Structural Requirements for Formyl Homooligopeptide Chemoattractants†

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ABSTRACT: Using solution peptide synthesis, we have made three series of N^{α}-formylated homooligopeptides, from the dipeptide to the heptapeptide, derived from L-methionine, L-norleucine, and *S*-methyl-L-cysteine and related to the chemotactic peptide N^{α}-formylmethionylleucylphenylalanine. Compounds were prepared to determine the combined effects of the main-chain length and the presence of a sulfur atom in side-chain γ- and δ-positions. Each peptide was tested for its ability to induce rabbit peritoneal polymorphonuclear leukocytes in the presence of cytochalasin B to secrete granule enzymes. In parallel, a conformational analysis was carried out in the solid state and in solution, using infrared absorption and circular dichroism. We examined these peptides in solvents of widely different polarities, i.e., chloroform, 2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoropropan-2-ol, and mixed organic–aqueous media. The tendencies to form antiparallel-chain β-associated and folded structures were determined. The biological and conformational data are described in terms of a model of the chemotactic peptide receptor of rabbit neutrophils recently proposed by Freer et al. (1982) [Freer, R. J., Day, A. R., Muthukumarswamy, N., Pinon, D., Wu, A., Showell, H. J., & Becker, E. L. (1982) *Biochemistry 21*, 257–263]. In the three N^{α}-formylated *C*-methoxy homooligopeptide series tested, the highest level of activity attained is at the tetrapeptide or pentapeptide stage, confirming the suggestion that the formylpeptide receptor is large enough to accommodate a peptide with at least four amino acid residues. All three homooligopeptide series, starting at the tetrapeptide, show a high propensity to give self-associated β-structures (certainly at least in part of the antiparallel type) in a solvent of low polarity ($CDCl_3$) which can mimic the environment at the receptor site. This supports the previously expressed view that this could be the biologically active conformation of the ligand.

The finding of Schiffmann et al. (1975) that N^{α}-formylmethionine is chemotactic stimulated the development of a new group of synthetic chemoattractants. These are small molecular weight N^{α}-acylated di-, tri-, tetra-, and hexapeptides. These compounds also induce the release of lysosomal enzymes (Showell et al., 1976), an effect which was absolutely correlated ($R = 0.95$) with their ability to stimulate chemotaxis. The biological response to the synthetic peptides is mediated through the interaction of the peptide with a specific receptor (Becker, 1979) residing on the neutrophil surface.

One of the most active chemotactic peptides is N^{α}-formylmethionylleucylphenylalanine (CHO-L-Met-L-Leu-L-Phe-OH),[1] the ED_{50} for chemotaxis being $(7.0 \pm 1.7) \times 10^{-11}$ M and for lysozyme secretion $(2.4 \pm 0.3) \times 10^{-10}$ M (Showell et al., 1976). The early studies demonstrated inter alia that the N^{α}-formyl group is essential for good activity since removal of the N^{α}-formyl group, N^{α}-acetylation, removal of the α-amino group, or replacement of the α-amino group by an ethyl group results in a 1000–10 000-fold loss of activity. On the basis of extensive structure–activity studies in which the various elements of CHO-L-Met-L-Leu-L-Phe-OH were systematically varied (Freer et al., 1980, 1982), as well as nuclear magnetic resonance spectroscopy (Becker et al., 1979), Freer et al. (1982) have proposed a working model of the rabbit neutrophil receptor topology. This model suggests that the chemotactic peptide exists on the receptor in a β-pleated sheet (antiparallel-chain arrangement); there are at least five critical areas of interaction of the tripeptide and receptor, and the receptor has sufficient "room" to accommodate a tetrapeptide, at least.

In an attempt to further explore the relationship of chemotactic peptide conformation in solution and biological activity, we have synthesized three N^{α}-formylated homooligopeptide series, namely, $CHO(L\text{-}X)_nOMe$ (where X = Met and $n = 2$–7), the isosteric Nle, and the side-chain lower homologue Cys(Me). Their conformational perferences have been studied in the solid state and in solution in a number of solvents with different capabilities of solvating the peptide chain, by using IR absorption and CD. With the biological and conformational data in hand, we discuss the working model of the chemotactic peptide receptor of rabbit neutrophil proposed by Freer et al. (1982).

Materials and Methods

The *t*-Boc amino acids were synthesized by the method of Moroder et al. (1976) using di-*tert*-butyl dicarbonate as the tert-butyloxycarboxylating agent. The amino acid methyl ester hydrochlorides were prepared by reaction of the free amino acid with methanol in the presence of thionyl chloride, as described by Boissonnas et al. (1956). Removal of the *t*-Boc group was carried out by treatment with a mixture of either TFA/$CHCl_3$ or HCl/MeOH. Peptide coupling was achieved by the mixed-anhydride method (Anderson et al., 1976) for the di-, tri-, and tetrapeptides and by the azide method as modified by Honzl & Rudinger (1961) for the penta-, hexa-, and heptapeptides. Both methods, as employed here, are known to be racemization free (Anderson et al., 1967; Klausner & Bodansky, 1974). Figure 1 gives the strategy of synthesis of the *t*-Boc homooligopeptide. The formyl group was introduced through conventional anhydride coupling as described

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[1] Abbreviations: CHO, formyl; *t*-Boc, *tert*-butyloxycarbonyl; OMe, methoxy; OBzl, benzyloxy; NHBzl, benzylamino; Nle, norleucine; Cys(Me), *S*-methylcysteine; MA, mixed anhydride; *n*-Hex, *n*-hexane; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; TFE, 2,2,2-trifluoroethanol; HFIP, 1,1,1,3,3,3-hexafluoropropan-2-ol; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; UV, ultraviolet; IR, infrared; CD, circular dichroism; Aib, aminoisobutyric; Cyl, cycloleucine; LDH, lactate dehydrogenase.

