Structure of Two Fragments of the Third Cytoplasmic Loop of the Rat Angiotensin II AT1A Receptor

IMPLICATIONS WITH RESPECT TO RECEPTOR ACTIVATION AND G-PROTEIN SELECTION AND COUPLING*

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The structural bases that render the third intracellular loop (i3) of the rat angiotensin II AT1A receptor one of the cytoplasmic domains responsible for G-protein coupling are still unknown. The three-dimensional structures of two overlapping peptides mapping the entire i3 loop and shown to differently interact with purified G-proteins have been obtained by simulated annealing calculations, using NMR-derived constraints collected in 70% water/30% trifluoroethanol solution. While the NH2-terminal half, Ni3, residues 213–231, adopts a stable amphipathic a-helix, extending over almost the entire peptide, a more flexible conformation is found for the COOH-terminal half, Ci3, residues 227–242. For this peptide, a cis-trans isomerization around the Lys6–Pro7 peptide bond generates two exchanging isomers adopting similar conformations, with an a-helix spanning from Asn9 to Ile15 and a poorly defined NH2 terminus. A quite distinct structural organization is found for the sequence EIQKN, common to Ni3 and Ci3. The data show that the extension and orientation of the amphipathic a-helix, present in the proximal part of i3, may be modulated by the distal part of the loop itself through the Pro232 residue. A molecular model where this possibility is considered as a mechanism for G-protein selection and coupling is presented.

The comprehension of the molecular details of G-protein coupled receptors (GPCRs) activation as well as of G-protein selection and coupling is still speculative. Similarly, several features of their three-dimensional structure still need to be defined. In this respect, while a large effort is being generated to define the orientation and three-dimensional organization of the transmembrane helices of GPCRs (1–5), not much has been done to describe the structural properties of the regions exposed to the extracellular or cytoplasmic media (6).

These considerations, and the results obtained on that subject for rhodopsin (7–11), for the a- and b-adrenergic receptors (12–14), and for the parathormone receptor (15, 16) have prompted us to undertake a study on some functionally relevant cytoplasmic domains of the angiotensin II AT1A receptor, mainly seeking to describe the structural and dynamic properties of the receptor surface that regulate its interaction with the various G-proteins.

In a previous work, we focused our attention on the conformational flexibility of a fragment of the AT1A COOH-terminal tail (17); here we show the existence and propose a model describing the dynamic features of the structural determinants that characterize the receptor third intracellular loop (i3) (Fig. 1).

As for various GPCRs such as the b-adrenergic, muscarinic, dopamine, and rhodopsin receptors (18–23), studies involving receptor chimeras and site-directed mutagenesis (24–28) indicate that i3 is one of the AT1A functional domains involved in G-protein interaction. In addition, comparison of the i3 of several GPCRs evidences a noticeable heterogeneity in amino acid sequence and size (29–32), suggesting that the secondary structure, rather than the primary sequence and/or the specific length of that domain, plays a key role in G-protein coupling.

Recent investigations revealed that a synthetic peptide representing the proximal part of i3 (residues 216–230) is able to activate purified Gq and G0 proteins, while the peptide comprising the distal part of that loop (residues 229–242) has no effect (33). Similar results have been obtained in a study focused on the activation of Gq, where it has been shown that the peptide encompassing residues 216–231 is active, while the one representing the i3 segment 230–241 does not exhibit any activity (34). Interestingly, the proximal half of i3 has been predicted to have a very high probability to adopt an amphipathic a-helical structure, whereas the distal half is predicted to form a short helix only at its COOH-terminal end (35, 36).

On the basis of these evidences, we have studied the solution conformation of two synthetic fragments mapping the entire i3 loop, the NH2-terminal 19-mer TSYTLIWKALKKAYEIQKN-NH2, N3 (residues 213–231), and the COOH-terminal 16-mer EIQKNKPRNDIFRII-NH2, C3 (residues 227–242). The underlined residues indicate the overlapping region between the two peptides.

The structures of the two peptides in 70% H2O/30% TFE were obtained by means of restrained molecular dynamics cal-

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calculations using, as restraints, the NMR-derived proton distances and \( \phi \) dihedral angles.

