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DEHYDROLYSINE CONTAINING ANALOGUES OF TUFTSIN: SYNTHESIS AND BIOACTIVITY

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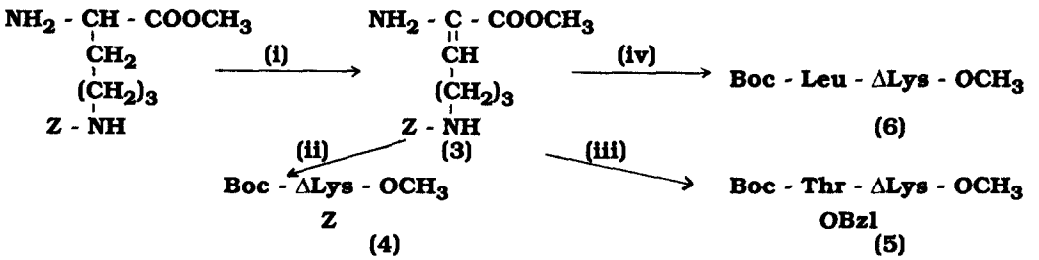
Abstract: Two analogues of tuftsin containing α,β-dehydrolysine, Thr-ΔLys-Pro-Arg (1) and Leu-ΔLys-Pro-Arg (2) have been synthesised. Presence of ΔLys moiety has been confirmed by ^{1}H NMR and aminoacid analysis. Analogue (1) and (2) have been checked for their phagocytic activity.

Tuftsin, a pluripotent bioregulator is a tetrapeptide, Thr-Lys-Pro-Arg[1] which activates the phagocytic cells, macrophages and polymorphonuclear leucocytes. Structural requirements for high activity of tuftsin appear very strict[2,3]. Very few active analogues of tuftsin have been reported so far; Leu-Lys-Pro-Arg retains full activity[4]. Thr-Lys bond in tuftsin is the primary site of enzymatic cleavage releasing the tripeptide, Lys-Pro-Arg which inhibits tuftsin's action by competing with it for cellular receptors[5]. The introduction of α,β-dehydroamino acid residues in peptides afford constrained and enzymatically stable analogues[6,7]. In order to stabilize the bond preceeding the lysine residue, we have synthesized two analogues of tuftsin, Thr-ΔLys-Pro-Arg (1) and Leu-ΔLys-Pro-Arg (2) and assessed their phagocytic activity. This is the first report where dehydrolysine has been incorporated in a bioactive compound.

Synthesis

Shin and coworkers have reported synthesis of N-protected dehydrolysine derivatives which involved a multistep preparation of a Wittig-Horner reagent and an appropriate aminoaldehyde derivative[8]. Our attempts to use this method to prepare dehydrolysine peptides gave poor results. The dehydrodipeptides, Boc-Thr-(OBzl)-ΔLys(Z)-OMe (5) and Boc-Leu-ΔLys(Z)-OMe (6) were synthesized according to scheme 1. Dehydrolysine moiety was generated using the N-chlorination dehydrochlorination method[9]. N^{ε}-benzyloxycarbonyl lysine methylester hydrochloride was subjected to N-chlorination using slightly less than an equivalent amount of t-butylhypochlorite. On dehydrochlorination of N^{α}-chloro-N^{ε}Z-Lys-OMe using 1,7, diazabicyclo-undec-7-ene (DBU), α,β-dehydrolysine methylester (3) was obtained in ~63% yield as an oil. To characterize it, (3) was converted to N^{α}-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-α,β-dehydrolysine methylester (4), obtained as an oil. A triplet at 6.6δ in NMR, characteristic of methyne protons confirmed the presence of ΔLys residue in (3) and (4). IR spectrum also showed a band at ~1665 cm^{-1}, diagnostic

of C=C. Without further purification (3) was coupled to Boc-Thr(OBzl)-OH and Boc-Leu-OH respectively to give dehydrodipeptides methylesters (5) and (6) as colorless oil which showed single spot on tlc and appropriate ^{1}H NMR spectra[10]. Compounds (5) and (6) were hydrolyzed to the corresponding dipeptide-free acids, Boc-Thr(OBzl)-ΔLys(Z)-OH and Boc-Leu-ΔLys(Z)-OH by alkaline hydrolysis. Arginine, the C-terminal residue was introduced unprotected, since this strategy is known to give the best yields in solution phase synthesis of tuftsin[11]. Moreover, in the present case catalytic hydrogenation, which is generally used to remove the nitro protection of arginine and other benzyl based protecting groups, could not be considered because of the presence of the double bond in ΔLys moiety. Thus, the C-terminal dipeptide fragment Z-Pro-Arg-OMe was obtained by coupling Z-Pro-OSu with Arg-OMe.2HCl. Deprotection with 30% HBr in acetic acid gave Pro-Arg-OMe which was coupled to Boc-Thr(OBzl)-ΔLys(Z)-OH and Boc-Leu-ΔLys(Z)-OH to give Boc-Thr(OBzl)-ΔLys(Z)-Pro-Arg-OMe (7) and Boc-Leu-ΔLys(Z)-Pro-Arg-OMe (8) by mixed anhydride procedure at pH 7. Compounds (7) and (8) were purified by column chromatography on silica gel and were fully charaterised by elemental analysis and H^{1}NMR[12]



