doi:10.1246/cl.2010.1240
Published on the web October 23, 2010

Prevention of Adsorption of Fluorescent Amino Acids to Gels by Using a Peptide Containing Dendritic Amino Acids

Mizuki Kitamatsu,* Kazumasa Akagi, and Masahiko Sisido
Department of Medical and Bioengineering, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushimanaka, Kita-ku, Okayama 700-0082

(Received September 21, 2010; CL-100805; E-mail: kitamatu@cc.okayama-u.ac.jp)

We synthesized two dendritic amino acids containing two or four carboxylic acids in the side chain. Using these amino acids, we prepared dendritic peptides that could wrap fluorescent amino acids. Because of the steric hindrance in the dendritic amino acid units present in the peptides, nonspecific adsorption of the fluorescent amino acid to gels was prevented.

Recently, we developed a method for screening peptides that bind to target proteins, by using multiple fluorescent amino acids as fluorescent tags.[1,2] In this method, protein-binding peptides can be quantified on the basis of the fluorescence patterns of fluorescent amino acids that have been converted to peptides: quantification is carried out after separation of the peptides in the protein–peptide mixture by gel filtration. Unlike the phage display method[3] and one-bead-one-compound method,[4] this method does not require the use of large carriers (phages and beads) that cause irregular binding to the target proteins; hence, precise identification of protein-binding peptides becomes possible.

This screening method essentially requires the use of many fluorescent tags having various excitation/emission wavelengths. Thus far, we have synthesized 50 fluorescent amino acids for use as fluorescent tags in the aforementioned screening method. However, 30 of these amino acids are unsuitable for use in screening because they adsorb on the gel used in the gel filtration step. For example, the fluorescent amino acid **Moc** is not adsorbed on the gel, but **Bad** and **Pyr** are readily adsorbed (These fluorescent amino acids were purchased from Watanabe Chemical (Hiroshima, Japan)). The possible reason for this is the nonspecific adsorption of the aforementioned fluorescent amino acids on the gel. Prevention of such nonspecific adsorption may help increase the number of fluorescent amino acids that can be employed as fluorescent tags in the present screening method; as a result, rapid screening of peptides may become possible.

In this study, we synthesized new amino acids having dendritic (bulky) side chains and used them for preventing the nonspecific adsorption of fluorescent amino acids on gels. The dendritic side chains cause steric hindrance in the amino acid molecules and thus help in preventing nonspecific interactions between the gel and the amino acid.

First, we synthesized dendritic amino acid derivatives Fmoc–Glu{Asp(O*t*-Bu)–O*t*-Bu}–OH (**1**) and Fmoc–Glu[Asp{Asp(O*t*-Bu)–O*t*-Bu}–Asp(O*t*-Bu)–O*t*-Bu]–OH (**2**), which contain two and four carboxylic acids in the side chain, respectively. The synthetic routes to **1** and **2** are shown in Figure 1. Fmoc–Glu–OBzl was allowed to react with H–Asp(O*t*-Bu)–O*t*-Bu to yield an intermediate. Subsequent deprotection of the Bzl group of this intermediate by catalytic hydrogenation afforded **1**[5] in 25% yield. Amino acid **2** was synthesized using Fmoc–Glu{Asp(O*t*-Bu)–O*t*-Bu}–OBzl (the intermediate formed in the synthesis of **1**) as the starting material. The *tert*-butyl ester group of Fmoc–Glu{Asp(O*t*-Bu)–O*t*-Bu}–OBzl was first removed, and the deesterified product was allowed to react with H–Asp(O*t*-Bu)–O*t*-Bu. Finally, catalytic hydrogenation was carried out in a manner similar to that described for **1**, and **2** was isolated in 16% yield. **1** and **2** were characterized by ^{1}H NMR (single species) analysis.[6]