The results show that Ni3 is characterized by a well-defined amphipathic \( \alpha \)-helix extending over almost the entire peptide sequence.

For Ci3, the data indicate the existence of cis-trans isomerization about the Lys 6—Pro 7 peptide bond giving rise to two slowly exchanging conformational states. The resulting isomers adopt very similar secondary structures characterized by a poorly defined NH\(_2\) terminus and by a flexible amphipathic \( \alpha \)-helix in the COOH-terminal stretch (Asn\(^{9} \)-Ile\(^{15} \)). Interestingly, the sequence EIQKN common to Ni3 and Ci3, and corresponding to the central part of i3, adopts a quite distinct structural organization in the two peptides suggesting, for Pro\(^{233} \), a functional role as structure breaker or modulator (37, 38).

The results of this work, together with our previous study (17), provide the basis for disclosing some conformational features of the native receptor cytosolic face. In particular, the data support the hypothesis that the capability of specific do-
mains of the receptor to form amphipathic α-helices is essential for receptor activation and G-protein selection and coupling.

RESULTS

CD Experiments—The CD spectra of both peptides did not indicate the existence of any preferential secondary structure in aqueous solution (Fig. 2), and they were not affected by changes in concentration within the 0.15–1.5 mM range.2 However, upon addition of small aliquots of TFE, it was found that NIII exhibited a transition to an α-helical conformation the extension of which increased up to 30% TFE (Fig. 2A), where it stabilized at an helical content of ~55%, as calculated according to the method of Chen et al. (40). For Ci3, instead, only a partial folding into an ill defined helical structure could be observed at high TFE concentration (Fig. 2B). In this case, however, due to the complexity of the spectrum suggesting the existence of a multiple conformation equilibrium, the use of the same method to evaluate the peptide α-helical content (Fig. 2B) appears not to be ideal. A detailed analysis of the CD data will be the subject of a work in preparation.2

1H-NMR Experiments—According to the CD results, all 1H-NMR experiments were recorded in 70% H2O/30% TFE-d3 (v/v) solution. The complete assignment of the proton resonances for the two peptides was obtained using standard two-dimensional methods (41). The spectra of Ci3 displayed two distinct sets of resonances for the stretch Lys6–Arg14 (Table I), suggesting the existence of a cis-trans isomerization about the Lys 6–Pro 7 bond. The correct assignments of the protons corresponding to the two conformers was based on the observation, however, due to the complexity of the spectrum suggesting the partial folding into an ill-defined helical structure could be evaluated the peptide

| Residue | 1H, ppm | 1H, ppm |
|---------|---------|---------|
| Glu1    | 7.2     | 6.4     |
| Ile2    | 8.6     | 8.5     |
| Gln3    | 7.2     | 7.2     |
| Lys4    | 7.2     | 7.2     |
| Asn5    | 6.4     | 8.5     |
| Lys6    | 8.5     | 6.4     |
| Pro7    | 7.9     | 7.3     |
| Arg8    | 8.1     | 8.4     |
| Asn9    | 8.6     | 8.6     |
| Asp10   | 8.4     | 8.4     |
| Asp11   | 8.1     | 8.1     |
| Ile12   | 7.8     | 7.8     |
| Phe13   | 7.7     | 7.7     |
| Arg14   | 7.7     | 7.7     |
| Ile15   | 7.0     | 7.3     |
| Ile16   | 7.1     | 7.1     |

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Evidence of Secondary Structure—The conformational features of the two peptides have been derived by the analysis of complementary NMR parameters such as the $\alpha$-proton chemical shift perturbation, the $\delta^{3}$HN,\text{a} coupling constants and the NOEs pattern.

