CIRCULAR DICHROISM SPECTRA OF BRADYKININ ANALOGS CONTAINING β-HOMOAMINO ACIDS

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SUMMARY

The CD spectra of β-homoprolyl^7-bradykinin (βHProB) and β-homophenylalanyl^8-bradykinin (βHPheB) were compared to those of bradykinin. The spectra were analyzed in terms of models that have been proposed for the solution conformation of bradykinin. Cann et al. (1) proposed 3 → 1 hydrogen-bonding across Pro^3, Pro^7 and Phe^8 in bradykinin, as shown by a 234 nm trough in the CD. The extra -CH_2- groups in the chains of the two bradykinin analogs would be expected to facilitate the proposed hydrogen-bonding, but in the case of βHProB the 234 nm trough is eliminated, and is reduced in magnitude for βHPheB. Ivanov et al. (2) proposed a cyclic conformation for bradykinin, stabilized by ionic attraction between the side-chain of Arg^1 and the carboxylate terminal. The extra -CH_2- groups of these two analogs would be expected to increase the stability of such a conformation, and there was some evidence that the ionic effects on the CD spectra of the two analogs were different from those on the bradykinin spectra. Alternatively, the effects could be attributed to cis-trans isomerizations around the prolyl peptide bonds.

In an effort to extend the biological half-life of the peptide bradykinin (BK), Ondetti and Engel (3) synthesized two analogs, containing extra -CH_2- groups one side or the other of Phe^8. The analogs, β-homoprolyl^7-bradykinin (βHProB) and β-homophenylalanyl^8-bradykinin (βHPheB), were designed to retain biological activity while conferring resistance to kininase II (angiotensin converting enzyme), a dipeptidyl carboxypeptidase thought to play a major role in terminating the actions of BK (4). The rationale behind the design of these analogs was to lessen the susceptibility of the peptides to proteolytic hydrolysis, by replacement of the relevant peptide bonds with β-aminoacyl bonds. This modification conserves the amino acid side-chains, which, presumably, are intimately involved in the mechanism of the biological activity. However, it is important in terms of biological activity, that not only should the chemical integrity of the peptide residue side-chains be maintained, but their spatial orientation, or ability to attain the correct biologically active conformation must be conserved.

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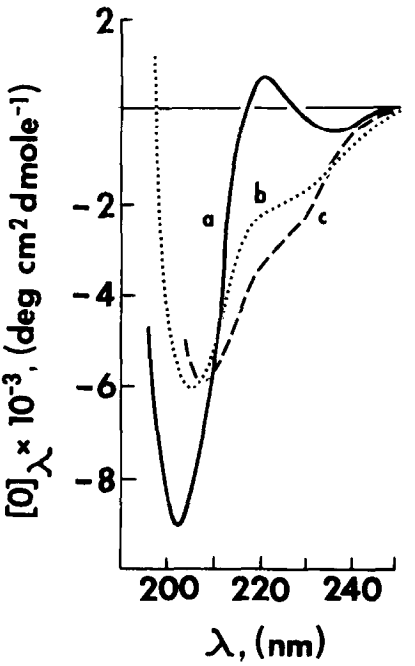

Figure 1. CD spectra of bradykinin in (a) ——, water; (b) ...., TFE and (c) ----, 4 M $CaCl_2$. The spectra were measured at an ambient temperature of 24°C.

The present study was begun to help define the solution conformations of these two analogs by examining their CD spectra and comparing them to those of BK. The CD spectrum of BK in aqueous solution (Fig. 1a) has been interpreted as representative of a disordered conformation (5,6) or a partially ordered conformation (1,2). Additionally, spectroscopic changes have been observed (1,7) for BK on changing the solvent environment, suggesting that some form of conformational response to environment occurs. The model proposed for the BK conformation by Cann et al. (1) has a 3 → 1 hydrogen bond across the Pro^7 with additional hydrogen bonding being introduced across Pro^3 and Phe^8 in organic solvents. This results in a hydrogen-bonded structure resembling a partial 2_7 ribbon or 2.2_7 helix (8). The conformation proposed by Ivanov et al. (2) is a pseudocyclic one, stabilized by an ionic interaction between the guanido group of Arg^1 and the carboxylate terminal.

