Role of β-Turn in Proteolytic Processing of Peptide Hormone Precursors at Dibasic Sites†

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ABSTRACT: Proteolytic activation of prohormones and proproteins occurs most frequently at the level of basic amino acids arranged in doublets. Previous predictions by Rholam et al. [Rholam, M., Nicolas, P., & Cohen, P. (1986) *FEBS Lett. 207*, 1–6] have indicated, on the basis of 20 prohormone sequences containing 53 dibasic potential processing sites, that dibasic sites situated in, or next to, β-turns were cleaved in vivo, whereas sites included in ordered structures like β-sheets or α-helices were not. We have used peptide analogs of the proocytocin/neurophysin processing domain and a purified preparation of the putative proocytocin convertase from bovine tissues as a model to demonstrate that (1) processing at dibasic sites is associated with a prohormone sequence organized in a β-turn structure; (2) the β-turn is an interchangeable motif since the original sequence could be replaced by an heterologous one possessing the ability to organize as a β-turn; and (3) this particular secondary structure participates in the catalytic reaction, most likely by favoring the interactions of the substrate with the processing endoprotease. It is concluded that, in addition to the dibasic and other amino acids around the cleavage loci, the β-turn constitutes a key feature in the proteolytic processing reaction in participating as the favorable conformation for optimal substrate–enzyme active site recognition.

Proteolytic processing of a number of protein precursors is an activation mechanism involving selective endoproteases. These enzymes have not been, as yet, unequivocally identified, and so far, limited information is available on their mechanism of action. Whereas a few possible candidates for processing endoproteases have been isolated and characterized, their precise relevance to the physiological process could not be assessed (Mizuno & Matsuo, 1984; Gluschankof et al., 1987; Maret & Fauchère, 1988; Plevrakis et al.,1989; Kuks et al., 1989; Darby & Smyth, 1990; Bourdais et al., 1991). By reference to the yeast pro-α-factor processing enzyme (Fuller et al., 1989), the furin/KEX2 substilisin-like gene product families were proposed to process enzymes in mammalian tissues (Thomas et al., 1988; Wise et al., 1990; Zollinger et al., 1990; Seidah et al., 1990). Since their action was shown in experiments using cell lines cotransfected with the relevant cDNAs, little as yet is known about the corresponding protein structures or about their substrate requirements. In contrast, a large number of prohormone and proprotein sequences are presently available, and examination of these structures clearly indicates that those loci where limited proteolysis occurs are predominantly basic amino acid doublets (rewieved in Cohen (1988) and Darby and Smyth (1990)).

Analysis of the sequences flanking these basic residues failed to reveal the occurrence of consensus amino acid stretches (Rholam et al., 1986; Bourdais & Cohen, 1991), but indicated that these sequences might participate in the recognition of the cleavage loci by providing accessible peptide segments constituted by β-turns (Rholam et al., 1986) or, alternatively, Ω-loops, as reinvestigated by Bek and Berry (1990)). Studies conducted with peptides reproducing the processing segment of proocytocin/neurophysin (pro-OT/Np) around the cleavage site $Lys^{11}Arg^{12}Ala^{13}$ have shown that the processing activity of the endoprotease, proocytocin/neurophysin convertase (Clamagirand et al., 1987), was affected by selective modifications of amino acid residues close to this site. In particular, amino-terminal extension of the octapeptide Leu^{8}–Leu^{15} (pro-OT/Np) by one amino acid favoring β-turn formation transformed this nonsubstrate into a substrate exhibiting a relative reactivity toward the protease (Brakch et al., 1989; Rholam et al., 1990).

The existence of such organized secondary structures was recently shown in the case of the processing sequences flanking the $Lys^{11}Arg^{12}$ doublet of the common precursor for ocytocin and neurophysin (pro-OT/Np) (Paolillo et al., 1992). Convergent observations obtained by circular dichroism (CD), infrared Fourier transform (FT-IR), and 1D and 2D proton nuclear magnetic resonance (NMR) spectroscopy indicated that the tetrapeptide sequence situated on the NH_2-terminal of the LysArg doublet (i.e., Pro^{7}–Gly^{10}) organizes as a β-turn and that the neighboring segment on the COOH-terminus of the dibasic pair tends to form an α-helical structure (Paolillo et al., 1992).