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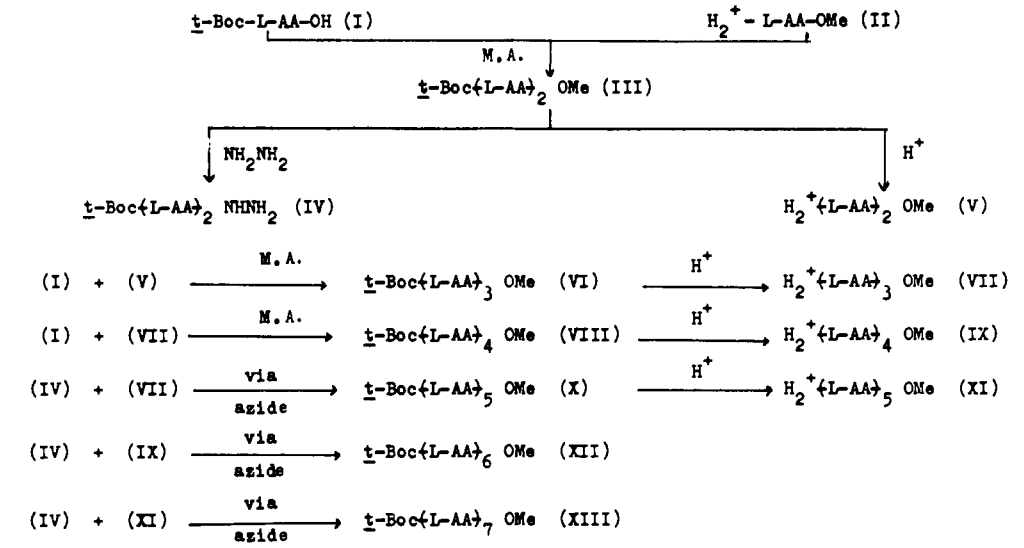

FIGURE 1: General synthetic scheme for a *t*-Boc(L-AA)OMe (n = 2–7) oligopeptide series. AA = amino acid.

Table I: Analytical Data

compd	n	mp (°C)	recrystallization solvent	$[\alpha]^{20}_D$[c] (deg)	TLC (R_f)[d] sys A	sys B	sys C	C, H, N, S calcd (obsd)
CHO(L-Met)$_n$OMe	2	103-103.5	$CHCl_3$/*n*-Hex	−39.2	0.75	0.90	0.60	44.7, 6.9, 8.7, 19.9 (44.4, 7.0, 8.5, 19.9)
	3	164-165	$CHCl_3$/*n*-Hex	−56.2	0.75	0.90	0.55	45.0, 6.9, 9.3, 21.2 (45.2, 7.0, 9.2, 21.2)
	4	200-201	DMF/H_2O	−63.9	0.70	0.90	0.55	45.2, 6.9, 9.6, 21.9 (45.4, 7.0, 9.5, 21.8)
	5	250-257[a]	DMF/H_2O	−71.0	0.70	0.90	0.50	45.3, 6.9, 9.8, 22.4 (44.8, 6.8, 9.7, 22.3)
	6	264-265[a]	DMF/H_2O	−78.8	*e*	0.90	0.10	45.4, 6.9, 9.9, 22.7 (44.8, 6.8, 9.8, 22.5)
	7	>300	DMF/H_2O	−89.5	*e*	0.90	0.00	45.4, 6.9, 10.0, 22.9 (44.7, 6.8, 10.0, 22.6)
CHO(L-Nle)$_n$OMe	2	105-105.5	$CHCl_3$/*n*-Hex	−76.5	0.90	0.75	0.50	58.7, 9.2, 9.8 (58.2, 9.1, 9.7)
	3	176-176.5	$CHCl_3$/*n*-Hex	−83.0	0.90	0.60	0.40	60.1, 9.3, 10.5 (59.3, 9.2, 10.4)
	4	228-229	$CHCl_3$/*n*-Hex	−89.4	0.90	0.55	0.30	60.9, 9.4, 10.9 (60.4, 9.3, 10.8)
	5	271-273[a]	hot DMF/H_2O	−112.1	0.90	0.35	0.20	61.4, 9.5, 11.2 (61.1, 9.6, 11.1)
	6	287-288	hot DMF[b]	−114.0	*e*	0.00	0.00	61.8, 9.5, 11.4 (61.2, 9.5, 11.3)
	7	315-316[a]	hot DMF[b]	−118.0	*e*	0.00	0.00	62.0, 9.6, 11.5 (61.2, 9.5, 11.4)
CHO{L-Cys(Me)}$_n$OMe	2	68-68.5	$CHCl_3$/*n*-Hex	−49.4	0.80	0.65	0.60	40.8, 6.2, 9.5, 21.8 (40.7, 6.1, 9.3, 21.6)
	3	142-144	$CHCl_3$/*n*-Hex	−71.3	0.75	0.50	0.45	40.9, 6.1, 10.2, 23.4 (41.1, 6.0, 10.1, 23.2)
	4	175-177	DMF/H_2O	−83.3	0.70	0.45	0.35	40.9, 6.1, 10.6, 24.3 (40.7, 6.0, 10.5, 24.6)
	5	228-229[a]	DMF/H_2O	−88.0	0.70	0.45	0.25	40.9, 6.1, 10.8, 24.8 (41.1, 6.0, 10.7, 24.9)
	6	253-254[a]	hot DMF[b]	−96.0	*e*	*e*	0.00	40.9, 6.1, 11.0, 25.2 (40.4, 6.0, 10.9, 24.9)
	7	>300	hot DMF[b]	−100.9	*e*	*e*	0.00	40.9, 6.1, 11.1, 25.2 (40.3, 6.0, 11.0, 25.2)

