Pergamon

Tetrahedron Letters, Vol. 37, No. 29, pp. 5199-5202, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01050-7

The Gramicidin Pore : Selective Tryptophan Replacement with Aspartic Acid

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Abstract: The chemoselective transformation of Trp^9 and Trp^{11}, located at the hydrophobic core of the antiparallel, double helical pore of O-acetyl gramicidin A(HCO-ValGlyAlaLeu°AlaVal°ValVal° TrpLeu° TrpLeu°TrpLeu°TrpNHCH$_2$CH$_2$OAc; o = D isomer) to Asp^9 gramicidin and Asp^9 Asp^{11} gramicidin has been demonstrated by oxidation with 0.25 eq. of *in situ* generated Ru(VIII), HPLC isolation, amino acid analysis and peptide sequencing.

The organic chemistry of the 20 coded amino acid side chains within the protein manifold, that play such an important role in protein structure, protein folding, protein design and protein function, has not received its due attention. Since the pioneering endeavours of Witkop in the fifties developments here have not been sustained. Experimental difficulties and skeptisism relating to our ability to perform selective operations within the confines of highly intricate protein structures continue to haunt this area. Yet, the delineation of chemoselectivity, arising from protein structural constraints will provide practical inputs across the entire domain and thus merits attention. Recent pioneering studies have shown that such selective operations are possible.[1]

We have endeavoured to develop during the past decade, in stages, the methodologies for coded amino acid side chain modification, the achievement of target side chain selectivity over competing sites and finally the delineation of preferences amongst the same side chain, as a function of its placement in the protein secondary/tertiary structure framework.[2]

The present communication reports the use of the dimeric gramicidin pore as the substrate and leading to *the unequivocal finding that, of the four tryptophan residues located at 9, 11, 13 and 15 sites, the 9-Trp, placed in the hydrophobic core, is most preferentially transformed to aspartic acid,* with *in situ* generated Ru(VIII) species, in a biphasic system.

Gramicidin A (GA) [HCO-ValGlyAlaLeu°AlaVal°ValVal°TrpLeu°TrpLeu°TrpLeu°TrpNHCH$_2$ CH$_2$OH] is produced by Bacillus brevis, during transition from vegetative to sporulation phase. This relatively small peptide (molecular weight ~ 1880), of non ribosomal origin, can, by dimer formation, generate either double helical pores or single channels, both having a hydrophilic interior and a hydrophobic surface. The pores sequester and transport monovalent ions. As could be seen from Figure 1, the pores have chirally alternating

side chains extending outwards and on the same side. The resulting vertical stacking of the indole rings of Trp^9 and Trp^{11}, permit, by interdigitation, helical clusters of pores. In addition, the residues act as splines enabling the nestling of lipids to form stable channels. The dynamic processes associated with pore and channel formation and the transformation of the former to the latter has been well studied. In solvents of low polarity, ranging from dioxan to methanol, GA and simple analogs exist almost exclusively as a double helical dimer, deriving stabilization from as many as 28 intramolecular hydrogen bonds. The predominant species here is an anti-parallel left handed double helix having 5.6 residues per turn, which crystallizes from methanol in the monoclinic form and whose structure has been established as 1 by X-ray crystallography. This representation brings out the unique stacking profile of Trp^9 and Trp^{11}, -*located in neighbouring strands*- which also was shown to play a role in the formation of helical clusters of pores by interdigitation.[3]

Clearly, Trp^9 and Trp^{11} are located in the hydrophobic region of 1 and this aspect can be taken advantage of, to bring about chemoselective changes. Thus, a reagent whose affinity lies towards this region, can be expected to selectively interact with these residues. We have experimentally verified this notion.

