Oxalopeptides as Core Motifs for Protein Design

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Oxalopeptides are demonstrated to be useful core motifs for bi-directional chain elongation leading to peptide homologues with interesting folding behaviour, thus constituting a useful strategy for protein backbone modification.

In spite of intense recent interest in protein design, sustained endeavours to change the nature of the peptide backbone have not been common.[1] Possibilities in this direction emerged during our recent studies relating to the chemical simulation of the mode of action of pituitary enzymes,[2] which led to the generation of the novel α-ketoamide (–CO–CO–NH–) unit in the peptide backbone.

We report here a new concept in peptide backbone design, with focus on the core, retropeptido-mimetic oxalamide unit (–NH–CO–CO–NH–), which acts as the cornerstone for programmed bi-directional elongation. The peptides so generated have appealing features and, in appropriate cases, form novel secondary structural elements of significance in protein design. Interestingly, the rather obvious strategy for introduction of the above motif in pre-formed peptides, failed.

Thus, the endeavours outlined here, consist of two parts, namely, the construction of the core element and further elongation of the central motif thus generated.

The core element, represented by, $(CO–A_{aa}–OMe)_2$ [A_{aa} = amino acid A] was constructed† using a modified general procedure[3] from the corresponding amino acid methyl ester and oxalyl chloride in dichloromethane admixed with triethyl amine, see Scheme 1.

$$Cl^-H_3N^+–A_{aa}–OMe \xrightarrow{i} MeO–A_{aa}–CO–CO–A_{aa}–OMe$$

Scheme 1 *Reagents and conditions*: i $(COCl)_2$,NEt_3,CH_2Cl_2,12 h

The bi-directional elongation of the core motif has been accomplished‡ (entries 1–9, Table 1).

† All amino acids used were of L-configuration. In the present work, the following typical procedure was followed for the preparation of the core element $(CO–A_{aa}–OMe)_2$ [A_{aa} = amino acid A].

A solution of oxalyl chloride (0.093 ml, 1 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise to a well-stirred solution of A_{aa}–OMe hydrochloride (2 mmol) in dry CH_2Cl_2 (30 ml) containing triethylamine (0.7 ml, 5 mmol) at 0 °C over 0.5 h and the mixture stirred at room temp. for 12 h. The reaction mixture was worked up by washing with 5% $NaHCO_3$ solution, drying the organic layer with anhyd. $MgSO_4$ and evaporating *in vacuo*. The residue was crystallized from methanol or ethyl acetate to give pure oxalopeptide. All analogues had non-zero rotation and gave satisfactory spectral and analytical data.

Selected data for $(CO–A_{aa}–OMe)_2$: A_{aa} [m.p./°C; yield (%)]: Gly (120–121; 86); Ala (159–160; 55); Phe (192–193; 60); Leu (114–115; 88); Met (95–96; 45); Tyr (232–233; 88); Trp (177–178; 60); Pro (148–149; 70); Aib (167–168; 70); N^{α}Z-Lys (124–125; 44).

Typical spectral data illustrated with $(CO–Leu–OMe)_2$: IR ν/cm^{-1} (KBr) 3345, 3299, 2958, 2871, 1741, 1664, 1516 and 1437; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.93 (12H, d, *J* 5.0 Hz, Leu Me), 1.64 (6H, m, Leu β-CH_2 + Leu γ-CH), 3.73 (6H, s, COOMe), 4.57 (2H, m, Leu α-CH), 7.73 (2H, d, *J* 7.5 Hz, NH); FAB MS (*m/z*) 345 (M + H)$^+$, 172 $(M/2)^+$.

Under conditions of the reaction, Ser afforded $(CO–\Delta\text{-}Ala–OMe)_2$ [m.p. 133–134 °C; 14%] and MeO–Ser–CO–CO–Δ-Ala–OMe [syrup; 10%]. This observation was exploited in the development of a general route to Δ-Ala peptides from serine containing precursors (D. Ranganathan, K. Shah and N. Vaish, *J. Chem. Soc., Chem. Commun.*, 1992, 1145).

N^{α}Z-Lys was largely recovered unchanged and His and Thr were transformed to intractable products.

‡ The bi-directional elongation of the core element was achieved by the following procedure, exemplified with $(CO–Leu–Ala–Gly–OMe)_2$ (entry 9, Table 1).