[a] With decomposition. [b] Washed. [c] Concentration, 0.5 g/100 mL of HFIP. [d] Thin-layer chromatography on silica gel (Merck): sys A = 1-butanol/acetic acid/water (3:1:1); sys B = chloroform/ethanol (9:1); sys C = benzene/ethyl acetate/methanol (15:15:1). [e] Not measurable, due to extensive tailing-off effect.

by Day et al. (1980). Peptides were considered homogeneous when a single spot was observed (100-μg load) in three TLC systems using various methods of detection, i.e., UV light, ninhydrin, hypochlorite/starch/iodide, picryl chloride/ammonia, or a $K_3Fe(CN)_6/FeCl_3$ solution. Each compound was also subjected to elemental analysis and melting point (Leitz Model Laborlux 12) determinations. The optical rotatory measurements were carried out on a Perkin-Elmer Model 214 polarimeter equipped with a thermostat. The analytical data of the formyl homooligopeptide series are included in Table I. The analytical data of the *t*-Boc homooligopeptide series have already been reported (Bonora & Toniolo, 1974, 1978; Bonora et al., 1975). The infrared absorption spectra were recorded by using a Perkin-Elmer Model 580 spectrophotom-

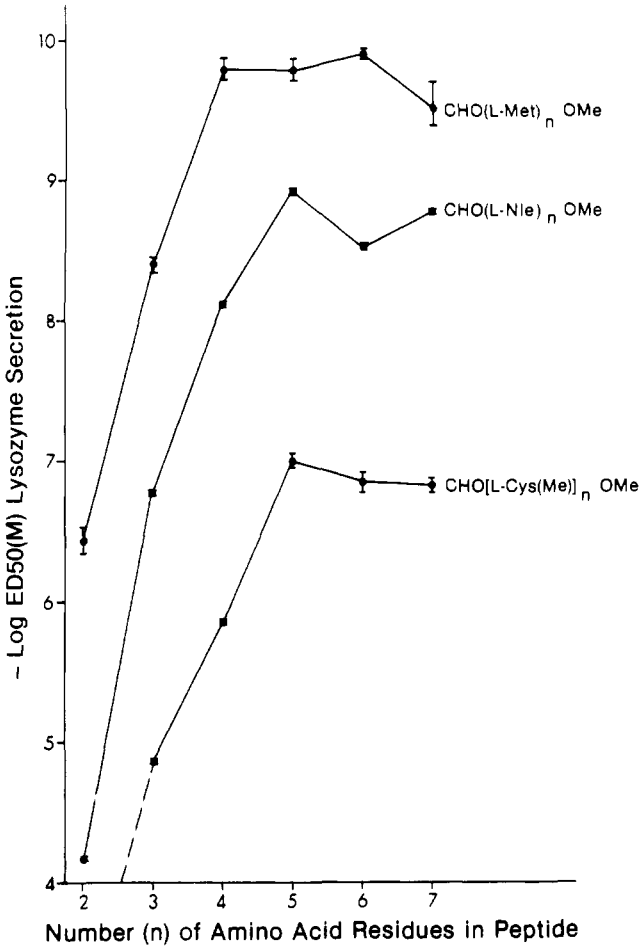

FIGURE 2: Lysozyme releasing activity of CHO(L-Met)$_n$OMe, CHO(L-Nle)$_n$OMe, and CHO[Cys(Me)]$_n$OMe (n = 2–7). The ED_{50} values for CHO(L-Met)$_n$OMe, CHO(Nle)$_n$OMe, and CHO[L-Cys-(Me)]$_n$OMe are the means ± SE (vertical bars) for five, four, and five independent experiments, respectively.

eter. The band positions are accurate to 1 cm^{-1}. For the solid-state measurements, the KBr disk technique was used. For the solution measurements, a cell of 10-cm path length was employed at low concentrations, whereas cells with path lengths of 0.2, 0.1, and 0.05 cm were used at high concentrations.

Circular dichroism spectra were recorded by using either a Cary Model 61 dichrometer without a computer (Figures 3 and 6) or a Jasco Model J-500A spectropolarimeter, the latter equipped with a DP-501N data processor (Figures 4 and 5). Cylindrical fused quartz cells of 0.5- and 1.0-mm path lengths were employed. The values are expressed in terms of $[\theta]_T$, the total molar ellipticity (degrees per square centimeter per decimole). Spectrograde chloroform, TFE, and HFIP were Fluka (Buchs, Switzerland) products.

Each compound was tested for its ability to induce the release of the lysosomal enzymes lysozyme and β-glucuronidase from cytochalasin B treated rabbit neutrophils. As before, because the release of the two enzymes paralleled each other, only the results for lysozyme are reported. Each compound was also tested as to whether it would induce leakage of LDH. None did, and these data are not reported. Details of these assays have been published previously (Showell et al., 1976).