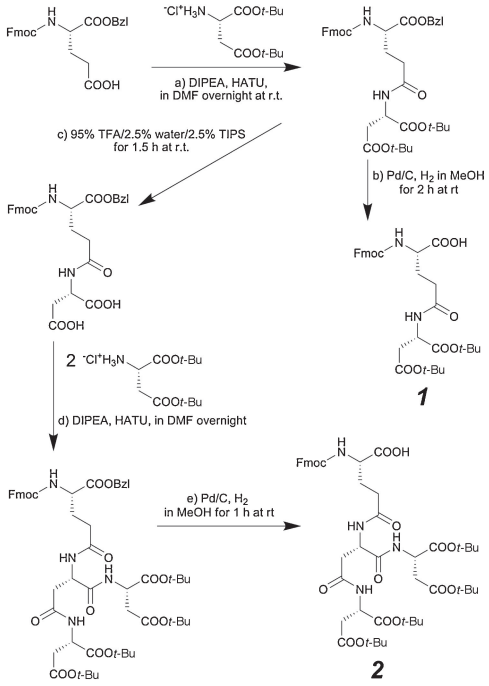

Figure 1. Synthetic routes to Fmoc-protected dendritic amino acids carrying two and four carboxylic acid *tert*-butyl esters in the side chain (**1** and **2**, respectively). a) HATU, DIPEA in DMF, overnight at rt (82%); b) Pd/C, H_2 in MeOH, 2 h at rt (31%); c) TFA/water/TIPS (95/2.5/2.5 (v/v/v)), 1.5 h at rt; d) HATU, DIPEA in DMF, overnight at rt (80%); e) Pd/C, H_2 in MeOH, 1 h at rt (78%).

To confirm whether these bulky amino acids can be incorporated into peptides, we synthesized peptides that consisted of these amino acids (Glu, **1**, and **2**) and a fluorescent amino acid by conventional solid-phase peptide synthesis (SPPS).[7] The amino acid sequences are Ac–E–E–**Pyr**–E–E–G–G–NH_2 (**E–Pyr**), Ac–**1**–**1**–**Pyr**–**1**–**1**–G–G–NH_2 (**1–Pyr**), and Ac–**2**–**2**–**Pyr**–**2**–**2**–G–G–NH_2 (**2–Pyr**), where Ac, E, and G indicate acetyl group, glutamic acid, and glycine, respectively. **Pyr** stands for 9-pyrenyl alanine, a fluorescent amino acid. The C-termini of these peptides are involved in amide bond

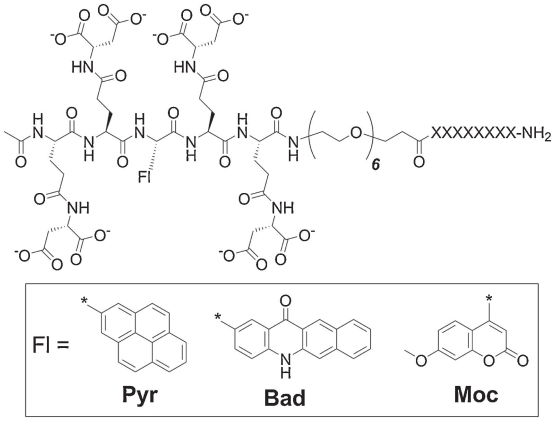

Figure 2. Chemical structures of **1–Fl–X** and fluorescent amino acids.

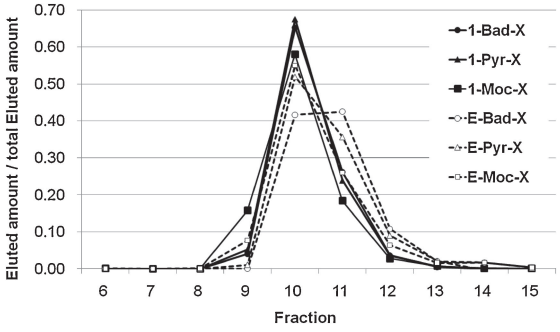

Figure 3. Amount of fluorescent-amino-acid-containing peptides in each fraction after gel filtration, as estimated from 2D-FL spectra of each fraction by least-squares analysis.

formation. These peptides have been successfully characterized by MALDI-TOF Mass.[6]

To examine whether wrapping of fluorescent amino acids by our dendritic amino acids can help prevent the nonspecific adsorption of the former on gels, we synthesized six peptides, Ac–E–E–Fl–E–E–Sp6–XXXXXXXX–NH_2 and Ac–**1**–**1**–Fl–**1**–**1**–Sp6–XXXXXXXX–NH_2 (**E–Fl–X** and **1–Fl–X**, **Fl**: **Pyr**, **Moc**, and **Bad**), by SPPS. The structures of fluorescent amino acids **Pyr**, **Moc**, and **Bad** are shown in Figure 2.[8] X indicates an equimolar mixture of 19 natural amino acids other than Cys.[9] Sp6 (Merck (Darmstadt, Germany)) indicates a long spacer consisting of ethylene glycol units.