In the present work, we have examined several questions concerning the role of the β-turn and its possible relevance to the catalytic process. Therefore, we have made use of a series of synthetic peptides either reproducing or mimicking the pro-OT/Np [1–20] processing domain to measure their reactivity toward a purified preparation of the putative proocytocin/neurophysin converting endoprotease isolated from bovine tissues (Clamagirand et al., 1987; Plevrakis et al., 1989). The results indicate that the β-turn situated on the amino-terminal side of the $Lys^{11}Arg^{12}Ala^{13}$ segment is both interchangeable and important in substrate recognition by the enzyme.

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Table I: Cleavage of Various Prohormone Sequences by the Proocytocin/Neurophysin Convertase[a]

substrates	cleavage site	% cleavage
pro-OT/Np (Cys^{1}–Ser^{104})	Cys^{1}-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-Gly-**Lys-Arg↓Ala**-Val-Leu-Asp-Leu-Asp-Val-Arg^{20}	80
proneuropeptide Y (Tyr^{1}–Trp^{70})	Ile^{28}-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-Gly-**Lys-Arg↓Ser**-Pro-Glu-Thr-Leu-Ile-Ser^{47}	90
proglucagon (His^{34}–Thr^{61})	Phe^{38}-Thr-Ser-Asp-Val-Ser-Lys-Val-Leu-Asp-Ser-**Arg-Arg↓Ala**-Gln-Asp-Phe-Val-Gln-Trp-Leu^{58}	40
proneurotensin (Glu^{154}–Tyr^{170})	Glu^{154}-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-**Lys-Arg↓Ala**-Ser-Tyr-Tyr-Tyr^{170}	100
proenkephalin B (Tyr^{228}–Thr^{240})	Tyr^{228}-Gly-Gly-Phe-Leu-**Arg-Arg↓Gln**-Phe-Lys-Val-Val-Thr^{240}	100

[a] Arrows indicate the peptide bond cleaved in the reaction.

MATERIAL AND METHODS

Secondary Structure Predictions. The secondary structure of different precursors was analyzed by a modification of Chou and Fasman's original method, which uses the multiplication approach instead of the arithmetic (Dufton & Hider,1977). α-Helices and β-sheets were deduced using tabulations of structure–formation parameters (Levitt, 1979). Chou and Fasman's numerical method for β-turn prediction was adopted (Chou & Fasman, 1978).

Peptide Synthesis. The peptides listed in Table III were synthesized by the solid-phase method (Merrifield, 1963) using an NPS 4000 semiautomated multisynthesizer (Neosystem, Strasbourg, France). They have been numbered in accordance with previous publications from this laboratory (Brakch et al., 1989). Peptide purification and analysis were conducted using a set of analytical techniques including HPLC, amino acid composition, amino-terminal sequencing, and fast atomic bombardment mass spectrometry, as in Nicolas et al. (1986).

Enzyme Assay. Each of the peptides described above was tested using a purified preparation of the proocytocin convertase isolated from bovine neurohypophysis, as described in Plevrakis et al. (1989).

K_m and V_{max} values of different peptide substrates (Table III) were determined from initial velocity measurements plotted vs various substrate concentrations using the Lineweaver–Burk representation. The standard assay was as follows: the peptide in the range 5–200 nmol was incubated with an aliquot of purified enzyme (1.6 μg of protein) in a final volume of 50 μL of 0.1 M ammonium acetate (pH 7.0) for 5 h. At the end of each incubation period, the reaction was stopped with 0.1 N HCl (10 μL), and the entire sample was subjected to HPLC analysis on a C_{18} column (ultrabase, SFCC-Shandon) eluted with a gradient of 5–40% or 15–40% acetonitrile/0.05% TFA over 30 min at a flow rate of 0.5 mL/min. Each of the fragments generated by the protease was identified by reference to standards and/or by amino acid composition analysis using a Waters Picotag station. Controls were conducted to show that under these reaction conditions the enzyme activity was stable. Each assay was run at least in triplicate and the mean value was evaluated (SD < 5%).