Gramicidin A (GA)[4] was protected as O-acetyl gramicidin A (GA-OAc) in 75% yields.[5] A suspension of GA-OAc (0.07 g, 0.036 mmol) in CCl_4 : MeCN : H_2O (1.5 : 1.5 : 3 mL) was admixed with $NaIO_4$ (0.648 mmol, 0.25 eq), $RuCl_3.3H_2O$ (< 1 mg), cooled in ice, sealed, left shaken at RT for 8 h, cooled, cautiously opened, filtered, residue washed with CCl_4 (2 mL), MeCN (2 mL), evaporated in vacuo, extracted with MeOH (3 x 5 mL) and evaporated. HPLC performed on the residue (0.038 g) as ten equal batches on a reverse phase column using a dual solvent gradient system [solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN; t(min) (A : B%): 0(100:0), 5(80:20), 45(70:30), 50(0:100)], led to the isolation of eluents of the two major peaks at retention times, respectively, 25 and 29 minutes (fractions II and I respectively).

Amino acid analysis of fraction I (0.007 g) showed the transformation of one of the four tryptophan residues to aspartic acid. Peptide sequencing showed that it was Asp^9 GA-OAc (**2**). The more polar fraction II (0.018 g), when similarly processed, was found to have the sequence $Asp^{9,11}$GA-OAc (**3**).

The yields of **2** and **3** are 10% and 28% respectively, based on GA-OAc used.

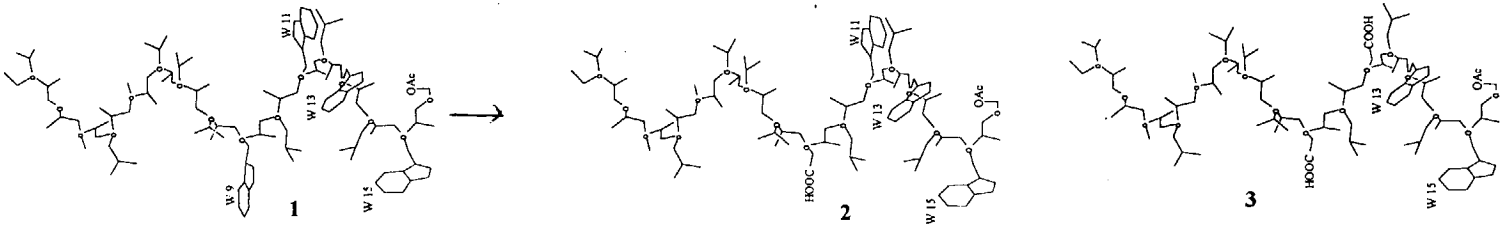
In Figure 1 are presented- along with the gross changes seen here- the profile of gramicidin pore as seen from X-ray crystallographic studies (**1**) and the possible secondary structures for Asp^9 gramicidin (**2**) and $Asp^{9,11}$ gramicidin (**3**).