The $\alpha$-proton residual chemical shifts (42) are reported in Fig. 3. In the case of Ni3 (Fig. 3A), it is evident that, except for Ser$^2$ and Leu$^3$, all the $\alpha$-proton resonances move upfield with respect to the random coil values, suggesting the existence of a helical structure extending from Ile$^6$ to Asn$^{19}$. The presence of sequential $d_{\text{HN,N}}(i,i+1)$ NOEs, spanning almost the entire Ni3 sequence in the 150-ms NOESY spectrum (Fig. 4A), indicates the existence of an $\alpha$-helical conformation whose stability is confirmed by the characteristic $d_{\text{HN,N}}(i,i+3)$ NOEs encompassing residues Tyr$^3$–Asn$^{19}$ (Fig. 4B). The summary of the interresidue NOEs is reported in Fig. 5A, showing a good correlation with the $\alpha$CH secondary shifts (Fig. 3A). Finally, except for Asn$^5$, the majority of the NH-$\alpha$CH coupling constants ($\delta^{3}$JHN,\text{a}) are in the range of 5.60–6.5 Hz (Table II), as expected for a peptide in an $\alpha$-helical conformation.

On the other hand, knowing that a minimum of 4 adjacent residues with a negative deviation of the $\alpha$CH chemical shifts is necessary to assess the presence of a stable helical organization, and that its stability is proportional to the intensity of such deviation (42), the secondary shifts plot for Ci3 (Fig. 3B) indicates that, in this case, a helical folding is possible only in the peptide COOH-terminal portion. In addition, except for Arg$^6$ and Asp$^{10}$, all the residues do not exhibit any significant difference between the trans and the cis isomers (Fig. 3B) suggesting that Ci3, in the two states, adopt very similar conformations.

Indeed, the analysis of the NOESY and rotating frame nuclear Overhauser effect spectra carried out for both the trans and cis isomers evidenced nearly no difference. Thus, Fig. 5B shows the summary of the interresidue NOEs only for the relatively more populated trans isomers. As for Ni3, a good correlation with the $\alpha$CH secondary shifts (Fig. 3B) can be observed. In fact, adjacent NH-NH correlations, together with medium and weak intensities $d_{\text{HN,N}}(i,i+3)$ and $d_{\text{HN,N}}(i,i+3)$ NOEs were found from Asp$^{10}$ to Ile$^{16}$, indicating the presence of a helical conformation only in the COOH-terminal portion of the peptide. Moreover, the coexistence of medium and weak intensity $(i,i+3)$ NOEs together with the intense $d_{\text{HN,N}}(i,i+1)$ correlations, suggests the presence of conformational fluctuations in that stretch.

The N-terminal half of Ci3, including the first five amino acids that belong to a well defined $\alpha$-helix in the COOH-terminal half of Ni3, clearly does not adopt any stable secondary structure. Except for the Asn$^5$ NH-Lys$^8$ NH connectivity, the other medium range NOEs associated to the presence of helical regions are unambiguously absent from the spectra. Finally, in the Pro-containing stretch, NOE connectivities reminiscent of the presence of a $\beta$-turn were not found.

Three-dimensional Structure Calculations of Ni3 and Ci3—Following the restrained molecular dynamics protocol described under “Materials and Methods,” 80 possible three-dimensional structures were calculated for both peptides. After minimization, 20 structures for each peptide were selected to represent their solution conformation. The structural statistics are reported in Table III.

Fig. 6A displays the Ni3 structures superimposed, for the minimum backbone deviation, between residues Tyr$^3$ and Lys$^{19}$. The existence of a stable and well defined $\alpha$-helical folding, involving most of the peptide, is also supported by the analysis of the main chain hydrogen bonds (Table III) and of the $\phi$ and $\psi$ dihedral angles (data not shown), carried out using the program DSSP (43).

In the case of Ci3, Fig. 6B refers to the trans-Pro conformers, and the superimposition of the backbone atoms has been made for the Asn$^5$–Ile$^{15}$ stretch. As previously mentioned, discussing the NMR data, the structures of the cis and trans isomers are quite similar, with an $\alpha$-helical conformation extending over the COOH-terminal peptide stretch, only. Because of the limited number of NMR constraints available, the NH$_2$-terminal region preceding Asn$^5$ is poorly defined and no elements of stable ordered secondary structure have been detected.

Fig. 6 (bottom) shows a schematic representation of the least energy structure for the two peptides evidencing the amphipathic nature of the $\alpha$-helical regions.