Results

Granule Enzyme Releasing Activity. In the CHO(L-Met)$_n$OMe series, the activity mounts steadily from the dipeptide [ED_{50} = (3.7 ± 0.9) × 10^{-7} M] to the tetrapeptide [ED_{50} = (1.6 ± 0.3) × 10^{-10} M], but increasing the number of Met residues beyond four causes no further change (Figure 2). Comparing the activities of the di-, tri-, and tetra-CHO(Met)$_n$ methyl esters with the activities of the corresponding free acids, CHO(L-Met)$_n$OH previously described (Showell et al., 1976), shows that the methyl esters are 10–20-fold more active. The activities in the CHO(L-Nle)$_n$OMe series increase from the dipeptide [ED_{50} = (6.9 ± 0.22) × 10^{-5} M] to the pentapeptide [ED_{50} = (1.2 ± 0.7) × 10^{-9} M]; increasing the number of residues beyond five causes no further increase in activity. CHO[Cys(Me)]$_2$OMe is inactive at 10^{-3} M, the highest concentration of dipeptide tested. The lysozyme-releasing capacity increases from CHO[Cys(Me)]$_3$OMe [ED_{50} = (1.4 ± 0.01) × 10^{-5} M] to CHO[Cys(Me)]$_5$OMe [ED_{50} = (1.0 ± 0.11) × 10^{-8} M]; the corresponding hexa- and heptapeptides show no further increase in activity. The members of the CHO(L-Met)$_n$OMe series are at least 10 times more active than the corresponding CHO(L-Nle)$_n$OMe peptides; the latter, in turn, have a 100-fold (or more) greater activity than the corresponding CHO[Cys(Me)]$_n$OMe peptides. In the CHO(L-Met)$_n$OMe series, the maximal enzyme activity for all the peptides was the same as that given by CHO-L-Met-L-Leu-L-Phe-OH. In the CHO(L-Nle)$_n$OMe series, the maximal enzyme activity of the dipeptide is 82% of that given by CHO-L-Met-L-Leu-L-Phe-OH, whereas the remaining peptides of the series have essentially the same maximum as the reference peptide. In the CHO[L-Cys-(Me)]$_n$OMe series, the maximal activity of the tri- and tetrapeptides is 78%, and that of the other peptides is 82% of the maximal activity of the reference tripeptide (data not shown). It appears that the smaller peptides of lower activity tend to be partial agonists relative to CHO-L-Met-L-Leu-L-Phe-OH, whereas the higher homologues tend to be full agonists.

Table II: Infrared Absorption Frequencies (cm^{-1}) of the CHO(L-X)$_n$OMe Peptides in the Solid State

compd	n	amide A	ester C=O	amide I
X = Met	2	3288	1745	1662,[a] 1645
	3	3278	1742	1688,[a] 1662,[a] 1636
	4	3278	1742	1690,[a] 1654,[a] 1634
	5	3284	1744	1690, 1631
	6	3282	1743	1690, 1631
	7	3282	1742	1691, 1630
X = Nle	2	3290	1742	1668,[a] 1640
	3	3284	1743	1664,[a] 1636
	4	3280	1748	1690,[a] 1660,[a] 1630
	5	3278	1746	1690,[a] 1666,[a] 1630
	6	3278	1744	1690,[a] 1664,[a] 1632
	7	3278	1746	1692, 1628
X = Cys(Me)	2	3328, 3308	1740	1672,[a] 1644
	3	3282	1742	1690,[a] 1666,[a] 1638
	4	3282	1742	1692,[a] 1666,[a] 1636
	5	3276	1742	1694,[a] 1664,[a] 1634
	6	3276	1743	1695,[a] 1668,[a] 1632
	7	3276	1744	1695,[a] 1664,[a] 1632

[a] Shoulder or very weak band.

Conformational Properties. The solid-state conformational preferences of the CHO(L-X)$_n$OMe [X = Met, Nle, Cys(Me); n = 2–7] series were examined by using IR absorption. The results obtained (Table II) strongly support the view that the penta-, hexa-, and heptapeptides form fully developed β-structures (Miyazawa, 1967). These β-structures are characterized by the amide A band at 3284–3276 cm^{-1} and the amide I band at 1636–1625 cm^{-1}. Tripeptides and tetrapeptides present evidence of the coexistence of β-structures and unordered associated structures, whereas in dipeptides the unordered associated structure is predominant. From the

Table III: Infrared Absorption Frequencies (cm^{-1}) of N-H Stretching Bands and A_H/A_F Ratios of CHO(L-X)$_n$OMe Peptides in $CDCl_3$ Solution

		$CDCl_3$ concn					
		3×10^{-2} M		10^{-3} M		2×10^{-4} M	
compd	n	N-H	A_H/A_F	N-H	A_H/A_F	N-H	A_H/A_F
X = Met	2	3416, 3330	1.10	3416, 3330	0.30	3416, 3328	0.15
	3	3432, 3316	1.60	3420, 3336	0.80	3420, 3332	0.30
	4	3272	*a*	3415, 3275	10.00	3416, 3332	2.00
	5	*b*	*b*	3280	*a*	3416, 3320	6.25
X = Nle	2	3420, 3328	0.70	3424, 3350	0.10	3424, 3328	<0.10
	3	3420, 3308	2.65	3424, 3320	0.74	3424, 3332	0.10
	4	3280	*a*	3416, 3284	10.00	3420, 3328	0.50
	5	*b*	*b*	3272	*a*	3280	*a*
X = Cys(Me)	2	3408, 3324	0.80	3408, 3320	0.40	3408, 3328	0.25
	3	3406, 3328	1.25	3408, 3340	0.80	3408, 3336	0.30
	4	3404, 3280	2.10	3404, 3344	1.70	3408, 3344	1.55
	5	*b*	*b*	3270	*a*	3408, 3336	2.70

