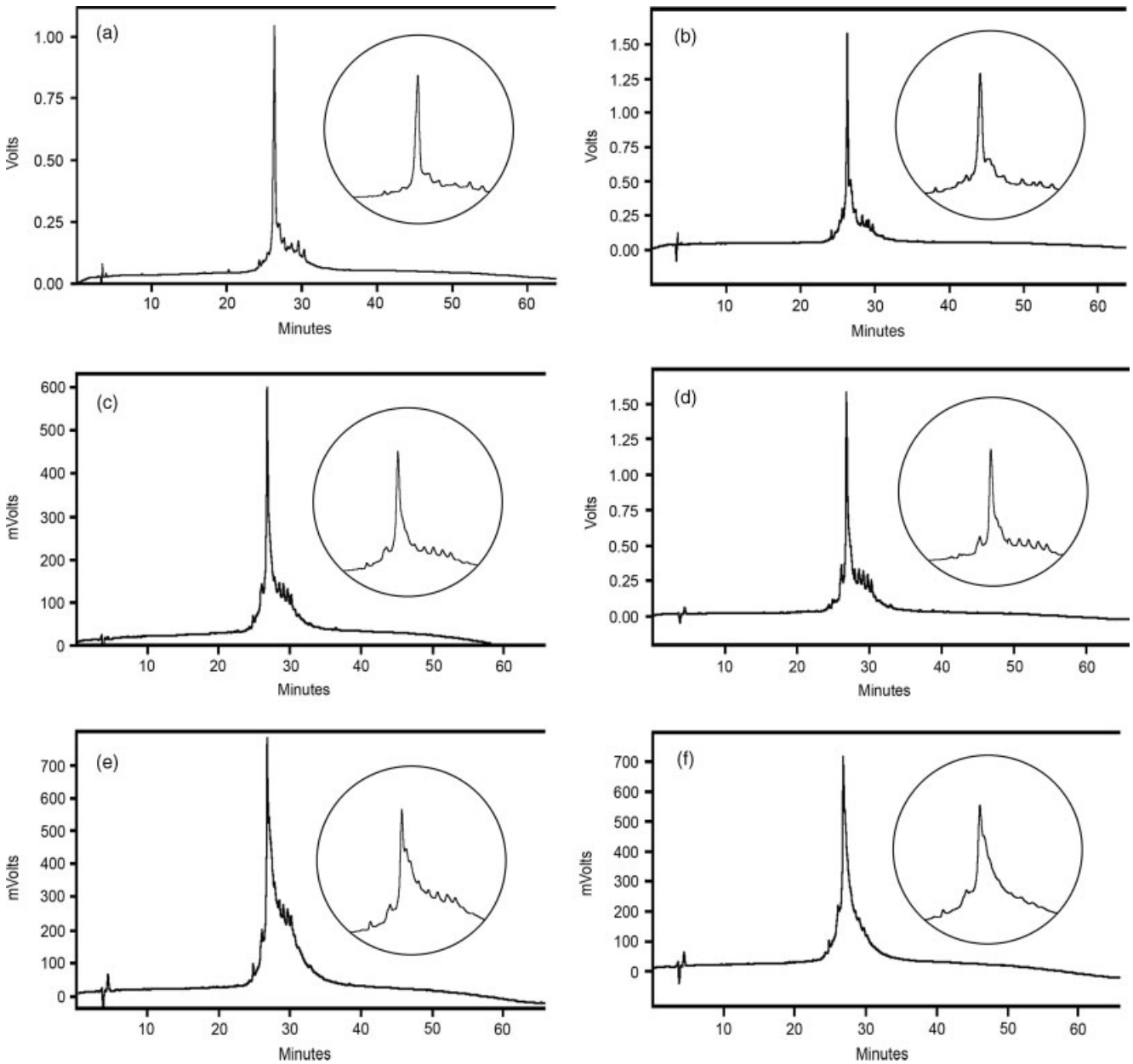

Figure 1 HPLCs of crude SDF-1α synthesized on Wang-PS resin (0.26 mmol/g) for (a) 101 h, (b) 22 h, (c) with DBU added to the deprotection mixture, (d) on HMPB-ChemMatrix resin (0.68 mmol/g), (e) on Wang-PS resin (0.55 mmol/g) and on (f) CLEAR resin (0.45 mmol/g). Blowup of main peak shown in circles.