The analogs described in this communication both have some bearing on the models of Cann et al. (1) and Ivanov et al. (2), since the $-CH_2-$ groups are introduced near the carboxyl terminal of the backbone chain, where, these models suggest some structural rigidity is present.

MATERIAL AND METHODS

β-homoprolyl^7-bradykinin (βHProB) and β-homophenylalanyl^8-bradykinin (βHPheB) were supplied by Dr. M.A. Ondetti of the Squibb Institute for Medical Research, Princeton, N.J. Bradykinin (BK) was purchased from Peninsula Inc., San Carlos, Ca. Trifluoroethanol (TFE) was purchased from Sigma Chemical Co., St. Louis, Mo., and calcium chloride dihydrate was purchased from Fisher Scientific Inc., Fairlawn, N.J. All of these chemicals were used without further purification. A JASCO ORD/UV-5 spectropolarimeter was used for the CD measurements. The molar ellipticity [θ] was expressed as deg. $cm^2 dmole^{-1}$. Path lengths of the cells varied from 0.2 mm to 10 mm. The peptide concentrations expressed as mg/ml ranged from 0.3 mg/ml to 1.5 mg/ml. Biological activities were tested in terms of effects on isolated rat uterus and Guinea-pig ileum, and for effects on mean arterial blood pressure (9). The effects on blood pressure were tested after intravenous and intra-arterial injection (via the ascending aorta). The two routes of administration were used to assess the relative rates of elimination in the pulmonary vascular bed.

RESULTS AND DISCUSSION

The additional $-CH_2-$ group of βHProB interspaces where the hydrogen bonding across Pro^7 is postulated to occur (Cann et al., 1). This interspacing should result in the bond angles and distances more closely approaching the optimum values described by Ramachandran et al. (10) for ideal hydrogen bonding; viz., a N to O distance between 2.6 and 3.2 Å and the angle between the N-H bond and the line N----O <30°. Cann et al. (1) used the criterion of a trough at 234 nm in the CD of BK as reflecting the hydrogen bonding across Pro^7. Thus, we should expect for βHProB an increased magnitude of the trough in the region of 230 nm. However, as shown in Fig. 2a, this is not the case. The trough is eliminated and the magnitude of the 220 nm peak is increased. The aqueous CD spectrum of βHProB is closely analogous to that of glycyl^6-BK (5) (an analog equipotent with BK), with the absence of a trough at 234 nm and an increased magnitude of the peak at 220 nm. The 220 nm peak has been attributed by Ivanov et al. (2) and Cann et al. (1), as arising from the orientation of the aromatic residues. If their interpretation is correct, the introduction of

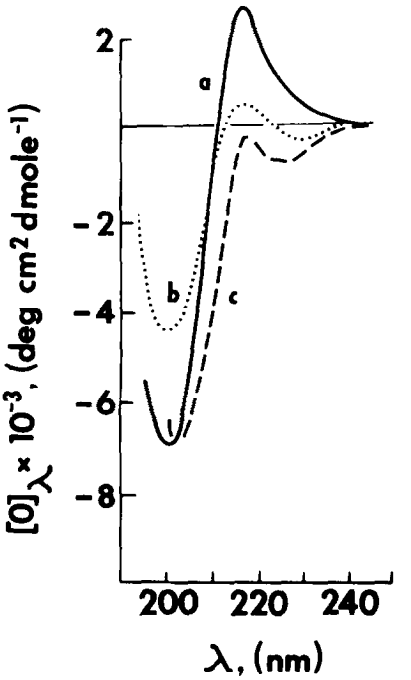

Figure 2. CD spectra of β-homoprolyl^7-bradykinin in (a) ——, water; (b) ...., TFE; (c) ----, 4 M $CaCl_2$. The spectra were measured at an ambient temperature of 24°C.

a $-CH_2-$ group on either side of Pro^7 apparently results in a similar disposition of the Phe residues.