[a] Not measurable. [b] Sparingly soluble.

absence of an intense absorption at 1650–1655 cm^{-1}, it is clear that even the heptapeptides have a chain length below the critical one for α-helix formation in the solid state (Toniolo et al., 1979a; Komoto et al., 1974). The observation of the absorption at 1690 cm^{-1} (Miyazawa, 1967) in the highest homooligopeptides indicates that the β-sheet formed is, at least in part, of the antiparallel type. The positions of the amide N–H and C=O stretching bands exclude the occurrence of folded, intramolecularly hydrogen-bonded structures (Toniolo & Palumbo, 1977). The diagnostic amide V region (750–600 cm^{-1}) was not examined, as absorptions due to S–C stretching modes of the thioether moiety present in the Met and Cys(Me) peptides also contribute to that spectral range (Nagami et al., 1975). From the position of amide A and amide I bands, it is safe to conclude that there is not a marked difference in the propensity of the three series to attain the β-conformation.

The solution conformational preferences of the three N^{α}-formylated homooligopeptides series were assessed by using IR absorption ($CDCl_3$) and CD (TFE, HFIP, and TFE/H_2O mixtures). The concentration dependence of the ratio of the hydrogen-bonded N–H absorption (>3400 cm^{-1}) to the non-hydrogen-bonded absorption (<3400 cm^{-1}) (A_H/A_F) in the IR asorption spectra is a useful tool in the search for intramolecularly hydrogen-bonded folded peptide conformations (Mizushima et al., 1952). As the concentration of the samples decreases, self-association also will decrease, and the A_H/A_F ratio will reach a limiting value which reflects the molecular situation at the monomeric level. Using this dilution technique, we investigated the three N^{α}-formylated homooligopeptides series from the dipeptides to the pentapeptides in $CDCl_3$, a solvent with a low ability to compete with peptide N–H···O=C hydrogen bonds. The higher oligopeptides are sparingly soluble in this halohydrocarbon. The results obtained are presented in Table III. In most cases at concentrations $>2 \times 10^{-4}$ M, the A_H/A_F ratios exhibit a significant concentration dependence, indicating the occurrence of substantial percentages of self-associated molecules in the conformational equilibrium mixtures.

At the lowest concentration examined (2×10^{-4} M), where in most cases it can safely be assumed that the intramolecular hydrogen bonds are those predominating (Shields et al., 1968; Ribeiro et al., 1979; Palumbo et al., 1976; Bonora et al., 1979; Toniolo et al., 1981), the di- and tripeptides show low percentages of folded forms (A_H/A_F values ≤ 0.30). These values, however, tend to increase rapidly with increasing peptide-chain length. There is an observable effect of the nature of the side chain on the propensity of the tetrapeptides to adopt folded forms: $(Met)_4 > [Cys(Me)]_4 >> (Nle)_4$. It is possible that intramolecularly hydrogen-bonded forms between main-chain amide N–H and side-chain sulfur atoms of Met and Cys(Me) residues abnormally stabilize the various N–H···O=C conformations (Ne'el, 1974; Palumbo et al., 1976).

Table IV: Infrared Absorption Frequencies (cm^{-1}) of C=O Stretching Bands of CHO(L-X)$_n$OMe Peptides in $CDCl_3$[a]

n	X = Met	X = Nle	X = Cys(Me)
2	1742, 1690,[b] 1674	1744, 1686,[b] 1674	1740, 1686,[b] 1674
3	1742, 1684,[b] 1670	1744, 1676	1740, 1686,[b] 1666, 1650[b]
4	1740, 1688,[b] 1654,[b] 1632	1762, 1674, 1630	1742, 1690, 1657,[b] 1632

[a] $CDCl_3$ concentration was 3×10^{-2} M. [b] Shoulder or weak band.

At the highest concentration examined (3×10^{-2} M), we have been able to investigate the spectral region of C=O stretching bands (Table IV). The most interesting finding is that the spectra of three tetrapeptides are dominated by an intense amide absorption at 1632–1630 cm^{-1} (β-structure). This conclusion is corroborated by the position of the corresponding N–H stretching absorptions (3280–3272 cm^{-1}) (Table III). Interestingly, the results in concentrated $CDCl_3$ solution match closely those reported above for the same peptide in the solid state (Table II).

Circular dichroism in the far-UV region is a sensitive tool for the determining the secondary structures of peptides (Beychok, 1967; Bonora & Toniolo, 1974, 1978; Toniolo et al., 1975, 1979b, 1981; Toniolo & Bonora, 1975; Becker & Naider, 1974; Naider et al., 1978). The CD spectra of the three N^{α}-formylated homooligopeptide series in TFE and HFIP (in the latter solvent only the heptapeptides have been examined) at 10^{-3} M concentration are illustrated in Figures 3–5. In the Met and Nle series (Figures 3 and 4), the intensities of the two negative maxima at 220–225 nm (peptide $\pi \rightarrow \pi^*$ transition) and at 197–200 nm (long-wavelength component of the split peptide $\pi \rightarrow \pi^*$ transition) increase gradually with increasing chain length. Concomitantly, the position of the band near 200 nm undergoes a progressive bathochromic shift. There is no evidence of CD curves resembling those of ordered secondary structures (Beychok, 1967; Bonora & Toniolo, 1974, 1978; Toniolo et al., 1975, 1979b, 1981).