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Table 1 Summary of resin and synthesis comparison of SDF-1α

Solid support	Linker	Substitution (mmol/g)	Deprotecting reagent	Deprotection time	Coupling time	Washes
PS	Wang	0.26	20% Pip	3 and 17 min	2×30 min	3 DMF 3 DCM
PS	Wang	0.26	20% Pip	2×2 min	2×2.5 min	1 DMF 1 DCM
PS	Wang	0.26	2% DBU/ 20% Pip	2×2 min	2×2.5 min	1 DMF 1 DCM
ChemMatrix	HMPB	0.68	20% Pip	2×2 min	2×2.5 min	1 DMF 1 DCM
PS	Wang	0.55	20% Pip	2×2 min	2×2.5 min	1 DMF 1 DCM
CLEAR	HMPA	0.45	20% Pip	2×2 min	2×2.5 min	1 DMF 1 DCM

activated with HCTU. The resins were washed three times with DMF and three times with DCM between all deprotection and coupling steps, resulting in a total synthesis time of 101 h (Figure 1(a) and Table 1). A small portion of the crude peptide was then purified by RP-HPLC and its mass was confirmed with MALDI-TOF (calculated: 7963.5 m/z, observed: 7959.9 m/z) (Figure 2). The observed mass may indicate oxidation of the cysteine residues in the peptide sequence.

From our earlier published research on fast Fmoc solid-phase peptide synthesis [7–9], we were confident that it would be possible to reduce the total synthesis time from 101 h. Deprotection times were reduced from 3 min followed by 17 min to 2×2 min, and coupling times were reduced from 2×30 min to 2×2.5 min. Washing times were also reduced from 3×30 s with DMF and 3×30 s with DCM to 1×30 s with DMF and 1×30 s with DCM. The total synthesis time with these reduced conditions was 22 h. Since there is a preshoulder and a postshoulder close to the main peak, it was difficult to determine a quantitatively accurate crude peptide purity with integration. However, the HPLCs of the crude peptides from the original and reduced syntheses are comparable (Figure 1(a) and (b)).

To investigate the effect of different resin types and loadings, we synthesized the peptide with reduced times on commercially available resins such as ChemMatrix resin, CLEAR resin and PS resin with a higher substitution. We also compared the common deprotecting reagent, 20% piperidine in DMF to a mixture of the stronger base, DBU and piperidine in DMF.

Initially, we compared the deprotecting reagent piperidine in DMF to a mixture of DBU and piperidine in DMF. Although both produced a significant postshoulder after the main peak, the postpeak impurities formed in the presence of DBU were significantly larger than when DBU was not present (Figure 1(b) and (c)). This result is different from our previous study. In our unpublished study on the synthesis of β-amyloid intermediates, we compared 20% piperidine, 2% DBU in DMF to 2% DBU in DMF and 20% piperidine in DMF. For that peptide, we found that the combination of DBU and piperidine in DMF resulted in a crude peptide that is more pure than either base alone.

We then compared resin materials and loadings. HMPB-ChemMatrix at a loading of 0.68 mmol/g (Figure 1(d)) produced a higher purity peptide than Wang-PS resin with a loading of 0.55 mmol/g (Figure 1(e)) but was less pure than the lower loaded 0.26 mmol/g Wang-PS resin (Figure 1(b)). This shows that, at comparable loadings, ChemMatrix produces a higher purity crude peptide than polystyrene. However, resin loading seems to be more influential than the resin material, for at a loading of 0.26 mmol/g polystyrene produces a higher purity crude peptide than the higher loaded ChemMatrix resin. This is similar to our previous findings [7,8]. CLEAR resin did not show an improvement over the Wang-PS-LL resin (Figure 1(f)). As it was made of a different material and had a higher loading, it is difficult to say whether one or both variables were responsible for the result.

CONCLUSIONS

A member of the chemokine family, SDF-1α, was successfully synthesized using HCTU and Wang-PS-LL resin. The total synthesis time was reduced from 101 to 22 h without special techniques. Although ChemMatrix resin was found to produce a higher purity peptide than polystyrene resin at higher loadings, low-loaded polystyrene resin was found to produce the highest purity peptide. The addition of DBU to the deprotection solution was found to produce more impurities. On the basis of our results, a combination of HCTU and low-loaded resins would be a good starting point for the synthesis of long, difficult peptides or small proteins such as chemokines at a low cost.