(i) $(CH_3)_3COCl$, DBU ; (ii) Di-t-butyldicarbonate ; (iii) Boc-Thr(OBzl)-OH (Mixed anhydride coupling) ; (iv) Boc-Leu-OH (Mixed anhydride coupling).

Scheme 1

Amino acid analysis on (7) and (8) showed the absence of lysine peak, which appeared in amino acid analysis, when (7) and (8) were subjected to catalytic hydrogenation (Pd/C, 10%). Alkaline hydrolysis (7) and (8) gave free acids Boc-Thr(OBzl)-ΔLys(Z)-Pro-Arg-OH and Boc-Leu-ΔLys(Z)-Pro-Arg-OH respectively. t-Butoxycarbonyl and the other side chain protecting groups were then removed using a mixture of trifluoromethanesulfonic acid and trifluoroacetic acid (1:10) in presence of dithiothreitol. The free peptides, obtained by precipitation with dry ether, were purified first by ion exchange chromatography (IRA-45) and finally by gel chromatography on biogel P-2. Fractions that gave positive Sakaguchi test[13] were pooled and lyophilized to obtain the desired tuftsin analogues (1) and (2).

Bioactivity of (1) and (2) was checked using phagocytic assay[14,15] on human leucocytes from fresh blood. Five experiments were carried out on leukocytes isolated from

fresh blood, using heat billed candida by nitroblue tetrazolium (NBT) method[16]. The optical density measured at 575 nm was used as a measure of the reduced formazan. Three experimetns were carried out again on human leukocytes, using staphylococus Aureus and cytochrome 'C'. Optical density measured at 550 nm gave an estimate of the reduced product. Data were normalised per mole of the tuftsin and activity is given with respect to unity for tuftsin (Table 1). Analogue (1) showed ~60-70% activity with respect to tuftsin whereas (2) retained almost the full activity. This indicates that the introduction of α,β-dehydrolysine does not effect the receptor binding ability of tuftsin. However, it is clear that stabilization of the Thr-Lys bond towards enzymatic degradation alone is not sufficient to produce analogues more active than tuftsin and that the structural requirements for tuftsin activity at this position may be quite specific[2,3].

Table 1: Phagocytic activity of Tuftsin analogues 1 and 2

Peptide	**Activity/mmol peptide for 2.6 x 10^6 Leukocytes**	
	NBT Method	Cytochrome "C" method
Tuftsin	1.00	1.00
$Thr^1\Delta Lys^2$Tuftsin 1	0.70 + 0.25 (5)	0.60 + 0.05 (3)
$Leu^1\Delta Lys^2$Tuftsin 2	1.0 + 0.05 (5)	0.90 + 0.10 (3)

Figure in brackets give the number of experiments performed.

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References:

1. Nishioka, L., Constantopoulas, A. and Najjar, V.A., Biochem. Biophys. Acta., 1973, 310, 203.
2. Najjar, V.A., Chaudhuri, M.K., Konopiniska, D., Beck, B.B., Layne, P.P. and Linehan, L., Augmenting Agents in Cancer Therapy, 1981, 459.
3. Stabinsky, Y., Gottlieb, P. and Fridkin, M., Mol. Cell. Biochem., 1980, 30, 165.
4. Matsumura, S., Takasaki, A., Hirotami, H., Kotere, T. and Fujiwara, S., Chem. Abs. 1975, 83:114937 3.g.
5. Spirer, Z., Zakuth, V., Bogair, N. and Fridkin, M., Eur. J. Immunol., 1977, 7, 69.