The percent cleavage [% = $100(A_0 - A_i)/A_0$] of each substrate peptide was evaluated from the absorbance (A_{215nm}) of each peptide incubated both in the absence (A_0) and in the presence (A_i) of enzyme under the same incubation time and pH conditions.

Circular Dichroism Measurements. All CD spectra were acquired on a Jobin-Yvon Mark IV high-sensitivity dichrograph attached to a Mink Digital 11 miniprocessor, using a cylindrical fused quartz cell of 0.1 cm path length at 25 °C. For experiments with 2,2,2-trifluoroethanol (TFE), samples were prepared by dissolving weighed quantities of the peptide in a minimum amount of water and then adding TFE up to a final content of 50 or 98% (v/v). CD spectra of peptides were acquired at three different peptide concentrations: 0.5, 2, and 5 mg/mL. No effect of peptide concentration on the spectral profiles was observed. Spectra, reported in units of mean residue ellipticity (peptide molecular weight/number of amino acids) $[\Theta]_R$ (deg·cm^2 dmol^{-1}), were base-line corrected and smoothed.

RESULTS

Prohormonal Dibasic Cleavage Sites Are Associated with β-Turns. Previous studies using secondary structure predictive methods (Rholam et al., 1986) conducted on a large number of prohormone cleavage sites have indicated that those dibasic processing sites which are cleaved in vivo are situated in, or immediately next to, β-turns. In contrast, those potential sites which are not cleaved in vivo are associated with ordered structures such as α-helices, β-sheets (Rholam et al., 1986), or higher order organization (Paolillo et al., 1992). In order to give experimental support to the concept that the β-turn structures might constitute a recognition signal for the processing enzyme machinery, several prohormone sequences were submitted to the action of the putative pro-OT/Np convertase.

Peptides representing either the entire prohormone sequence (pro-OT/Np and proneuropeptide Y) or fragments of the precursor (proglucagon, proneurotensin, and prodynorphin) were used (Table I). These molecules contained dibasic moieties of the LysArg or ArgArg type which are known to be processed in vivo. The results indicated that, in all cases, in vitro cleavage occurred at the COOH-termini of those basic doublets (see arrows in Table I), i.e., a locus identical to the in vivo cleavage point. On the basis of this finding, and since there is a great divergence in peptide primary structures around the scissile bonds (see Table I), these experimental data suggested that these proteolytic processing sites might share one, or more, common structural feature(s), i.e., the β-turn structure which was predicted in the vicinity of these cleavage loci (Rholam et al., 1986; Bek & Berry, 1990). Therefore, in view of the special role played by this structural organization in a number of biological processes (Rose et al., 1985; Tinker et al., 1988; Duffaud & Inouye, 1988; Ni et al., 1989; Ball & Alewood, 1990; Collawn et al., 1990; Eberle et al., 1991; Laczkö et al., 1992), we hypothesized that this widespread structural motif could be replaced by a nonhomologous tetrapeptide stretch possessing equivalent secondary structure forming propensity without significant changes in the enzyme reaction.

The β-Turn Is an Interchangeable Structural Motif. In order to establish the relevance of the β-turn structure with the endoproteolytic reaction, analogs of the Pro^{7}–Leu^{15} segment of pro-OT/Np were synthesized. Since it has been

Table II: Amino Acid Sequences of Proocytocin/Neurophysin Related Peptide Substrates

XXV	*Pro-Leu-Gly-Gly*-Lys-Arg-Ala-Val-Leu
XXXVIII	*Ser-Ser-Asn-Gly*-Lys-Arg-Ala-Val-Leu
XXXIX	*Tyr-Lys-Gly-Gln*-Lys-Arg-Ala-Val-Leu
XXXX	*Asn-Asn-Ile-Ala*-Lys-Arg-Ala-Val-Leu
XXXXI	*Ile-Ala-Val-Leu*-Lys-Arg-Ala-Val-Leu
XXXXII	*Ile-Ala-Val-Leu*-Lys-Arg-Ala-Val-Leu-Asp-Leu-Asp-Val-Arg
[Pro^7]XXIII	*Pro-Leu-Gly-Gly*-Lys-Arg-Ala-Val-Leu-Asp-Leu-Asp-Val-Arg
XXXXIII	*Ala-Val-Leu-Asp-Asp-Leu-Asp-Val*-Lys-Arg-Ala-Val-Leu-Asp-Leu-Asp-Val-Arg
I	*Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-Gly*-Lys-Arg-Ala-Val-Leu-Asp-Leu-Asp-Val-Arg