In TFE solvent BK gives the CD spectrum shown in Fig. 1b. Compared to the CD spectrum in water (Fig. 1a), the 234 nm trough has been replaced by a shoulder at 228 nm, presumably by a blue-shift of the responsible transition, while the trough at 204 nm is decreased in magnitude. βHProB shows similar CD behavior in TFE (Fig. 2b), with a shallow trough, $[\theta] = -410°$ appearing at 233 nm and the 220 nm peak changing from $[\theta] = +2607°$ in water to $[\theta] = +443°$ in TFE. The resultant spectrum of βHProB in TFE is quite close to that of BK in water, although the trough at 204 nm is still significantly lower for βHProB than for BK. Since the trend of the CD spectra for both BK and βHProB on going from water to TFE is similar, it appears

likely that the conformational changes is similar for the two peptides.

A recent paper by Lo and Mattice (11) indicated that the CD of poly-L-proline is affected by high concentrations of $CaCl_2$; an effect which they attributed to isomerization about the prolyl peptide bonds induced by Ca^{++} binding. Marlborough et al. (7,12) have suggested that the CD of BK, particularly in the 220 nm region, may be reflected by the poly-L-proline character of BK. This suggestion has also been made in the case of BK potentiating peptides and their analogs from Bothrops jararaca snake venom (13). The CD spectrum of BK in the presence of high concentrations of $CaCl_2$ undergoes a change similar to that of poly-L-proline, with the peak at 220 nm disappearing. A spectrum similar to that of BK in TFE is obtained (Fig. 1c). βHProB in a similar concentration of 4 M $CaCl_2$ yields a CD spectrum that in the 220 nm region is like that of BK in $CaCl_2$. However, the spectrum of βHProB remains unchanged in the 204 nm region (Fig. 2c). Thus, the conformational change induced by $CaCl_2$ is different for BK and βHProB, possibly due to configurational differences around the Pro^7.

In the case of βHPheB, the interspacing $-CH_2-$ group occurs across Phe^8. Again applying the criteria proposed by Ramachandran et al. (10), the bond lengths and angles are more favorable for hydrogen bonding across Phe^8 in βHPheB than in BK. Thus, following the arguments of Cann et al. (1), we would also expect a stronger 230 nm trough in organic solvents. The CD spectrum of βHPheB in TFE (Fig. 3b) shows that the trough in the region of 230 nm in Fig. 3a has altered to a stronger transition with a shoulder at 210 nm while the trough at 204 nm has not changed. However, it should also be noted that the behaviour of the 204 nm trough in the CD spectrum of βHPheB in TFE is different from both that of BK and βHProB. There is no decrease in the magnitude of the 204 nm trough for βHPheB on going from water (Fig. 3a) to TFE, unlike the situation with both BK and βHProB. Similarly, the CD spectrum of βHPheB in 4 M $CaCl_2$ (Fig. 3c) demonstrates different behavior to that of βHProB, but is similar to that of BK in 4 M $CaCl_2$. All

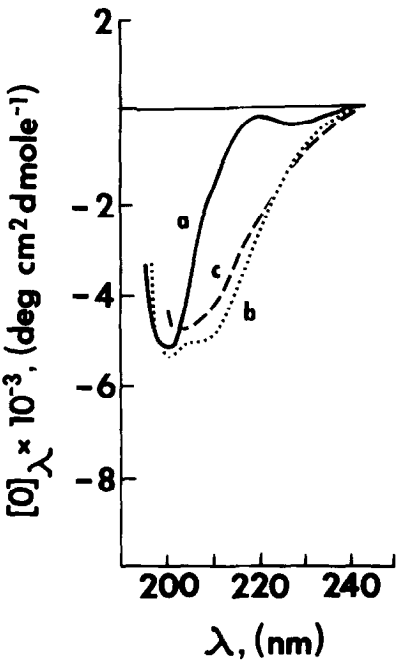

Figure 3. CD spectra of β-homophenylalanyl^{8}-bradykinin in (a) ——, water; (b) ...., TFE; (c) ----, 4 M $CaCl_2$. The spectra were measured at an ambient temperature of 24°C.

