Synthesis of Peptide Dendrimer

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We report a facile and specific method to ligate the 1,2-amino thiol moiety of N-terminal cysteine of an unprotected 24-residue peptide to a glyoxylyl scaffolding to yield a dense octabranched thiazolidinyl peptide dendrimer with a MW of 24 205. Peptide dendrimers[1,2] with their characteristic branched structures (Figure 1) represent a class of artificial proteins assembled on a scaffolding or template and which would attain the macromolecular bulk as proteins, but have the advantages that they self-assemble and obviate the need of extensive folding required for biochemical activity. The flexibility of designing scaffoldings and the attendant dendritic peptides has led to successful engineering of artificial proteins which function as enzymes,[3] ion channels,[4] antibiotics,[5] diagnostic reagents,[6] and vaccines.[7]

While these peptide dendrimers present exciting opportunities, they also present a demanding challenge to their synthesis. Current methods of stepwise solid-phase synthesis of peptide dendrimers are inadequate to yield such macromolecular products with high purity. Although the use of protected peptide monomers offers improvements,[8] it suffers the limitations of poor solubility and slow coupling reactions. A more direct and efficient approach is the use of nonpeptidyl linkages for the ligation between the unprotected peptide segments and scaffoldings. Examples of this approach include conjugation through thioalkylation,[9] thioester,[10] and oxime.[11] Other applicable but yet untried methods include hydrazone,[12] reverse proteolysis,[13] and domain ligation.[14]

Domain ligation is particularly appealing because it utilizes the facile and chemoselective reaction between a weak base such as 1,2-amino thiol and an alkyl aldehyde to give thiazolidine ring under acidic conditions.[15] With small peptides, this reaction is usually completed within 10 min and highly specific for the N-terminal of Cys.[16] Unprotected side chains of lysine, arginine, and other amino acids are excluded from this reaction to allow totally unprotected peptides to be ligated to an aldehyde-containing scaffolding. To illustrate the utility of this reaction in the synthesis of peptide dendrimers, we used an octavalent lysinyl scaffolding, popularly known as MAP (multiple antigen peptide) which consists of three levels of sequentially branched lysine[6] (Lys_4-Lys_2-Lys-β-Ala). This scaffolding has been found to be useful in the design of peptide-based vaccines,[7] artificial enzymes,[3] and triple helix structures.[17] For these applications, dendrimers containing three or four branches of peptides are usually sufficient. To meet the challenge of this reaction, we used a MAP model containing eight branches that gives a highly compact dendrimer (Figure 1). The alkyl aldehyde in the scaffolding was generated by oxidizing the 1,2-amino ethanol moiety of the N-terminal Ser on the scaffolding,[18] [Ser_8Lys_4-Lys_2-Lys-β-Ala (Ser_8-MAP)], with sodium periodate at pH 7 to yield a glyoxylyl derivative of $(HCO)_8$-MAP in nearly quantitative yield.[19] For the weak base, we made use of the 1,2-amino thiol group of Cys at the N-terminal of a purified and unprotected 24-amino acid residue peptide, CI-24 (CNYNKRKRIHIG-PGRAFYTTKNII) obtained by the solid phase method.[20] CI-24 contains the principal neutralizing determinant of the surface coat protein gp120 of HIV-1, MN strain and which is a target for the development of HIV-1 vaccines.

The thiazolidine ligation was adequately performed at pH 4.2 in H_2O. However, we found that the use of an organic cosolvent and elevated temperature (37 °C) provided consistently better results than in H_2O alone because they enhanced the rate of formation and prevented various intermediate dendrimers aggregating or precipitating during the course of the reaction. The best combination was found to be *N*-methylpyrrolidinone (NMP): H_2O,[21] (1:1, v/v). Other organic cosolvents such as DMSO or DMF were not suitable. DMSO was shown to be a mild oxidant that led to disulfide formation of cysteinyl containing peptides and DMF led to formylation (M + 28) of the unprotected peptide as shown by MS analysis of products containing M + 28 peaks. Using the optimized NMP:H_2O mixture, the less-hindered tetra- and pentameric MAPs were completed in <2 h, while the hexa- and heptameric MAP required 7 and 30 h, respectively (Figure

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(18) Peptide CI-24 and octameric MAP core were synthesized by the solid phase method using Boc chemistry according to our previous method.[21,7a] N-terminal Ser residues on MAP core were oxidized to glyoxylyl moiety according to Greoghegan et al.[19b] LD-MS analysis of CI-24 (calcd) 2864.4, (found) 2868 and amino acid analyses of these compounds agreed with the calculated values.