previously shown by spectroscopic studies that the tetrapeptide Pro^{7}LeuGlyGly^{10} sequence tends to form a β-turn under certain solution conditions (Paolillo et al., 1992), this tetrapeptide was replaced in each case by a different sequence. Three tetrapeptide sequences (Table II), Ser^{7}SerAsnGly^{10}, Tyr^7-LysGlyGln^{10}, and Asn^{7}AsnIleAla^{10}, respectively, were chosen on the basis of their capacity to organize as β-turns, as previously shown by X-ray crystallography (Hynes et al., 1989) or NMR spectroscopy (Aumelas et al., 1989).

Direct verification that these peptides (i.e., peptides XXXVIII, XXXIX, and XXXX) possess the propensity to organize in β-turn conformers was performed using circular dichroism. CD spectra of these derivatives, recorded in different solvents, are shown in Figure 1A-D. In aqueous solution, the main feature of the spectra of the Pro^7–Leu^{15} segment of pro-OT/Np and of its derivatives is an intense negative band between 190 and 195 nm, typical of polypeptides with a predominance of unordered conformer populations (Woody, 1974, 1985). In the presence of increasing amounts of TFE, the intensity of the band around 195 nm decreased and its maximum was red-shifted. Indeed, in solution containing 98% TFE, the CD curves of these nonapeptides exhibited two minima around 202 and 225 nm and a small maximum around 190 nm. According to the classification of Woody (1974, 1985), these CD spectra can be accurately described as a mixture of types C and D spectra characteristic of the type I(III) β-turn (Gierasch et al., 1981) and of a distorted turn structure (Ötvös et al., 1981), respectively. Moreover, the spectrum of peptide XXXIX (Figure 1C) was affected by the contribution of the tyrosine chromophore, as observed in other peptides (Fermandjian et al., 1981, 1983). On the basis of these spectroscopic data, it can be concluded that these pro-OT/Np peptide analogs have the potential to form structures in organic solvents. Therefore, the CD profiles of these nonapeptides, as observed previously for other pro-OT/Np peptide analogs (Rholam et al., 1990), represent in aqueous solution a conformational equilibrium between aperiodic structures and folded conformations (β-turns), which is shifted to ordered structures by hydrogen-bonding-promoting solvents like TFE.

The three resulting nonapeptides also were tested for their ability to be cleaved by pro-OT/Np convertase at the Arg^{12}Ala^{13} bond, i.e., the locus where cleavage occurs both in vitro and in vivo on the full length precursor (Clamagirand et al., 1987; Brakch et al., 1989; Camier et al., 1991). The results in Table III indicate that pro-OT/Np convertase can cleave all three modified peptides (peptides XXXVIII, XXXIX, and XXXX) as well as the reference (peptide XXV) at the COOH-terminus of the LysArg motif without important effects on the kinetic parameters. Indeed, the values of K_m and V_{max} were in the ranges 150–450 μM and 700–2800 pmol h^{-1}, respectively (Table III). Although the efficacy of peptide XXXIX was higher, it should be noted that the overall magnitude of these changes affected both K_m and V_{max} similarly (see Table III).