the spectra of these peptides show a slight red shift of the 204 nm trough on going from water to 4 M $CaCl_2$, while no significant change in wavelength is seen on going from water to TFE.

Although the spectra obtained from βHProB and βHPheB are not entirely consistent with Cann et al.'s model of hydrogen bonding (as reflected by the intensity of the 230 nm trough), it may be that the orientation of the aromatic residues, and their subsequent effects on the 220 nm transition in the CD spectra superimpose on any transitions arising from hydrogen bonding.

In the pseudocyclic BK conformation proposed by Ivanov et al. (2), there is a turning point around the Gly^{4} residue. Presumably the stability of this particular turn in the chain arises from the small steric disruption of Gly. It has been pointed out in a recent review article (14) that cyclic peptide

Table I. Biological Activities (% of Bradykinin)

Analogs	Rat Uterus	G.P. Ileum	Rat Blood Pressure	
			IV	IA
Bradykinin	100	100	100	100
β-homoPro^{7}-BK	28.4	5.5	66.7	10
β-homoPhe^{8}-BK	1.7	2.8	<1.0	2.8

Biological activities of bradykinin and the analogs βHProB and βHPheB measured by effects on rat uterus and Guinea-pig ileum and rat blood pressure (intravenously, IV and intra-arterially, IA). Bradykinin = 100.

structures are favored by the presence of Pro and Gly in a peptide chain; residues around which the backbone may turn. Thus, the introduction of extra $-CH_2-$ groups into the chain could result in an even more favorable disposition to adopt a cyclic conformation. However, it must also be considered that with these two analogs, the side-chains, and particularly the aromatic side-chains, might be disposed in a different orientation with respect to the receptor binding site compared to BK.

Ondetti and Engel (3) reported that βHProB, injected intravenously, is as potent as BK in its ability to lower mean arterial blood pressure and βHPheB is 30 to 100 times less potent. We have confirmed these results but have found that βHProB is only 1/3 to 1/20th as active as BK, when injected via the root of the aorta or when tested on Guinea-pig ileum or rat uterus. These data indicate that βHProB has relatively little intrinsic biological activity but is much more resistant to degradation by lung enzymes than is BK. This is supported by the observation by Ondetti and Engel (3) that βHProB is not cleaved by rat or rabbit angiotensin converting enzyme. Our results are shown in Table I.

The lower biological activity of βHProB and the almost negligible biological activity of βHPheB could be a result of the pseudocyclic conformation being

disturbed. The nearer the end of the chain the extra $-CH_2-$ group occurs, the more the biological activity is reduced. Possibly the introduction of the extra $-CH_2-$ group nearer the carboxyl terminal in βHPheB (compared to βHProB) displaces the carboxylate terminal perturbing the ionic interaction postulated by Ivanov et al. (2).

The CD spectroscopic behavior of BK in the presence of high concentrations of $CaCl_2$ is consistent with the trans to cis isomerization around the prolyl bonds, suggested by Marlborough et al. (12) to be involved in the conformational changes of BK. However, the differing effects of $CaCl_2$ on the CD of βHProB and βHPheB are consistent with differing effects upon the ionic attraction involved in the conformation proposed by Ivanov et al. (2). The extra turns introduced into the BK backbone chain by the additional $-CH_2-$ group would be expected to perturb the ionic interaction stabilizing the cyclic conformation, and thus may produce different CD spectra in high ionic strength media.

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