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(21) The procedure used for thiazolidine ring formation was as follows. All solutions used for this reaction were deaerated and purged with argon and EDTA (0.02 M) and were added to prevent disulfide oxidation. Peptide CI-24 (7.18 mg) dissolved in 0.02 M NaOAc (0.13 mL) containing 0.02 M EDTA to give a 20 mM solution at pH 4 were added to a 11.6 mM solution of MAP core (0.166 mg) in H_2O (0.01 mL) and diluted with an equal volume of NMP:HOAc (9:1, v/v) to give a final volume of NMP:H_2O at 1:1. The reaction was performed at 37 °C for 80 h. The progress was followed by HPLC. The product was purified by semipreparative HPLC and characterized by LD-MS.

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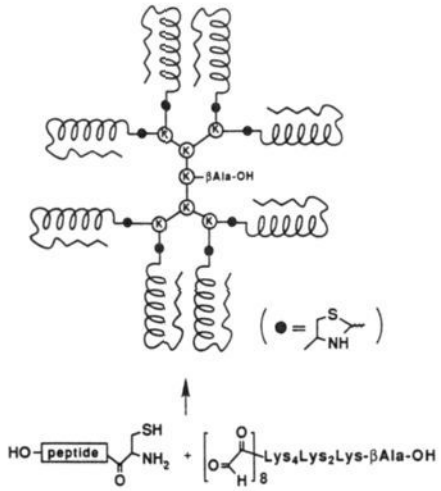

Figure 1. A schematic representation of a peptide dendrimer containing eight peptidyl branches anchored on a scaffolding of oligolysine (indicated by circled K) via a thiazolidine linkage (solid circles) which is obtained by reacting the N-terminal cysteine with a glyoxylyl scaffolding. Note, a new assymmetric carbon is generated in the thiazolidine ring. The peptide CI-24 is derived from the V3-loop, the principal neutralizing determinant of gp120, HIV-1 and whose crystal structure shows helix-turn-β-sheet structure.[22]

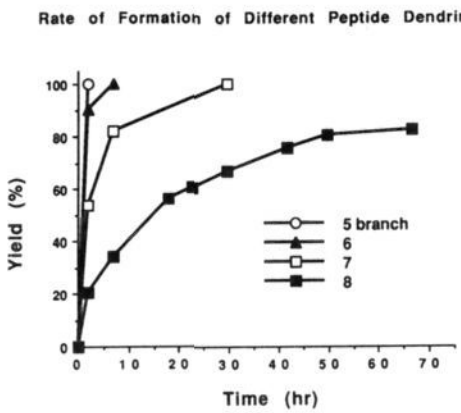

Figure 2. Rate of formation of different peptide dendrimers in NMP: H_2O (1:1, v/v) at 18 °C.

2). The more hindered, fully substituted octameric MAP was found to give 82% in 67 h.

The advantages of this approach were apparent in the purification and characterization of the end products. Each form of dendrimers was clearly identified by RP-HPLC despite their large molecular weights (Figure 3). More importantly, their order of elution was in the same order of their increased MW, which allowed easy monitoring and optimization. Furthermore, their MW [octamer (calculated/found):24 205 D/24 211 ± 24 u; heptamer: 21 358.8/21 361 ± 21.4 u; hexamer: 18 512/18 517 ± 18.5 u; pentamer: 15 666/15 658 ± 15.7 u) were unambiguously established by mass spectral analysis in addition to other conventional characterization (Figure 4).