$(CO–Leu–Ala–OMe)_2$ **3**: A solution of $(CO–Leu)_2$ bis-hydrazide (1 mmol, m.p. 219–220 °C, prepared from the core diester with $NH_2NH_2·H_2O$ in dry ethanol) in a mol dm^{-3} HCl (6 ml), at 0 °C, was treated, under vigorous stirring conditions, with $NaNO_2$ (3 mmol in 1 ml H_2O), neutralized with ice cold satd. K_2CO_3 solution, extracted with cold ethyl acetate (2 × 30 ml), dried (anhyd. $MgSO_4$) and admixed, without delay, with freshly prepared Ala–OMe (from 2 mmol ester hydrochloride and triethyl amine in CH_2Cl_2 at 0 °C). The reaction mixture was stirred at 0 °C for 2 h and at room temp. for 24 h, washed with ice cold 2 mol dm^{-3} H_2SO_4, water, aq. bicarbonate (10 ml each), dried (anhyd. $MgSO_4$) and evaporated *in vacuo* to give the title peptide **3** as colourless needles.

$(CO–Leu–Ala–Gly–OMe)_2$ **9**: Transformation of **3** (2 mmol) to bis-hydrazide (m.p. 223—224 °C, 78%) followed by azide condensation (generated *in situ*, at 0 °C, from 1.6 mmol hydrazide, 9 ml 1 mol dm^{-3} HCl and 4.8 mmol $NaNO_2$ as above) with freshly prepared Gly-OMe (3.2 mmol) at room temp. for 48 h and work-up as in the case of **3**, yielded the title hexapeptide as colourless needles from methanol.

Selected spectral data for **9**: IR ν/cm^{-1} (KBr) 3280, 3045, 2945, 2870, 1739, 1628, 1520, 1500; ^{1}H NMR (400 MHz, $CDCl_3$ + 2% $(CD_3)_2SO$) δ 0.95 (6H, d, *J* 5.0 Hz, Leu Me), 1.00 (6H, d, *J* 5.0 Hz, Leu CH_3), 1.31 (6H, d, *J* 6.5 Hz, Ala Me), 1.70 (6H, m, Leu β-CH_2 + Leu γ-CH), 3.75 (6H, s, COOMe), 3.87 (2H, dd, Gly CH), 4.20 (2H, dd, Gly CH), 4.37 (2H, m, Leu α-CH), 4.58 (2H, m, Ala α-CH), 7.34 (2H, br, Gly NH), 7.47 (2H, d, *J* 7.5 Hz, Ala NH), 7.96 (2H, d, *J* 7.5 Hz, Leu NH); ^{13}C NMR (100.57 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 17.42 (Leu Me), 21.36 (Ala Me), 22.75 (Leu β-CH_2), 24.58 (Leu γ-CH), 40.80 (Gly CH_2), 48.32 ($COOCH_3$), 52.03 (Leu α-CH), 52.95 (Ala α-CH), 159.86 (CO-CO), 170.53, 171.04 (Leu CO, Ala CO), 172.45 (Gly CO).

Table 1 Bi-directional elongation of the core elements

Entry	Extended oxalopeptide[m.p./°C; yield[a](%); FAB[b]MS, *m/z*$(M + H)^+$, $(M/2)^+$]
1	MeO-Ala-Ala-CO-CO-Ala-Ala-OMe[216–217; 63; 403, 201]
2	MeO-Leu-Leu-CO-CO-Leu-Leu-OMe[200–201; 65; 571, 285]
3	MeO-Ala-Leu-CO-CO-Leu-Ala-OMe[c] [184–185; 65]
4	MeO-Ser-Leu-CO-CO-Leu-Ser-OMe [205–206; 55; 519, 259]
5	MeO-Thr-Leu-CO-CO-Leu-Thr-OMe[197–198; 80; 547, 273]
6	MeO-His-Leu-CO-CO-Leu-His-OMe[c] [124–126; 43; 619, 309]
7	MeO-Leu-Leu-Leu-CO-CO-Leu-Leu-Leu-OMe [283–284; 76; 797]
8	MeO-Ala-Leu-Leu-CO-CO-Leu-Leu-Ala-OMe [156–157; 72]
9	MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe[c] [235-236; 30; 601]

[a] Isolated yield(%). [b] Fast atom bombardment using *m*-nitrobenzyl alcohol matrix. [c] Prepared by azide coupling.

$$MeO–A_{aa}–CO–CO–A_{aa}–OMe \xrightarrow{i,\ ii} MeO–B_{aa}–A_{aa}–CO–CO–A_{aa}–B_{aa}–OMe \xrightarrow{iii} MeO–C_{aa}–B_{aa}–A_{aa}–CO–CO–A_{aa}–B_{aa}–C_{aa}–OMe$$