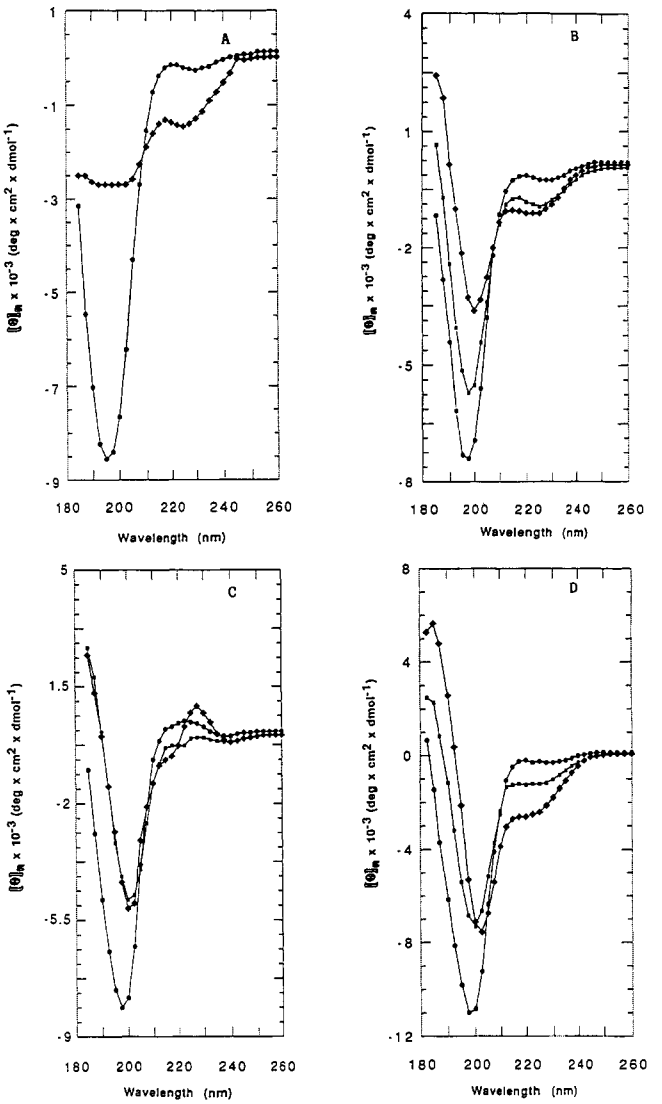

FIGURE 1: CD spectra of pro-OT/Np-related peptides in H_2O (●), 50% TFE (■), and 98% TFE (◆): (A) peptide XXV; (B) peptide XXXVIII; (C) peptide XXXIX; (D) peptide XXXX.

Table III: Kinetic Parameters for Proocytocin/Neurophysin Related Peptide Cleavage by the Convertase[a]

	K_m (μM)	V_{max} (pmol h^{-1})	V_{max}/K_m (pmol h^{-1} μM^{-1})
XXV	150	720	4.8
XXXVIII	350	990	2.8
XXXIX	370	2810	7.6
XXXX	430	1420	3.3
XXXXI	6450	4730	0.7
XXXXII	2880	4280	1.5
[Pro^7]XXIII	140	550	3.9
XXXXIII	4760	480	0.1
I	100	260	2.6

[a] In all cases where cleavage was observed, it occurred at the Arg–Ala bond.

Therefore, since the K_m and V_{max} values obtained with heterologous β-turn-forming sequences were of the same magnitude, these observations argue in favor of the interchangeability of the β-turn next to the LysArg doublet. However, the data did not allow us to correlate the processing reaction with a single β-turn subtype. This might explain the

observed variations in efficacies measured with those different β-turn-containing peptide substrates.

The β-Turn Is Essential for Substrate Recognition. The previous observations indicated that the tetrapeptide sequence specifying a β-turn could be replaced by another one with similar properties without drastic changes in the cleavage reaction occurring on the COOH side of the dibasic moiety. If this particular secondary structure was important for the cleavage by the processing endoprotease, one would expect that its replacement by an ordered structure of a different type, like a β-sheet or an α-helix, might affect either the thermodynamics or the kinetics of the reaction or both. This hypothesis was assessed by synthesizing different peptide derivatives of the Pro[7]–Leu[15] segment of pro-OT/Np. Three peptides were chosen on the basis of theoretical or experimental considerations (Table II).