The same observation holds true for the Cys(Me) series to the pentapeptide (Figure 5). Conversely, the CD curves of

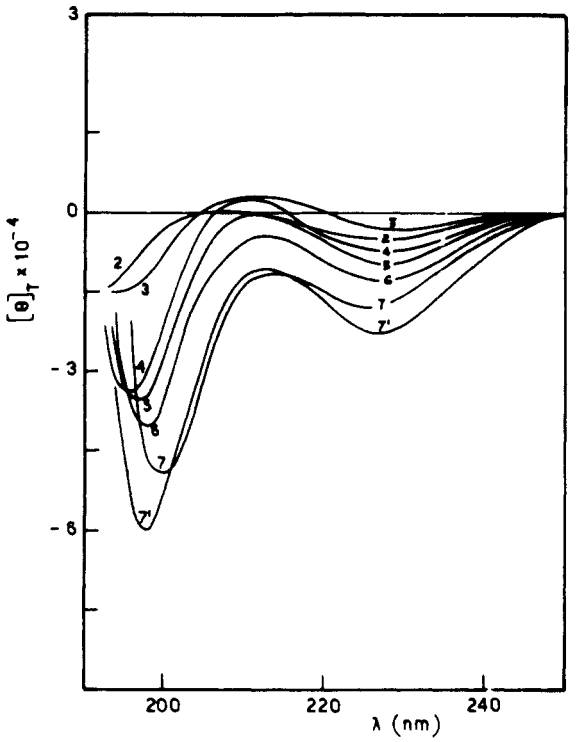

FIGURE 3: Circular dichroism spectra of CHO(L-Met)$_n$OMe in TFE at 20 °C (concentration 10^{-3} M); curve 7′ = heptapeptide in HFIP.

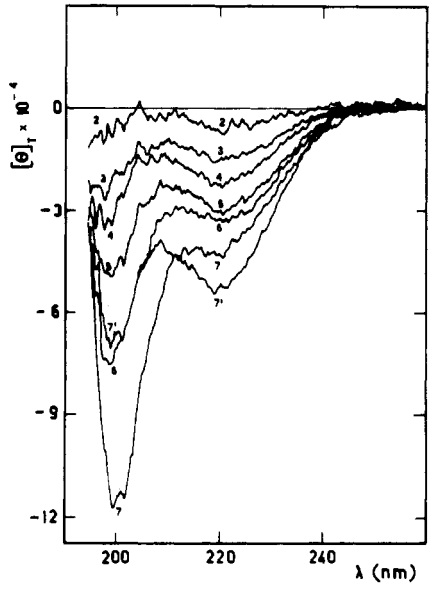

FIGURE 4: Circular dichroism spectra of CHO(L-Nle)$_n$OMe in TFE at 20 °C (concentration 10^{-3} M); curve 7′ = heptapeptide in HFIP.

the hexa- and heptapeptides are dramatically different from those of the lower members of the series, being characterized by a negative maximum at approximately 230 nm followed by a stronger positive ellipticity below 200 nm. The overall shape of these spectra is reminiscent of those reported for oligo- and polypeptides in the β-conformation (Beychok, 1967; Bonora & Toniolo, 1974, 1978; Toniolo et al., 1975, 1979b, 1981).

In HFIP, however, the CD patterns of the three heptapeptides, including that from Cys(Me), are those typical of unordered peptide conformations. This observation is in accord with the known characteristics of the strong hydrogen-bonding donor of HFIP (Middleton & Lindsey, 1964); consequently, HFIP requires a critical chain length for the onset of ordered secondary structures in peptides longer than TFE (Bonora &

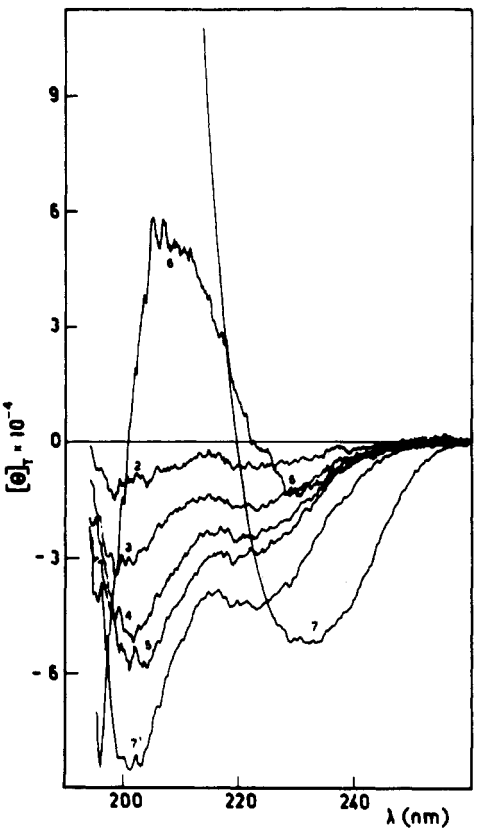

FIGURE 5: Circular dichroism spectra of CHO[L-Cys(Me)]$_n$OMe in TFE at 20 °C (concentration 10^{-3} M); curve 7′ = heptapeptide in HFIP.

Toniolo, 1974; Toniolo et al., 1975, 1979b, 1981; Toniolo & Bonora, 1975).