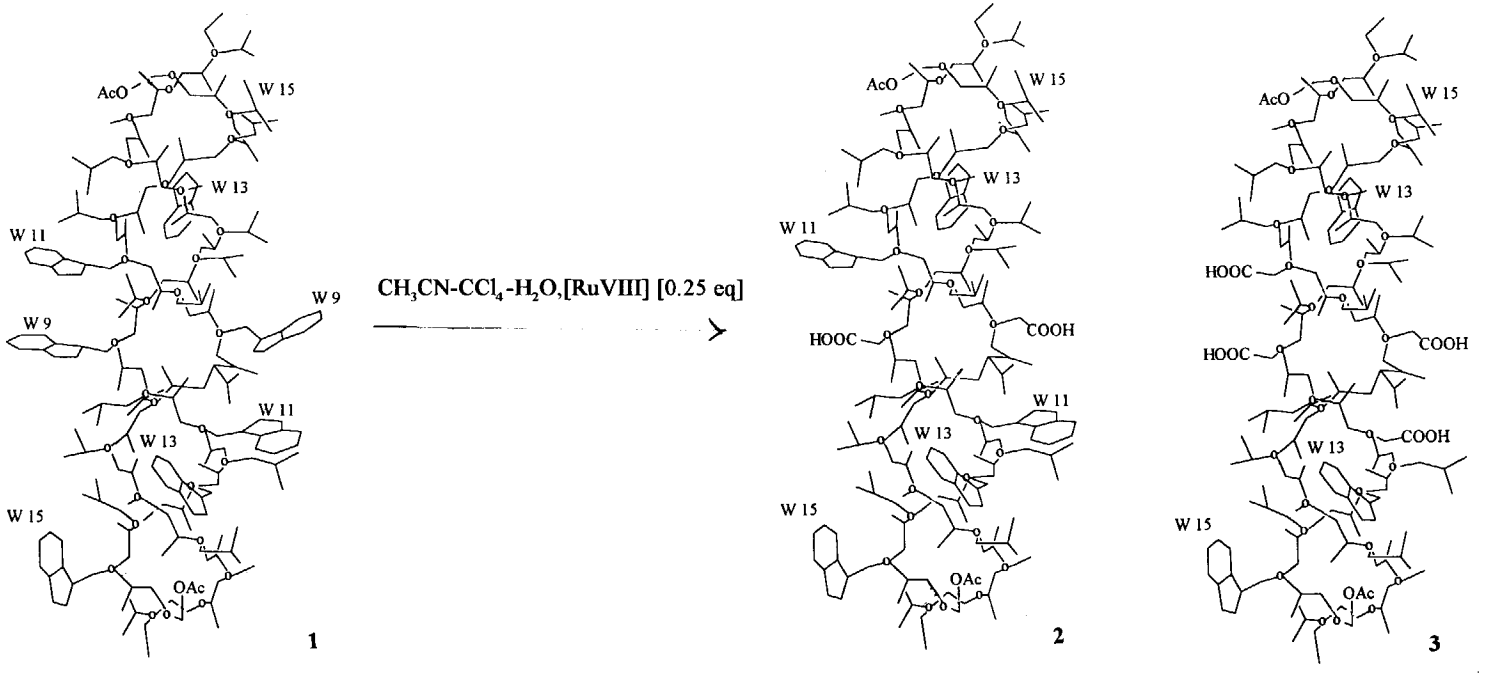
The selectivity profile seen here is strongly suggestive of the envisaged pore conformations for **2** and **3**. In any event, chemoselectivity is possible only when the interaction with the reagent takes place in the pore model of gramicidin A (**1**).

Oxidation of GA-OAc with equivalent of the reagent afforded poor yields of crude products, whose HPLC profile was quite complex.

A surprising number of criteria had to be met in the transformation of **1**-->**2** and **3**. The Ru(VIII) mediated Trp-->Asp change has been shown by us to proceed via sequence, N-formyl kynurinane --> kynurinane --> γ-oxo glutamic acid -->Asp.[6] In order to achieve selectivity, this cascade should effectively compete over attack on the Trp^{11} site.[7] Of the four Trp residues located at 9, 11, 13, and 15 positions in GA, the former pair are at the hydrophobic core. Indeed, crystallographic studies tend to show that polar molecules can reach and envelop Trp^{13} residues.[3d] Since in Ru(VIII) oxidations in bi-phasic media, the reagent is partitioned heavily in favour of the organic phase, the observed preference for Trp^9 and Trp^{11}, is understandable. The exposed profile of Trp^9 over Trp^{11} clearly seen in **1**, would explain the preference shown

Figure 1


W 11
W 13
OAc
W 9
W 15
1
HOOC
2
COOH
3


AcO
W 15
W 13
W 11
W 9
W 9
W 11
W 13
W 15
OAc
1
CH3CN-CCl4-H2O,[RuVIII] [0.25 eq]
AcO
W 15
W 13
W 11
HOOC
COOH
W 11
W 13
W 15
OAc
2
AcO
W 15
W 13
HOOC
HOOC
COOH
COOH
W 13
W 15
OAc
3

for Trp^9 oxidation.[8]

From a practical vantage, compounds **2** and **3** are excellent substrates, particularly pertaining to possibilities for the formation of pore clusters with bi-valent cations. Since the procedure presented here offers a simple route to **2** and **3** from the commercially available gramicidin, diverse studies relating to the understanding of the profile of these are planned.