On the basis of the modified Chou and Fasman method (Dufton & Hider, 1977), the sequence of peptide XXXXI is predicted to adopt a β-sheet ($\langle P_\beta \rangle = 2.06$) or an α-helix ($\langle P_\alpha \rangle = 2.86$) structure. Although its CD curve exhibits, in aqueous solution, a spectrum characteristic of a predominantly random structure (Woody, 1974, 1985), CD measurements in different percentages of TFE confirmed its propensity to organize as a β-sheet (Figure 2B). Indeed, as compared to standard spectra (Greenfield & Fasman, 1969), the spectrum exhibited by peptide XXXXI in TFE is most typical of a β-sheet with a negative band at, or near, 216 nm and a positive one between 195 and 200 nm. The design of the other two peptides was based on recent spectroscopic data obtained with peptides reproducing the pro-OT/Np [7–20] sequence, which demonstrated that the Ala[13]–Arg[20] segment (the eight NH_2-terminal residues of neurophysin), as previously predicted (Crémino et al., 1988), organizes in an α-helix (Paolillo et al., 1992). Therefore, in peptide XXXXII, the LysArg doublet was surrounded by IleAlaValLeu on the NH_2-terminus and by the α-helix-promoting sequence on the COOH-terminus. For peptide XXXXIII, the octapeptide sequence Ala[13]–Arg[20] was positioned on both sides of the LysArg doublet. CD spectra confirmed the ability of these peptides to organize as an ordered structure containing a notable proportion of conformers in the α-helix (Figure 2C,D). Indeed, all of the spectra showed, in TFE, two minima at 222 nm (helical n→π* transition) and between 202 and 208 nm (overlapping helical and random coil π→π* transition at 208 and 200 nm, respectively) and a maximum at 192 nm. The helix content of these peptides, estimated from the ellipticity at 222 nm (Chen et al., 1974), increased when compared to reference peptide I (Figure 2A).

The behavior of those substrate analogs toward the pro-OT/Np endoprotease was also analyzed (Table III). Replacement of the β-turn by an α-helix (peptides XXXXII and XXXXIII) or a β-sheet (peptide XXXXI) resulted in considerable increases in both K_m and V_{max}, the values for which were found in the ranges 2000–7000 μM and 300–5000 pmol h^{-1}, respectively (Table III). However, careful comparison of the kinetic parameters obtained with substrates of the same length indicated that the replacement of the β-turn by an ordered structure (β-sheet or α-helix) produced a more pronounced effect on the K_m than on the V_{max}. Indeed, pairwise comparisons of peptides XXXXI and XXV, peptides XXXXII and [Pro[7]]XXIII, and peptide XXXXIII with reference peptide I indicated increases in K_m of 43-, 20-, and 47-fold, whereas the corresponding V_{max} values were modified only by factors 6, 7, and 2, respectively (Table III).