A mixture of three peptides, **1–Pyr–X**, **1–Bad–X**, and **1–Moc–X** (final concentration in each case: 5 μM, 300 pmol), was fractionated by gel filtration (column volume: 0.7 cm (φ); column height: 20 cm; Superdex™ 75 prep grade, GE Healthcare (London, U.K.)). A 50 mM HEPES–NaOH (pH 7.4) mixture was used as the elution buffer, and elution was carried out at room temperature. Each fraction eluted from the column was diluted with 50 mM HEPES–NaOH (pH 7.4) containing 50% MeOH and analyzed by two-dimensional fluorescence (2D-FL) spectroscopy. Then, the concentration of each fluorescent-amino-acid-containing peptide in the mixture was estimated by least-squares analysis of the fluorescence intensity in the 2D-FL spectrum of the mixture, after comparing the 2D-FL spectrum with the spectra of the component fluorescent peptides. The concentrations of three peptides, **E–Pyr–X**, **E–Bad–X**, and **E–Moc–X** (controls), in another mixture were estimated by using the same protocol. The amounts of 80% of every peptide applied on gels were eluted by gel filtration (these amounts were estimated from fluorescence intensities of each peptide).

A wide concentration distribution was observed in the case of **E–Bad–X** and **E–Pyr–X**, as shown in Figure 3, while a narrow concentration distribution was observed in the case of **E–Moc–X**. These results indicated that **Bad** and **Pyr** adsorbed on the gel more strongly than did **Moc**. On the other hand, **1–Bad–X** and **1–Pyr–X** showed the same narrow distribution as did **E–Moc–X**. **1–Moc–X** too showed a narrow distribution, as in the case of **E–Moc–X**. To determine the degree of distribution, we estimated FWHM/f_{max} (FWHM: full width at half-maximum, f_{max}: the maximum ratio of the amount of the peptide in a given fraction to the total amount of peptide eluted) for each fluorescent-amino-acid-containing peptide in Figure 3. The FWHM/f_{max} values of **E–Bad–X**, **E–Pyr–X**, and **E–Moc–X** were 1.37, 0.98, and 0.36, respectively. The FWHM/f_{max} values of **1–Bad–X**, **1–Pyr–X**, and **1–Moc–X** were 0.26, 0.27, and 0.32, respectively. These results confirmed that **1** modified to the fluorescent amino acids in peptides prevents the nonspecific adsorption of the fluorescent amino acids on gels.

In summary, we successfully synthesized new dendritic amino acids having two or four carboxylic acids in the side chain. Nonspecific adsorption of fluorescent amino acids on gels could be prevented by wrapping the fluorescent amino acids with the synthesized dendritic amino acids. The method of prevention of nonspecific adsorption of fluorescent compounds by these dendritic amino acids can be utilized to the number of fluorescent amino acids adsorbed on gels and proteins. This method does not need special modifications on the fluorescent compounds. The method also gives water solubility to hydrophobic fluorescent compounds by multiple glutamic acids on the dendritic amino acids. By this method, the number of fluorescent amino acids will be employed in our peptide-screening method by using multiple fluorescent amino acids.

References and Notes

1 M. Kitamatsu, M. Futami, M. Sisido, *Chem. Commun.* **2010**, *46*, 761.
2 M. Kitamatsu, T. Yamamoto, M. Futami, M. Sisido, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5976.
3 J. K. Scott, G. P. Smith, *Science* **1990**, *249*, 386.
4 K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, R. J. Knapp, *Nature* **1991**, *354*, 82.
5 Hydrogenation of the compounds must be carried out under carefully controlled conditions because prolonged hydrogenation may lead to deprotection of the Fmoc group compound.
6 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
7 M. Kitamatsu, T. Kashiwagi, R. Matsuzaki, M. Sisido, *Chem. Lett.* **2006**, *35*, 300; M. Kitamatsu, M. Shigeyasu, M. Saitoh, M. Sisido, *Biopolymers (Pept. Sci.)* **2006**, *84*, 267; M. Kitamatsu, M. Shigeyasu, M. Sisido, *Chem. Lett.* **2005**, *34*, 1216; M. Kitamatsu, M. Shigeyasu, T. Okada, M. Sisido, *Chem. Commun.* **2004**, 1208.
8 Abbreviations used are as follows: Fmoc, 9-fluorenylmethoxycarbonyl; Bzl, benzyl; **Pyr**, 1-pyrenyl alanine; **Bad**, [benzo[*b*]-acridin-12(5*H*)-on-2-yl]alanine; **Moc**, 4-(7-methoxycoumaryl)-alanine.
9 Cys was removed to avoid intracyclization, dimerization, or polymerization of peptides.