DISCUSSION

Peptide Structural Features—There is a general consensus (6, 44–46) on the hypothesis that the specific conformational changes of GPCRs cytosolic domains, induced by agonist binding, are critical for their activation. Since experimental observations with substitution or deletion in receptor mutants cannot rule out the induction of indirect conformational effects, the use of synthetic peptides representing defined receptor regions allows the performance of experiments in a more conformationally controlled manner. The validity of this approach is based on the recognition that if the isolated fragments retain the biological function they have in the receptor (e.g., G-protein activation), it is likely that their folding and conformational dynamics are similar to the ones they undergo in the native
Due to the recognized relevance of the i3 loop as a critical region for GPCRs to express their activity, many attempts are being carried out to identify the domains involved in the complex molecular process of G-protein coupling. In the specific case of the rat AT1A receptor, i3 has been found to play a pivotal role, with distinct biological functions exerted by its proximal and distal portions.

In this study, to verify the existence of structural determinants that may help to describe the conformation and dynamics of the i3 domains involved in G-protein coupling, we have characterized the solution structure of two overlapping fragments of the AT1A i3 loop, corresponding to its proximal and distal halves (residues 213–231 and 227–242, respectively).
were the same as reported in containing isomers of Ci3, the sequential and medium range NOEs showing over almost the entire peptide. Since most of the helical terminal domain of the two isomers is not significant, being solely the result of the limited number of NOEs available.

Thus, the conformational and energetic similarities of the trans and cis isomers suggest that the cis-trans isomerization process is likely to occur in the native receptor as well. In the case of the i3 loop, it is also known that its proximal portion is essential for G-protein coupling (24), while there are evidences that the distal portion of i3 is essential for receptor activation and G-protein selectivity (25). In fact, more generally, there is a tendency to locate the critical motif for GPCRs activation and G-protein selection in that region of the i3 loop (6, 44, 45).

Recognizing that G-protein coupling and activation require the presence of amphipathic α-helices in specific receptor cytosolic regions (17, 18, 25, 62–64), although theoretical predictions indicate that the two i3 terminal portions must have a helical conformation, we propose that, in the receptor resting state (65, 66) the cis-trans isomerization produces a conformational perturbation throughout i3, leading to a significant reduction of the extent of the helical structure and to an increase of conformational instability. Consequently, we believe that the possibility to control the rate of the cis-trans interconversion can be envisaged as a modulator of the conformation and/or exposition of the preceding flexible region of i3.

The Angiotensin II ATR Receptor—Combining these results with a previous study on the peptide encompassing residues 300–320 (17), we may extend the description of the conformational and dynamical properties of the receptor cytosolic domains involved in G-protein coupling.

Besides accepting that ligand binding to GPCRs causes not yet well defined conformational changes (6, 44–46), it is now recognized that reorientation of the transmembrane helices (TM) should play a crucial role in affecting the conformation of the cytoplasmic surface. In particular, experimental evidence suggests that, for rhodopsin (67), β-adrenergic receptor (6), and also for angiotensin II ATR receptor (5), upon ligand binding

| Ni3 (A) | 1 T S Y T 2 | 5 | 10 | 15 | N-NH₂ |
|--------|-------------|---|----|----|-------|
|        | 213         | 217| 222| 227|       |

| d_α (i,i+1) | * |
| d_α (i,i+1) | * |
| d_α (i,i+1) | * |
| d_α (i,i+1) | * |
| d_α (i,i+3) | * |
| d_α (i,i+3) | * |
| d_α (i,i+4) | |

| Ci3 (B) | 1 E I O K 2 | 5 | 10 | 15 | N-NH₂ |
|--------|-------------|---|----|----|-------|
|        | 227         | 231| 236| 241|       |

| d_α (i,i+1) | * |
| d_α (i,i+1) | * |
| d_α (i,i+1) | * |
| d_α (i,i+1) | * |
| d_α (i,i+3) | * |
| d_α (i,i+3) | * |

**Fig. 5.** Summary of sequential and medium range NOEs for Ni3 (A) and the trans-Pro-containing isomers of Ci3 (B). Data were obtained from a 600-MHz NOESY experiment with a mixing time of 150 ms and collected in 70% H₂O/30% TFE-d₃, pH 4, at 25 °C. The thickness of the bars corresponds to NOE intensities, classified into (59, 60) or in the presence of amphipathic TFE as a cosolvent (55–58).