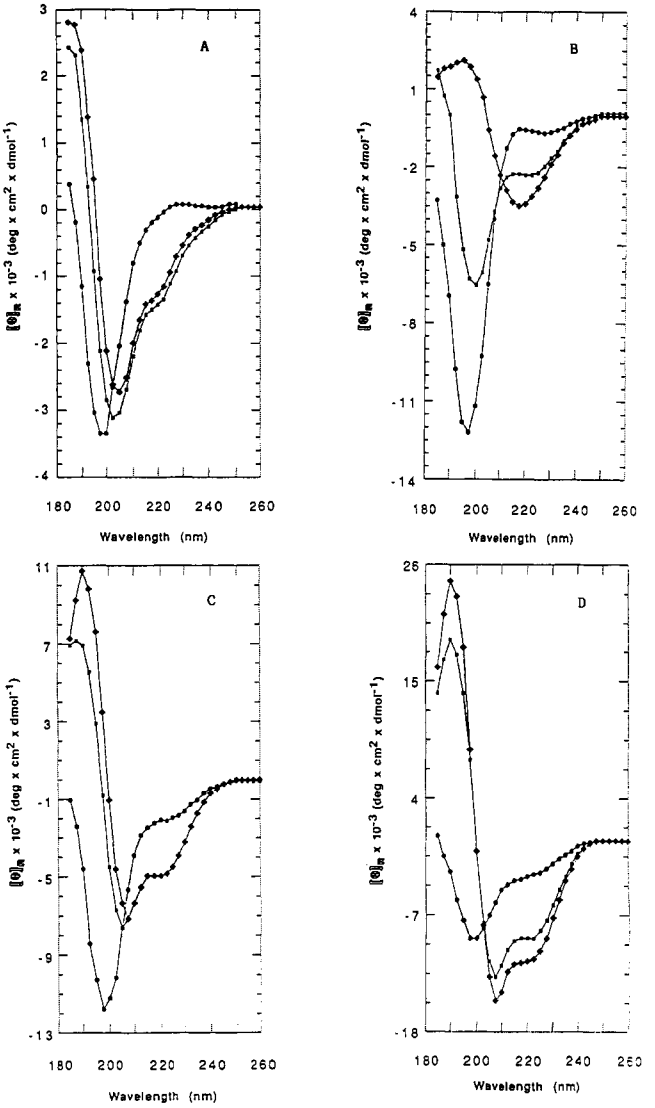

FIGURE 2: CD spectra of pro-OT/Np-related peptides in H_2O (●), 50% TFE (■), and 98% TFE (◆): (A) peptide I; (B) peptide XXXXI; (C) peptide XXXXII; (D) peptide XXXXIII.

On the basis of these data, it can be inferred that the nature of the secondary structure at the cleavage loci is a major determinant of the K_m value. Therefore, it can be concluded that the β-turn structure is an important feature in substrate recognition by the enzyme. Although these large modifications in K_m essentially reflect the replacement of a given secondary structure by another, we cannot exclude the possibility that part of these changes may be a consequence of modifications in substrate–subsite interactions with the enzyme resulting from primary structure modifications. However, it should be noted that replacement of the native β-turn-forming sequence, by a heterologous one containing different amino acid residues did not produce effects of comparable magnitudes (Table III).

DISCUSSION

Most often, proteolytic processing of peptide hormone and protein precursors proceeds by cleavage of peptide bonds situated around basic amino acid residues arranged as doublets (Cohen, 1988; Darby & Smyth, 1990). Single basic amino acids (Devi, 1991; Bourdais & Cohen, 1991) as well as higher order arrangements such as triplets, quadruplets, and quintets are also encountered but are less frequent. Conservation of such signals for enzyme recognition does not appear to be correlated with the existence of consensus primary sequences,

and examination of a large number of putative cleavage sites has failed to reveal striking features capable of providing a rational basis for a heuristic interpretation of processing selectivity.

The idea that some privileged secondary structures might provide part of the substrate binding site for processing enzyme recognition was first proposed by Geisow and Smyth on the basis of a limited number of prohormone sequences (Geisow, 1978; Geisow & Smyth, 1980). This earlier concept was revived by an examination of 20 prohormones containing 56 dibasic processing sites cleaved in vivo (Rholam et al., 1986). Indeed, although this particular secondary structure, i.e, β-turns, or larger structures such as Ω-loops (Rholam et al., 1986; Bek & Berry, 1990) may provide accessible sites for endoprotease action on macromolecular precursors, evidence that this type of structural organization may indeed play a role in these biological processes has only recently been supported by direct experimental data. The functional role of these particular structures in the endoproteolytic reaction was first suggested by preliminary observations indicating the importance of peptide segments situated on both the amino- and carboxy-terminal sides of the LysArg doublet involved in the enzyme reaction (Brakch et al., 1989; Rholam et al., 1990). Using a combination of spectroscopic techniques, it has recently been shown that the pro-OT/Np[7–20] processing domain can organize in solution as a turn–helix arrangement (Paolillo et al., 1992). Secondly, a series of observations using site-directed mutagenesis of various prohormones has underlined the particular importance of amino acid residues other than the dibasic ones in the specification of the correct processing at the cleavage sites (Gomez et al., 1989; Brakch et al., 1991). Consequently, the possible participation of proline and other residues that are situated next to the basic sites possess the propensity to favor β-turn formation was demonstrated using the somatostatin precursor as a model (Gomez et al., 1989; Brakch et al., 1991).