Acknowledgements: We are grateful to Professor F. Westheimer, Department of Chemistry, Harvard University, Cambridge, USA for encouragement, Dr. Darshan Ranganathan, RRL, Trivandrum and Dr. D. Balasubramanian, CCMB, Hyderabad, for valuable discussions. Financial assistance from DST and INSA, New Delhi is gratefully acknowledged.

References and Notes

1. a. Barton, D. H. R.; Herve, Y.; Potier, P.; Thierny, J. *J. Chem. Soc., Chem. Commun.* **1984**, 1298; b. Kaiser, E. T.; Lawrence, D. S. *Science* **1984**, *226*, 505; c. Offord, R. E. *Prot. Eng.* **1987**, *1*, 151; d. Kaiser, E. T. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 913; e. West, J. B.; Scholten, J.; Stolowich, N. J.; Hogg, J. L.; Scott, A. I. *J. Am. Chem. Soc.* **1989**, *111*, 4513; f. Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1990,** *112*, 2457.

2. a. Ranganathan, S.; Ranganathan, D.; Bamezai, S.; Singh, W. P.; Bhattacharyya, D.; Singh, G. P.; Rathi, R.; Saini, S.; Jayaraman, N.; Patel, B. K. *Pure & Appl. Chem.* **1990**, *62*, 1437 and references cited therein; b. Ranganathan, S.; Ranganathan, D.; Bhattacharyya, D. *Tetrahedron Lett.* **1991**, *32*, 5615; c. Ranganathan, S.; Jayaraman, N. *J. Chem. Soc., Chem. Commun.* **1991**, 934; d. Ranganathan, S.; Patel, B. K. *Tetrahedron Lett.* **1993,** *34*, 2533.

3. a. Veatch, W. R.; Blout, E. R. *Biochemistry* **1974,** *13*, 5257; b. Sychev, S. V.; Nevskaya, N. A.; Jordanov, St.; Shepel, E. N.; Miroshnikov, A. T.; Ivanov, V. T. *Bioorg. Chem.* **1980**, *9*, 121 [solution studies]; d. Wallace, B. A.; Ravikumar, K. *Science* **1988**, *241*, 182; e. Langs, D. A. *Science* **1988**, *241*, 188; f. Langs, D. A.; Smith, G. D.; Courseille, C.; Precigoux, G.; Hospital, M. *Proc. Natl. Acad. Sci. USA.* **1991**, *88*, 5345 [X-ray crystallographic studies].

4. Commercial gramicidin [gramicidin D (Dubos)] from Sigma was used.

5. Sarges, R.; Witkop, B. *J. Am. Chem. Soc.* **1965**, *87*, 2011.

6. a. Ranganathan, S.; Ranganathan, D.; Bhattacharyya, D. *J. Chem. Soc., Chem. Commun.* **1987**, 1085; b. Bystrov, V. F.; Arseniev, A. S. *Tetrahedron* **1988**, *44*, 925.

7. None of the several HPLC fractions examined had Asp at all locations. We believe that the initially formed kynurinane is preferentially transformed to Asp over attack on a fresh Trp residue. The formation of Asp^9 GA as well as $Asp^{9,11}$ GA strongly supports the above notion. Oxidation on a large scale followed by esterification of Asp residues and column chromatography are planned to isolate **2** and **3** for conformation studies by NMR, metal complexation and X-ray crystallography. The initial objective reported here, was to unequivocally demonstrate chemoselectivity of like residues, based on secondary structures.

8. The Trp^9 was also found to be the most sensitive to degradation, of GA incorporated into sodium dodecyl sulfate micelles, when subjected to UV irradiation. Surprisingly, this study showed that Trp^{11} was the least sensitive. Trp^{13} and Trp^{15} showed intermediate sensitivity (McKim, S.; Hinton, J. F. *Biochim. Biophys. Acta* **1993**, *1153*(2), 315-21.

(*Received in UK* 10 *April* 1996; *revised* 23 *May* 1996; *accepted* 31 *May* 1996)