Since biological activity is tested in water, it is important to investigate peptide conformational preferences in aqueous solution. Unfortunately, these oligopeptides are only sparingly soluble in water. Nevertheless, we did examine their conformational properties in TFE/water mixtures. We were able to perform an extensive investigation only in the case of the most soluble Met series. The addition of 80% water (v/v) to solutions of the hexapeptides in TFE (concentration 2×10^{-4} M) induces a substantial variation in the CD properties. Under these experimental conditions, the spectrum (Figure 6) indicates a well-developed β-conformation (Beychok, 1967; Bonora & Toniolo, 1974, 1978; Toniolo et al., 1975, 1979b, 1981). Comparable results were obtained for the Nle analogue, where, however, only 65% water (v/v) is required to produce the trans conformational change. These results are not surprising, since it is known that water is a poor solvent for hydrophobic peptides and causes them to aggregate (Bonora & Toniolo, 1974, 1978; Toniolo et al., 1975, 1979b, 1981; Toniolo & Bonora, 1975).

Discussion

The conformational analysis described in this work indicates that the N^{α}-formylated homooligopeptides derived from L-Met, L-Nle, and L-Cys(Me) tend to form intermolecular β-structures with antiparallel-chain arrangement in the solid state and in concentrated $CDCl_3$ solution beginning at the tetrapeptide level. In TFE, the β-structure is shown only by the Cys(Me) hexa- and heptapeptides. Addition of water to solutions of Met and Nle hexapeptides in TFE forces them to assume well-developed β-type associated structures. We have been able to disrupt peptide aggregation by diluting their $CDCl_3$ solutions. Under these conditions, the true tendency to form

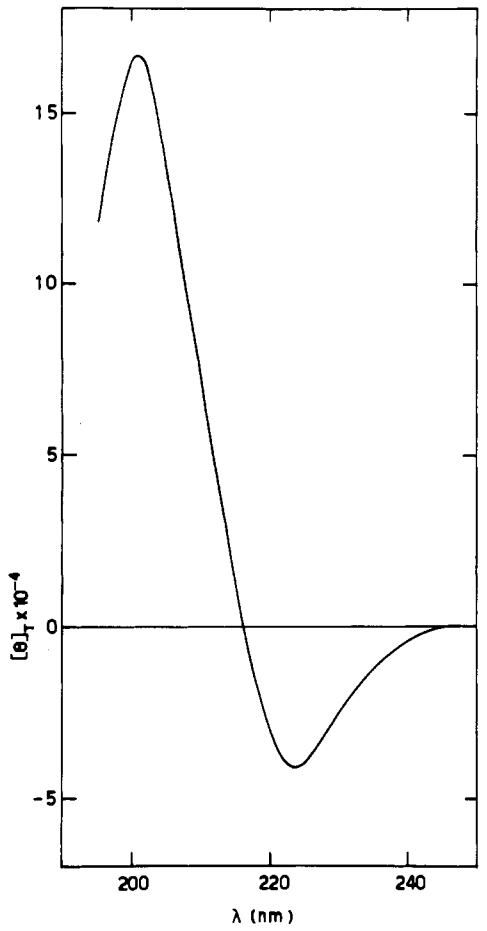

FIGURE 6: Circular dichroism spectrum of CHO(L-Met)$_6$OMe in TFE/water (20:80 v/v) (concentration 2×10^{-4} M).

intramolecularly hydrogen-bonded folded species could be detected. Finally, in HFIP, an acidic fluoro alcohol, all the peptides examined here exist essentially in an unordered conformation. These results are strongly indicative of the importance of solvent/solute interactions in determining peptide secondary structure. The structural role of the amino acid side chains is demonstrated by (i) the stabilizing effect of the sulfur atom of Met and Cys(Me) peptides on the N–H···O=C intramolecularly hydrogen-bonded folded forms in $CDCl_3$ at high dilution, (ii) the positive influence of the presence of a sulfur atom in the γ-position [Cys(Me) peptides], but not in the δ-position (Met peptides), on the formation of β-associated structures in TFE, and (iii) the correlation between the high hydrophobicity of the saturated hydrocarbon side chains of the Nle peptides and their strong propensity to aggregate in organic/aqueous solutions.

The only difference observed in the conformational behavior of N^{α}-formylated peptides and their N^{α}-*tert*-butyloxycarbonylated analogues (Palumbo et al., 1976; Bonora & Toniolo, 1974, 1978; Toniolo et al., 1975, 1979b, 1981; Toniolo & Bonora, 1975; Becker & Naider, 1974; Naider et al., 1978; Paskowski et al., 1978; Coffey et al., 1981) is a higher tendency to aggregate shown by the former.

Freer et al. (1982) have previously suggested that the rabbit neutrophil receptor for chemotactic formyl peptides is large enough to accommodate an oligopeptide with at least four amino acid residues. These authors also have proposed a working model for the chemotactic receptor of rabbit neutrophils. In this model, the peptide ligand exists on the receptor in an antiparallel β-structure. The tendency of CHO-L-Met-L-Leu-L-Phe-OH and its carboxyl derivatives to adopt that ordered secondary structure has been demonstrated in recent investigations by nuclear magnetic resonance (Becker et al., 1979; Bleich et al., 1979) and IR absorption [C. Toniolo and G. M. Bonora, unpublished results; cf., however, Bakir & Stevens (1982)]. Furthermore, five critical areas of ligand/receptor interactions have been postulated (Freer et al., 1980, 1982). These are in the vicinity of (i) the N-terminal formyl group, (ii) the Met side chain, with a region of positive charge around the electron-rich sulfur atom in the δ-position, (iii) the hydrophobic Leu and (iv) Phe side chains, and (v) the carbonyl group of the amino acid residue in position 3.