Turns, or loops, are often associated with exposed regions in proteins (Thornton et al., 1988; Wilmont & Thornton, 1988; Chothia et al., 1989; Ring et al., 1992). These types of structures, which are flexible and mobile, favor both the accessibility and the segmental adaptability of a given sequence, as compared with other ordered structures such as α-helices and β-sheets (Thornton et al., 1988; Chothia et al., 1989). Demonstration of the important role played by the β-turn in prohormone processing was first provided by substituting other β-turn-promoting segments for the native sequence without drastic changes in the kinetic parameters of the endoproteolytic reaction. If these observations favor the interchangeability of this structural motif type (Hynes et al., 1989), it can be inferred that the small differences observed in both kinetic parameters (see Table III) could explain the in vivo differential proteolytic processing of prohormones. Secondly, it could be shown that replacement of the amino acid sequences which favor β-turn formation by segments organized in an α-helix or in a β-sheet led instead to endoprotease substrates with largely modified kinetic parameters. Obviously, the observed large increase in K_m values reflects the fact that these dibasic residues situated in α-helix or β-sheet structures may represent poorer substrates than the native ones. However, it should be noted that only the smaller peptides, i.e., XXXXI (β-sheet) and XXXXII (α-helix), were cleaved with abnormally high V_{max} values, whereas the larger peptide XXXXIII (α-helix) exhibited a low V_{max}. Considering the fact that these three substrates bear common P_2, P_1, and P_1' subsites, which are known to be essential for enzyme reaction (Plevrakis et al., 1989; Brakch et al., 1991; Resnick et al., 1991; Thorne et al., 1991; M. Rholam, N. Brakch, H. Boussetta, and P. Cohen, in preparation), it is tempting to speculate that the source of V_{max} differences could be attributed to both peptide size (9 and 14 residues compared with 18 residues) and/or to the proportion of ordered conformers of the corresponding peptides in solution. On the basis of these considerations, it may be anticipated that, in vivo, those dibasic sites which are in the immediate vicinity of β-turns are preferentially recognized by the processing endoprotease and might be cleaved first. This conclusion may also hold true for plurifunctional proproteins exhibiting multiple potential cleavage sites. Moreover, although this concept provides a rational basis for understanding the discrimination between in vivo cleaved and uncleaved dibasic sites, it does not permit a simple interpretation of the observed hierarchy between the various dibasic amino acid combinations (LysArg > ArgArg > LysLys = ArgLys) (M. Rholam, N. Brakch, Simon, El Autri, Doucet, and P. Cohen, in preparation).

It is worth noting at this point that the conformation of the model peptides used in this work was determined by spectroscopic methods under solution conditions in which an organic solvent promoting ordered structures was used. In all cases, an equilibrium was observed between ordered and aperiodic forms, which shifts to the disordered conformations in water (Rose et al., 1985). Therefore, it is reasonable to assume that, under the conditions of the assay, interactions with the enzyme active site would favor ordered conformers (Fersht, 1985), shifting the equilibrium toward the observed β-turn or helix conformations.

On the basis of the data presented here, it is attractive to conclude that proteolytic processing may require the involvement of exposed and flexible structures in prohormones and/or proproteins, i.e., β-turns or larger loop structures. Since these particular secondary structures represent a significant fraction of a globular protein surface (Rose et al., 1985; Ring et al., 1992), it is not surprising that they are found to play an important role in determining the functional conformation and/or specificity parameters in enzyme activity (Rose et al., 1985), antibody recognition sites (Kuntz, 1972), peptide hormone active forms (Ball & Alewood, 1990), signal receptor-mediated internalization of lipoproteins (Collawn et al., 1990; Eberle et al., 1991), protein phosphorylation (Tinker et al., 1988), signal peptide excision (Duffaud & Inouye, 1988), thrombin–fibrinogen interactions (Ni et al., 1989), and glycosylation of precursors (Laczkö et al., 1992). Since our data strongly indicate that this model study might be generalizable to a number of prohormones or proproteins (see Table I), it should therefore also be extended to other processing endoproteases for which an in vivo involvement has been established. This should tell us more about the functional role and the universality of this particular secondary structure in prohormone processing.

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