Preparation and Properties of Trimethylammonium Group-containing Analogues of [D-Ala^2,Leu^5]Enkephalin and Gramicidin S

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Quaternization of primary amino groups of derivatives of [D-Ala^2,Leu^5]enkephalin and gramicidin S with MeI–$KHCO_3$ in MeOH afforded new biologically active analogues possessing trimethylammonium group(s).

Syntheses of analogues of biologically active peptides containing *N*-methylamino acids in place of normal α-amino acids have been reported frequently in structure–activity studies. Few reports, however, have dealt with peptide analogues possessing the trimethylammonium group. We were interested in the efficient conversion of a primary amino group into the trimethylammonium group using MeI–$KHCO_3$ in MeOH described by Chen and Benoiton.[1] Preparation of analogues having a trimethylammonium group in place of the primary amino group would provide compounds with the following characteristics. (i) Retention of positive charge on the N atom which is lost by the usual acylation-type modification. (ii) Absence of HN which is sometimes essential to the interaction with receptor sites through H-bonding. (iii) Steric bulkiness provided by the $N(Me)_3^+$ group (compared to the NH_3^+ group). Thus, by means of quaternization using MeI–$KHCO_3$, preparation of trimethylammonium group-containing analogues of some biologically active peptides has been attempted.

To prepare analogues of the opioid pentapeptide [D-Ala^2,Leu^5]enkephalin (**1**) (Tyr-D-Ala-Gly-Phe-Leu), the protected pentapeptide (**2**) (Boc-Tyr-D-Ala-Gly-Phe-Leu-OEt) (m.p. 110—112 °C) was synthesized by conventional methods. Removal of the N-protecting group of (**2**) gave the trifluoroacetate of pentapeptide ethyl ester (**3**) (m.p. 119—123 °C), which was subjected to the quaternization reaction: *i.e.*, a mixture of (**3**) (0.11 mmol), MeI (2.2 mmol), and $KHCO_3$ (1.1 mmol) in MeOH (2.2 ml) was stirred for 38 h at room temperature. Silica gel and Sephadex LH-20 column chromatography afforded the trimethylammonium group-containing analogue (**4**) (m.p. 139—142 °C, yield 65%), which on saponification furnished the zwitterionic trimethyl

Table 1. Biological activities of analogues of [D-Ala^2,Leu^5]enkephalin (**1**).

(Tyr-D-Ala-Gly-Phe-Leu)			GPI contraction[a]	Receptor binding[a]
Analogue	N-terminal	C-terminal	(% inhibition)	(I.C.$_{50}$/M)
(**1**)	H_3N^+	CO_2^-	78	3.1×10^{-10}
(**7**)	H_3N^+	$CONH_2$	75	1.7×10^{-10}
(**3**)	H_3N^+	CO_2Et	63	5.7×10^{-10}
(**5**)	Me_3N^+	CO_2^-	76	1.0×10^{-8}
(**8**)	Me_3N^+	$CONH_2$	65	1.7×10^{-8}
(**4**)	Me_3N^+	CO_2Et	7	8.0×10^{-8}
(**6**)	Me_3N^+	CO_2Me	22	1.0×10^{-7}

[a] See text.

analogue (**5**) (Me_3Tyr^+-D-Ala-Gly-Phe-Leu-O$^-$) (m.p. 190—193 °C, anal: $C_{32}H_{45}N_5O_7 \cdot \frac{5}{2}H_2O$). Transesterification of (**4**) with methanolic HCl gave the corresponding methyl ester, possessing a trimethylammonium chloride group, (**6**) (m.p. 124—128 °C). Quaternization of [D-Ala^2,Leu^5]enkephalinamide (**7**), derived from (**2**) by treatment with methanolic NH_3 followed by deprotection, afforded the trimethylammonium group-containing analogue (**8**) (m.p. 158.5—161.5 °C as hydrogen carbonate). All the analogues synthesized exhibited reasonable 200 MHz ^{1}H n.m.r. spectra. The pharmacological action of these analogues (10^{-5} M) was tested on guinea pig ileum (GPI) stimulated transmurally at 0.05 Hz with pulse duration of 1 ms and the potency to inhibit contraction of the ileum is shown in Table 1. The effect of these analogues on the binding of [^{3}H][D-Ala^2,Met^5]enkephalinamide (2 nM) to rat brain membranes was also examined by the filter method using Whatman GF/B glass filters[2] and the I.C.$_{50}$ (concentration producing 50% inhibition) values are also listed in Table 1. The low opioid potency of (**4**) and (**6**) having C-terminal ester functions is consistent with the result reported by DiMaio *et al.*[3] for Me_3Tyr^+-Gly-Gly-Phe-Leu-OMe. On GPI assay the trimethylammonium analogues (**5**) and (**8**), however, exhibited activity only slightly lower than the corresponding (**1**) and (**7**), respectively, having unmodified N-terminals. The moderate opioid potency of (**5**) and (**8**) discovered in this study is interesting considering the low activity of (**4**) and (**6**) and also the loss of activity of the 3-hydroxy-*N*-methylmorphinan opiates on quaternization.[4] The result seems to indicate that the presence of an H atom on the terminal N atom, which enables H-bonding interaction, is not essential for the interaction of enkephalin molecules with opioid receptors.

The quaternization reaction was then applied to gramicidin S (**9**), a cyclic decapeptide antibiotic with the primary structure cyclo[-(Val-Orn-Leu-D-Phe-Pro)$_2$-]. The resulting hexamethyl analogue having two trimethylammonium iodide groups on Orn sidechains, namely [Orn$^+$(Me)$_3^{2,2'}$]GS·2I$^-$ (**10**) (m.p. 228—231.5 °C, yield 75%), gave satisfactory results in amino acid analysis and elemental analysis ($C_{66}H_{106}N_{12}O_{10}I_2 \cdot 5H_2O$). The c.d. spectrum of (**10**) recorded in MeOH was quite similar to that of (**9**) and the 270 MHz ^{1}H n.m.r. spectrum of (**10**) measured in [$^2H_6$]Me_2SO solution was also similar to that of (**9**), having a shielded NH signal for Val (δ 7.25) and a small coupling constant for the NH of D-Phe (δ 9.16, d, *J* 3.1 Hz), which indicated that (**9**) and (**10**) adopt the same intramolecularly H-bonded β-sheet conformation. The bis(trimethylammonium)-type analogue (**10**) exhibited essentially the same antimicrobial activity as the parent (**9**) against several micro-organisms tested including *Staphylococcus aureus* and *Bacillus subtilis*. This result demonstrated that when these antibiotics bind to bacterial membrane or receptor molecules in order to manifest their activity, the function of the charged Orn sidechains does not involve interaction through H-bonding but is simply electrostatic in nature.

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