6. English, M.L. and Stammer, C.H., Biochem. Biophys. Res. Commun., 1978, 85, 780.
7. Fischer, G.H., Berryer, P., Ryan, J.K.L., Chauhan, V.S. and Stammer, C.H., Arch. Biochem. Biophys., 1981, 211, 269.
8. Shin, C., Obara, T., Segami, S. and Yonezawa, Y., Tetrahedron Letters, 1987, 33, 3827.
9. Schimdt, U. and Ohler, E., Angew. Chem. Int. Engl., 1977, 16, 327.
10. Compound (5): R_f=0.66, $CHCl_3$:CH_3OH (9:1); $[\alpha]_D^{25}$ -8.4° (C, 0.83, in CH_3OH). ^1H NMR (90 MHz, $CDCl_3$), δ: 8.02 (1H, s, NH of ΔLys), 7.25 (10H, br, aromatic proton of Z and OBzl), 6.62 (1H, t, -$C^{\beta}H$ of ΔLys), 5.2 (1H, d, NH of Thr), 5.08 (2H, s, CH_2 of Z), 4.6 (2H, s, CH_2 of Bzl), 4.2 (1H, br, $C^{\alpha}H$ Thr), 3.6 (3H, s, OCH_3), 3.1 (2H, br, $C^{\varepsilon}H_2$ΔLys), 1.65 (2H, br, $C^{\delta}H_2$ of ΔLys), 1.4 (9H, s, 3 x CH_3Boc), 1.24 (2H, br, ΔLys $C^{\gamma}H_2$), 1.0 (3H, m, Thr $C^{\gamma}H_3$)
Compound (6) R_f=0.58, $CHCl_3$:CH_3OH (9:1); $[\alpha]_D^{27}$-7.0° (C, 0.75 in CH_3OH) ^1H NMR (90 MHz, $CDCl_3$) δ: 8.17 (1H, s; NH of ΔLys), 7.35 (5H, br, aromatic proton of Z), 6.63 (1H, t, -C = $C^{\beta}H$ of ΔLys), 5.25 (1H, d, NH of Leu), 5.12 (2H, s, CH_2 of Z), 4.1 (1H, $C^{\alpha}H$ of Leu), 3.62 (3H, S, -$COOCH_3$), 3.1 (2H, br, -$C^{\varepsilon}H_2$ of ΔLys), 1.97 (2H, br, $C^{\gamma}H_2$ of ΔLys), 1.65 (2H, br, -$C^{\delta}H_2$ of ΔLys), 1.5 (m, 3H, $C^{\beta}H_2$ and $C^{\gamma}H$ of Leu), 1.36 (9H, s, 3 x CH_3 of Boc), 0.95 (6H, d, 2 x CH_3 of Leu).
11. Gottilieb, P., Stabinsky, Y., Zakuth, V., Spirer, Z. and Fridkin, M., Ann. N.Y. Acad.Sci., 1983, 12, 419.
12. Compound (7) R_f=0.42, n-Butanol:pyridine:acetic acid:water (4:1:1:7); m.p. 160-162°C; $[\alpha]_D^{27}$ -7.05° (c, 0.55, MeOH); ^1H NMR (90 MHz, $CDCl_3$) δ: 8.05 (1H, br NH of ΔLys), 7.3 (10H, m, aromatic protons), 6.76 (1H, t, -C=$C^{\beta}H$ of Lys), 5.02 (2H, s, CH_2 of Z), 4.6 (2H, s, CH_2 of benzyl ester), 3.56(3H, s ,OCH_3), 3.15 (5H, m, CH_2 of Arg and $C^{\varepsilon}H2$ of ΔLys), 1.7 (br,2H, -$C^{\delta}H_2$ of ΔLys), 1.42 (9H, s, 3xCH_3 0f Boc), 1.22 (3H, br, $C^{\gamma}H_3$ of Thr); Elemental analysis:- found: C, 59.15; N, 13.14; H,7.04; required: C, 60.0; N, 13.153; H, 6.9.
Compound (8) R_f=0.42, n-Butanol:pyridine:acetic acid:water (4:1:1:7); m.p. 145-147°C; $[\alpha]_D^{27}$ -4.8° (c, 0.85, MeOH); ^1H NMR (90 MHz, $CDCl_3$) δ: 8.17 (1H, br NH of ΔLys), 7.3 (5H, s, aromatic protons of Z), 6.7 (1H, t, C=$C^{\beta}H$ of Lys), 5.02 (2H, s, CH_2 of Z), 3.3 (3H, s, -$COOCH_3$), 3.12 (2H, m, -CH_2 of Arg), 2.3-1.45 (8H, m, CH_2 of Pro and Arg), 1.4 (9H, s, 3xCH_3 of Boc), 0.92 (6H, d, 2xCH_3 of Leu) ; Elemental analysis:- found: C, 58.0; H, 8.0; N, 14.8; required: C, 57.72; H, 7.9; N, 14.5.
13. Archer, R. and Crocker, C., Biochim. Biophys. Acta, 1952, 9, 704.
14. Bachner, R.Z. and Nathan, D.K., New Engl. J. Med., 1968, 274, 971.
15. Gordon, J., Clin. Path., 1973, 26, 52.
16. Tzehoval, T., Sefal, S., Stabinsky, Y., Fridkin, M., Spirer, Z. and Feldman, M., Springer's Seminar on Immunopathology, 1979, 42, (2), 205-221.

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