Secondary structure prediction indicates that the proximal part of i3 has a high propensity to adopt a helical structure in the receptor (35, 36). Indeed, the data presented in this study (Figs. 2A and 6A) clearly show that upon addition of small amounts of TFE, Ni3 folds into an amphipathic α-helix extending over almost the entire peptide. Since most of the helical peptides do not present medium range NOE connectivities to their termini, at room temperature, either in aqueous solution (59, 60) or in the presence of the helix-stabilizing solvent TFE (55, 61), the observation that for Ni3 the helical structure in those regions is not extensively frayed strongly suggests that, in this case, when formed the helix is highly stable. In fact, only the first two amino acids, residues 213 and 214 in the receptor, appear to be flexible (Fig. 6A).

For the distal part of i3, instead, the prediction (35, 36) indicates the existence of a helical organization only in the COOH-terminal portion. The experimental data show that, in the presence of TFE, not only the NH₂-terminal portion of Ci3 is characterized by the absence of any preferential secondary structure (Fig. 6B), but also that the helix present in the COOH-terminal region is quite flexible.

**Structure-Function Correlation**—In an attempt to correlate the observed structural determinants with their possible functional role, it is important to accept the hypothesis that conformational adaptability is an essential feature.

The comparison of the secondary structure of Ni3 and Ci3 shows that the common sequence EIQK adopts a quite different organization in the two peptides: it is part of a well defined α-helix in Ni3, while it is highly disordered in Ci3, indicating that the flanking amino acids, in this case Pro⁷ (38), play a critical role as secondary structure modulators.

The cis-trans isomerism observed in the NMR spectra of Ci3 reveals a remarkable feature with respect to the results reported for the majority of the peptides undergoing that isomerization. While the cis isomer population is usually significantly smaller than the trans one, in our case the percentages of the two isomers as well as their conformation are quite similar, with an α-helix spanning residues 9–15 in both of them (Fig. 7). The dissimilar structural organization observed for the NH₂-terminal domain of the two isomers is not significant, being solely the result of the limited number of NOEs available.

Not surprisingly, in aqueous solution both peptides turned out to be unstructured, as clearly indicated by their CD spectra (Fig. 2). The tendency of small peptides to adopt spontaneously secondary structure prediction indicates that the proximal portion is essential for G-protein coupling (24), while there are evidences that the distal portion of i3 is essential for receptor activation and G-protein selectivity (25). In fact, more generally, there is a tendency to locate the critical motif for GPCRs activation and G-protein selection in that region of the i3 loop (6, 44, 45).
the helix-helix interaction between TM7 and TM3 is removed and a new one between TM7 and TM6 is favored. Furthermore, recently, the TM6 of the AT1A receptor has been shown to play an essential role in triggering the response to angiotensin II binding (68).

Previously (17), we proposed that the agonist-induced lateral shift of the receptor (69) can favor the formation of an amphipathic helix in the receptor COOH-terminal tail, the hydrophilic side of which is one of the anchoring points of AT1A with the G-protein \(\alpha\)-subunit. Indeed, on the basis of the models proposed (5, 6, 67), we may add that the suggested TMs movements, associated to receptor activation, imply a decrease of TM6 flexibility that ought to be sensed by the distal part of the \(i^3\) loop. This event should be sufficient to reduce the rate of the \(\text{cis-trans}\) isomerization at the Lys232—Pro 233 peptide bond. Such a conformational signal might be converted into a stabilization of the amphipathic \(\alpha\)-helix throughout the \(i^3\) loop and/or the exposure of the correct surface of the proximal portion of the loop, necessary for the interaction/coupling with the proper G-protein (70, 71).