The biological and conformational results described here are in agreement with the proposed model. In particular, the most relevant biological finding is that in the three N^{α}-formylated *C*-methoxy homooligopeptide series tested the highest level of activity is attained at the tetrapeptide or pentapeptide stage. Work is currently in progress to synthesize and to test the chemotactic activity of the CHO(L-Met)$_n$Y (Y = OH, OBzl, NHBzl) series. The lowest members of the Y = OH series have already been examined by Showell et al. (1976). CHO-L-Met-L-Leu-L-Phe-Y (Y = OBzl, NHBzl) have been found to be >10 times more active than the parent Y = OH tripeptide (Freer et al., 1982).

In addition, the most significant conformational result is the high propensity to give self-associated β-structures (certainly, at least in part, of the antiparallel type), exhibited by all three homooligopeptide series in a solvent of low polarity ($CDCl_3$), which can mimic the environment at the receptor site, starting at the tetrapeptide level. This conclusion supports the view that this could be the biologically active conformation of the ligand, favored by the high local concentration of –CO–NH groups of the polypeptide chain of the receptor. M. Iqbal et al. (unpublished results) have found that the biological activity of the stereochemically hindered CHO-Met-Aib-Phe-OH and CHO-Met-Cyl-Phe-OH is high but less than that of CHO-Met-Leu-Phe-OH. This suggests that folded structures may be compatible with high although not optimal biological activity.

As a corollary, the multiple, specific points of interaction between the amino acid side chains of the ligand and the receptor might be the factors modulating the different biological activities observed for the three series. This latter consideration could explain the finding that the CHO(L-Met)$_n$OMe peptides are more active biologically than the corresponding CHO(L-Nle)$_n$OMe peptides which, in turn, are more active than the corresponding CHO[L-Cys(Me)]$_n$OMe peptides. This rank order is the same as that found for CHO-L-Met-L-Leu-L-Phe-OH, CHO-L-Nle-L-Leu-L-Phe-OH, and CHO-L-Cys(Me)-L-Leu-L-Phe-OH (Freer et al., 1980). This suggests the possibility that the N-terminal amino acid is the predominant amino acid in determining the binding of the peptide to the receptor. This is not unreasonable, considering the dominating influence of the N-terminal formyl group on binding.

The rank order for the tendency to self-associate found for the three tetrapeptides in concentrated $CDCl_3$ solutions is Met > Nle > Cys(Me) (Table III). As a consequence, it is quite possible that the propensity to aggregate might be a concomitant factor which helps to explain, at least in part, the observed rank order of biological activity.

Acknowledgments

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Registry No. I (AA = Met), 2488-15-5; I (AA = Nle), 6404-28-0; I [AA = Cys(Me)], 16947-80-1; II (AA = Met)·HCl, 2491-18-1;

II (AA = Nle)·HCl, 3844-54-0; II [AA = Cys(Me)]·HCl, 34017-27-1; III (AA = Met), 51529-23-8; III (AA = Nle), 67509-51-7; III [AA = Cys(Me)], 58623-55-5; IV (AA = Met), 88211-78-3; IV (AA = Nle), 67509-49-3; IV [AA = Cys(Me)], 58623-54-4; V (AA = Met), 52056-86-7; V (AA = Nle), 88211-79-4; V [AA = Cys(Me)], 88211-80-7; VI (AA = Met), 53298-44-5; VI (AA = Nle), 67509-52-8; VI [AA = Cys(Me)], 58623-56-6; VII (AA = Met), 52056-90-3; VII (AA = Nle), 76492-09-6; VII [AA = Cys(Me)], 76492-27-8; VIII (AA = Met), 53298-45-6; VIII (AA = Nle), 67509-53-9; VIII [AA = Cys(Me)], 58623-57-7; IX (AA = Met), 68731-91-9; IX (AA = Nle), 76492-10-9; IX [AA = Cys(Me)], 76492-28-9; X (AA = Met), 53415-27-3; X (AA = Nle), 67509-54-0; X [AA = Cys(Me)], 58623-58-8; XI (AA = Met), 76492-31-4; XI (AA = Nle), 76492-11-0; XI [AA = Cys(Me)], 76492-29-0; XII (AA = Met), 53298-46-7; XII (AA = Nle), 67509-55-1; XII [AA = Cys(Me)], 58623-59-9; XIII (AA = Met), 53298-47-8; XIII (AA = Nle), 67509-56-2; XIII [AA = Cys(Me)], 58623-60-2; CHO(L-Met)$_2$OMe, 88211-81-8; CHO(L-Met)$_3$OMe, 88211-82-9; CHO(L-Met)$_4$OMe, 88211-83-0; CHO(L-Met)$_5$OMe, 88211-84-1; CHO(L-Met)$_6$OMe, 88211-85-2; CHO(L-Met)$_7$OMe, 88211-86-3; CHO(L-Nle)$_2$OMe, 88211-87-4; CHO(L-Nle)$_3$OMe, 88211-88-5; CHO(L-Nle)$_4$OMe, 88211-89-6; CHO(L-Nle)$_5$OMe, 88211-90-9; CHO(L-Nle)$_6$OMe, 88229-03-2; CHO(L-Nle)$_7$OMe, 88211-91-0; CHO[L-Cys(Me)]$_2$OMe, 88211-92-1; CHO[L-Cys(Me)]$_3$OMe, 88211-93-2; CHO[L-Cys(Me)]$_4$OMe, 88211-94-3; CHO[L-Cys(Me)]$_5$OMe, 88211-95-4; CHO[L-Cys(Me)]$_6$OMe, 88229-04-3; CHO[L-Cys(Me)]$_7$OMe, 88229-05-4.

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