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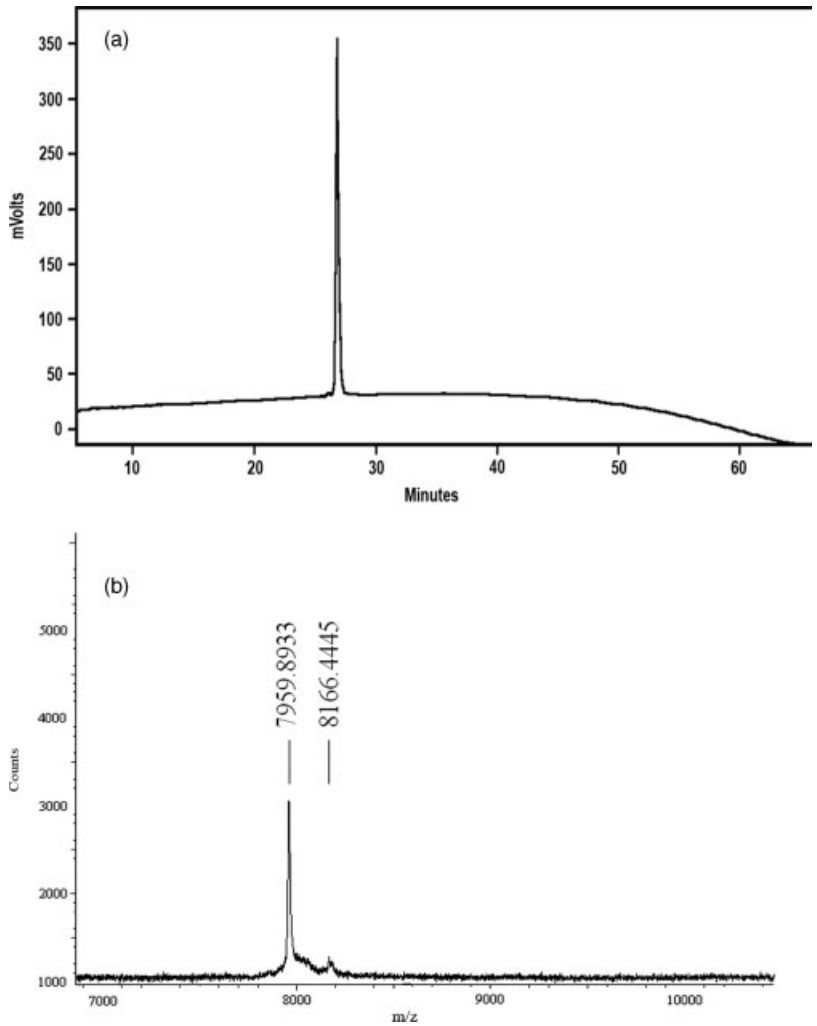

Figure 2 (a) HPLC and (b) mass spectrum of purified SDF-1α.

REFERENCES

1. Kunkel SL, Godessart N. Chemokines in autoimmunity: from pathology to therapeutics. *Autoimmun. Rev.* 2002; **1**: 313–320.
2. Berson JF, Long D, Doranz BJ, Rucker J, Jirik FR, Doms RW. A seven-tranmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. *J. Virol.* 1996; **70**: 6288–6295.
3. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metathesis. *Nature* 2001; **410**: 50–56.
4. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological azix in non-small cell lung cancer metastases. *Am. J. Respir. Crit. Care Med.* 2003; **167**: 1676–1686.
5. Cotton G, Geffroy D. Chemokines & chemical protein synthesis. *Spec. Chem. Mag.* 2006; **26**: 38–39.
6. Ueda H, Siani MA, Gong W, Thompson DA, Brown GG, Wang JM. Chemically synthesized SDF-1α analogue, N33A, is a potent chemostactic agent for CXCR4/fusin/LESTR-expressing human leukocytes. *J. Biol. Chem.* 1997; **272**: 24966–24970.
7. Hood CA, Fuentes G, Patel H, Page K, Menakuru M, Park JH. Fast conventional Fmoc solid phase peptide synthesis with HCTU. *J. Pept. Sci.* 2008; **14**: 97–101.
8. Fuentes G, Hood CA, Patel H, Page K, Menakuru M, Park JH. Fast automated conventional Fmoc solid phase peptide synthesis of human β-amyloid (1–42). In *Proceedings of the 10th Chinese Peptide Symposium*, 2008 (Submitted).
9. URL: http://www.peptideinstruments.com/doc_files/doc_5.pdf. [last accessed February 2008].

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