### Table II

1H-NMR chemical shifts and \(J_{\text{HN-\alpha}}\) coupling constants for Ni3, (2.0 mM) in 70% H\(_2\)O/30% TFE-d\(_3\) (v/v), pH 4, at 25 °C

| Residue | \(J_{\text{HN-\alpha}}\) | \(\delta_{\text{H}}\) | \(\gamma_{\text{H}}\) | Others |
|---------|----------------|----------|----------|--------|
| Thr\(^1\) | 3.88 | 4.08 | 1.24 |
| Ser\(^2\) | 8.66 | 4.60 | 3.95, 3.88 |
| Tyr\(^3\) | 6.2 | 8.30 | 4.42 | 2.97, 2.86 |
| Thr\(^4\) | 5.6 | 7.78 | 4.05 | 4.21 | 1.29 |
| Leu\(^5\) | 6.2 | 7.67 | 4.22 | 1.80, 1.68 | 1.74 |
| Ile\(^6\) | 6.5 | 7.50 | 3.86 | 1.94 | \(\gamma_{\text{H}}\) 1.60, 1.22 |
| Trp\(^7\) | 5.6 | 8.96 | 4.43 | 3.36, 3.34 |
| Lys\(^8\) | 5.9 | 7.96 | 3.90 | 2.00, 1.93 | 1.44 |
| Ala\(^9\) | 5.9 | 7.99 | 4.15 | 1.57 |
| Leu\(^10\) | 6.0 | 8.50 | 4.12 | 1.89 |
| Lys\(^11\) | 5.7 | 8.15 | 4.00 | 1.82, 1.74 | 1.35 |
| Ala\(^12\) | 5.6 | 8.19 | 4.10 | 1.54 |
| Tyr\(^13\) | 5.9 | 8.40 | 4.23 | 3.21 |
| Glu\(^14\) | 5.9 | 8.17 | 2.95 | 2.32, 2.21 | 2.64, 2.50 |
| Ile\(^15\) | 6.5 | 8.26 | 3.85 | 2.01 | \(\gamma_{\text{H}}\) 1.60, 1.23 |
| Gln\(^17\) | 6.3 | 8.06 | 4.11 | 2.16 | 2.54, 2.38 |
| Lys\(^18\) | 5.9 | 8.17 | 4.10 | 1.72, 1.69 | 1.33 |
| Asn\(^19\) | 7.6 | 7.90 | 4.69 | 2.90, 2.81 |
| Thr\(^21\) | 3.88 | 4.08 | 1.24 |

### Table III

Structural statistics of Ni3 and Ci3

| Constraints | Ni3 | Ci3 |
|-------------|-----|-----|
| Intraresidue | 21 | 32 |
| Sequential | 57 | 61 |
| Medium range | 10 | 10 |
| Total | 164 | 103 |
| RMSD\(^a\) | 0.2–0.8 (residues 3–18) | 0.3–1.2 (residues 9–15) |
| Heavy atoms | 0.3–1.6 (residues 3–18) | 0.5–2.0 (residues 9–15) |
| \(\langle E\rangle\)\(^b\) | 42 | 88 |

| Hydrogen bonds | Donor NH\(^d\) | Acceptor CO | Donor NH | Acceptor CO |
|----------------|----------------|-------------|----------|-------------|
| Trp\(^7\) | Tyr\(^3\) |
| Ala\(^9\) | Leu\(^5\) | Ile\(^6\) |
| Lys\(^11\) | Trp\(^7\) |
| Lys\(^12\) | Ile\(^6\) |
| Ala\(^13\) | Ala\(^9\) |
| Glu\(^15\) | Lys\(^11\) |
| Ile\(^16\) | Lys\(^12\) |
| Gln\(^17\) | Ala\(^13\) |
| Lys\(^18\) | Tyr\(^14\) |
| Asn\(^19\) | Ile\(^16\) |

\(^a\) RMSD, root mean square deviation from pairwise comparison between all the structures (Å).
\(^b\) In Kcal/mol.
\(^c\) Hydrogen bonds were searched using the Measure Hbond facility of INSIGHT and were regarded as present if the following criteria were satisfied simultaneously: 1) the distance between the donor H and the acceptor O was <2.5 Å; 2) the angle between the heavy atom donor, the hydrogen and the heavy atom acceptor (NH—O) was >120 degrees; 3) the hydrogen bond occurred in at least 50% of the energy minimized structures.