Our work is significant in several regards. First, the thiazolidine ligation adds a new reaction to the repertoire of currently known methods applicable to the synthesis of peptide dendrimers.[8-12] In contrast to the linkage such as thioester, it was found to be stable in pH 3–8 for 3 days without observable degradation. It differs from other known linkages to give a five-membered ring structure and offers possibility of conformational rigidity. Furthermore, it also is the only known method that is capable of producing an amide bond linkage when the aldehyde component is a glycoaldehyde ester through the intramolecular O,N-acyl transfer

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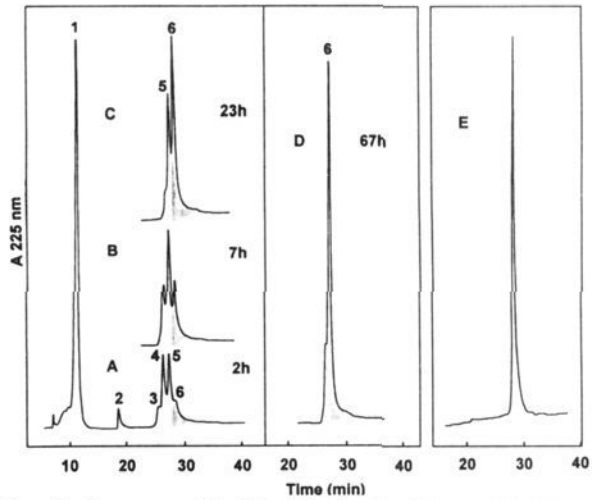

Figure 3. Time course of the thiazolidine ligation of Cys-peptide (CNYNKRKRIHIGPGRAFYTTKNII) and the glyoxylyl scaffolding [$(HCOCO)_8Lys_4Lys_2Lys$-βAla] analyzed by RP-HPLC (Vydac analytical column 218TP54). Panel A = 2 h, peak 1 is the excess Cys-peptide; peak 2 is a side product of peak 1 in the disulfide form; peak 3, 4, 5, and 6 correspond to penta-, hexa-, hepta-, and octameric forms. The MW of these forms were confirmed by LD-MS. Panels B to D show the progression and completion of each dendrimer. Panel E shows the purified octameric peptide dendrimer. HPLC condition: linear gradient of 0.67% B/min from 30% buffer B. Buffer A = 100% H_2O with 0.05% CF_3CO_2H and buffer B = 60% CH_3CN with 0.039% CF_3CO_2H; flow rate at 1.5 mL/min.

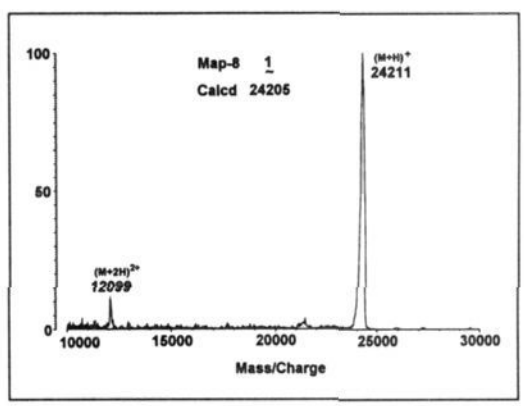

Figure 4. LD-MS (laser desorption mass spectrometry) analysis of the octameric form of peptide dendrimer.

reaction previously developed in our laboratory.[14] Second, both components of the reactions were derived from readily accessible amino acids. Different variations of placing the 1,2-aminothiol and aldehyde at side chains, N- or C-terminus provide the versatility of this reaction. Third, our peptide dendrimer is a compact octamer with a MW of 24 205. It surpasses in size the octaoxime peptide dendrimer prepared by K. Rose[11] which has a MW of 19 916 and is believed to be the largest artificial protein obtained by controlled synthesis. Utilizing improved methodology, larger and more complicated peptide dendrimers with interesting properties will undoubtedly be prepared in the future.

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Supplementary Material Available: Tables of heptamer and octamer yields and LD-MS analysis of dendrimic intermediates (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.