Scheme 2 *Reagents and conditions*: i, aq. NaOH 2 mol dm^{-3}, 4 h, room temp.; ii, DCC, HOBT, DMF, CH_2Cl_2, B_{aa}-OMe, 24 h, iii azide coupling with C_{aa}-OMe, 24 h

The symmetrical nature of peptides **1–9** is reflected in their ^{1}H NMR and FAB mass spectra. Thus, the individual residues on either side of the core appear identical in the ^{1}H NMR spectra. Fortunately, interactions involving the oxalamide NH can be easily monitored because of the appearance of these NH protons as doublets at significantly lower fields (δ 7.5–8.0) and their ready exchangeability. Each of the peptides exhibited in the FAB MS, peaks corresponding to $(MH)^+$, which, in many cases was the base peak and, interestingly ones corresponding to $(M/2)^+$.

The changing profiles in structures arising from elongation of the core motif have been probed by ^{1}H NMR spectroscopy. The correlation of the development of secondary structures as a function of bi-directional elongation was the focus of such studies.

Temperature dependence of NH chemical shifts (VT studies)§ as observed in ^{1}H NMR spectra of MeO–Leu–Leu–CO–CO–Leu–Leu–OMe (entry 2, Table 1) showed that both the oxalamide and the Leu NH group of protons are solvent exposed and hence not involved in any intramolecular hydrogen bonding.

Bi-directional elongation with two residues resulted in the emergence of secondary structures. Thus, VT studies conducted in $CDCl_3$ between 296 and 326 K with $(MeO–Gly–Ala–Leu–CO–)_2$ [entry 9, Table 1] demonstrated for the Leu NH protons—which form an integral part of the oxalopeptide core—a $d\delta/dT$ value of −1.6 ppb K^{-1}, positively showing their involvement in intramolecular hydrogen bonding. Interestingly, the remaining 2 NH protons of Ala and Gly residues exhibited divergent behaviour exhibiting $d\delta/dT$ value, respectively, −9.00 and −5.60 ppb K^{-1}. These data show that while the Ala NH protons are truly solvent exposed, the nature of the Gly NH remains ambiguous.

§ The temperature dependence of the amide proton shifts was determined in $CDCl_3$ using a 400 MHz (Bruker-WM 400) instrument, between 218 and 273 K. A minimum of twelve temperature steps were recorded in each experiment. In both cases, the chemical shifts were found to vary linearly with temperature. The $d\delta/dT$ values of −6.3 and −10.0 ppb K^{-1} for oxalamide and Leu NH, respectively, excluded any intramolecular hydrogen bonding in **2** (Table 1).

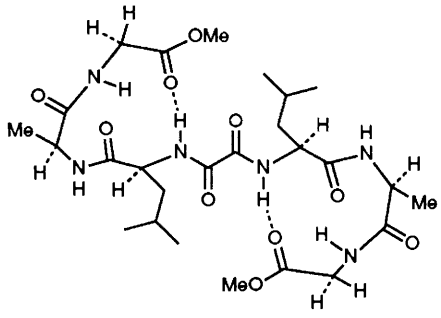

Fig. 1

Based on the above, the novel C_2 symmetric structure (Fig. 1), is proposed¶ for MeO–Gly–Ala–Leu–CO–CO–Leu–Ala–Gly–OMe (entry 9, Table 1). The C_2 symmetric secondary structural motif arising from intramolecular H-bonding involving the Leu–NH and the Gly–CO is strongly supported by the chemical shift non-equivalence of the two Gly α protons due to restricted rotation around the ϕ, ψ torsion angles resulting in their appearance as two sets of double doublets at, respectively, δ 3.87 and 4.20.

¶ The secondary structure envisaged here is reminiscent of γ-turns in peptides (G. Nemethy and M. P. Printz, *Macromolecules*, 1972, **5**, 755), excepting for the fact that the required second seven-membered intramolecular hydrogen bonding involving Gly-NH has not been decisively seen.

The circular dichroism (CD) spectrum of the compound in MeOH, demonstrating distinct negative ellipticity at 231 nm, is supportive of the envisaged secondary structure.

Regardless of finer details, the notion that the bi-directional elongation of the core motif can lead to secondary structural elements seems secure. The systems and methodologies presented here will have ramifications right across the protein domain. Their potential in the modulation of protein function and protein design and their utility, in inter- or intra-strand cross linking, design of inhibitors, crafting of transition-state mimics and the preparation of hormone antagonists would constitute some obvious options. Facets of these are currently receiving our attention.

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