\(^d\) NH and CO represent backbone atoms.

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\(\text{\(\text{cis-trans}\) Pro—Pro}\) peptide bond. Such a conformational signal might be converted into a stabilization of the amphipathic \(\alpha\)-helix throughout the \(i^3\) loop and/or the exposure of the correct surface of the proximal portion of the loop, necessary for the interaction/coupling with the proper G-protein (70, 71). Interestingly, the receptor residues we suggest to be responsible for that conformational transition (residues 235–241) are close to the NH\(_2\)-terminal side of TM6, and this region has been shown to play a critical role for G-protein selectivity and acti-

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conformation also in the muscarinic receptor (25, 29, 44).

Finally, Shirai et al. (33) and Sano et al. (34) showed that only the peptide representing the proximal portion of i3 was able to activate G\(_i\) and G\(_q\) proteins. We believe that we may justify those results based on the idea of a distinct role played by the two regions of i3, at least in the case of the AT\(_{1A}\) receptor, with the distal region being involved in receptor activation and G-protein selectivity, and the proximal one being essential for G-protein coupling.

Receptor activation and G-protein selection should not require interaction with G-protein; instead, they seem to imply conformational modifications and exposition of specific receptor cytoplasmic domains such as the proximal half of i3 that, on the contrary, is expected to selectively interact with the proper G-protein.

Thus, consistent with the hypothesis that peptides representing fragments of a protein can retain their functional properties, only Ni3 is expected to interact and activate purified G\(_i\) and G\(_q\) proteins. In fact, Ni3, free in solution, can reach the G-protein and be induced to assume the conformation necessary for binding and activation. When inserted in the receptor, due to sterical hindrance, this process cannot be spontaneous; in fact, from the more general view of receptor activity, it must be controlled. It is this the step in which the distal portion of the loop comes into play, regulating the conformational stabilization and proper exposition of the required loop region without any specific need to interact with the protein G, thus justifying the lack of activity that characterizes Ci3 in solution.

FIG. 6. Top, final 20 Molecular Dynamics selected structures of N3 (A) and the trans-Pro-containing isomers of C3 (B) calculated from NMR-derived distance and \(\phi\) angle restraints at 25 °C. The superimposition of the backbone atoms has been made from Tyr\(^{11}\) to Lys\(^{18}\) for Ni3 and from Asn\(^{9}\) to Ile\(^{15}\) for Ci3. In both peptides the NH\(_2\) terminus is shown at the top. In the case of the cis-Pro-containing isomers of Ci3, the 20 Molecular Dynamics selected structures are not shown because they are nearly identical with those obtained for the trans isomers, with an \(\alpha\)-helix extending over the peptide stretch Asn\(^{9}\)–Ile\(^{15}\). Bottom, least energy structure showing the amphipathic character of the helix for both peptides. Within positions 3–18 for Ni3 and 9–16 for Ci3, hydrophilic residues are located on one side of the molecule, while hydrophobic residues are located on the other side of the molecule.

FIG. 7. Superposition of the least energy structure of the trans and the cis isomers of C3 for residues 9–15. The different organization observed in the NH\(_2\)-terminal portion (top) between the two isomers is not significant because no structural information could be obtained in that region from the NMR data.

\[\text{CONCLUSIONS}\]

G-protein-coupled receptor activation is definitely associated with exposition of a previously buried region to G-protein.

The data obtained thus far on fragments of the i3 loop and of the COOH-terminal tail (17) of the angiotensin II AT\(_{1A}\) receptor seem to support that model. In fact, they indicate the existence of structural and dynamic features that we believe relevant to gain further insights on the structure-dynamics-function relationship of the cytoplasmic regions involved in the process of receptor activation and G-protein selection and coupling.

Studies are under way for a complete description of the extramembranous domains of AT\(_{1A}\) and of the dynamics of receptor/G-protein activation.

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