BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **230,** 133–135 (1997)
ARTICLE NO. RC965900

Circular Dichroism Spectra of Calcium Channel Antagonist ω-Conotoxins

Jae Il Kim, Atsuko Ohtake, and Kazuki Sato[1]
Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan

Received November 25, 1996

The circular dichroism (CD) spectrum of ω-conotoxin GVIA is quite different from those of ω-conotoxin MVIIA and MVIIC, despite their distinct similarity in three dimensional structures. In order to characterize the unique CD spectrum of ω-conotoxin GVIA, we focused our attention on the aromatic chromophore and analyzed the CD spectra of three synthetic analogs, in which Tyr^{13}, Tyr^{22}, and Tyr^{27} were individually replaced by alanine. Replacement of Tyr^{27} caused a significant change in both the near- and far-ultraviolet CD spectrum of ω-conotoxin GVIA and resulted in the ω-conotoxin MVIIA/MVIIC-like pattern, suggesting that Tyr^{27} has a dominant contribution to the unique CD profile of ω-conotoxin GVIA.

The venom of fish-hunting cone snail contains several peptide antagonists, referred to as ω-conotoxins (ω-CTXs), which are useful for the study on the functional diversity of neuronal calcium channels (1, 2). ω-CTX GVIA and MVIIA specifically block the N-type calcium channels, and ω-CTX MVIIC mainly targets the P/Q-type calcium channels (Fig. 1) (3, 4). Both mutational and structural studies on ω-CTXs are essential for understanding the molecular basis of the toxin/channel interaction.

Previously, we reported that Tyr^{13} is essential for the activity of ω-CTX GVIA (5), MVIIA (6), and MVIIC (7), suggesting that Tyr^{13} is a common binding site in ω-CTXs irrespective of the calcium channel subtypes that they target. It was also found that the CD spectra of ω-CTX MVIIA (6) and MVIIC (7) are quite different from that of ω-CTX GVIA (5). According to the three-dimensional structure analysis of ω-CTX GVIA (8-11), MVIIA (12, 13), and MVIIC (14, 15) by NMR spectroscopy, the polypeptide chain framework consisting of a short triple-stranded antiparallel β-sheet and several reverse turns is conserved in all ω-CTXs. Therefore, it is interesting to address an origin of unique CD profile of ω-CTX GVIA in terms of the correlation between CD and NMR studies.

In the present study, we focused our attention on the effect of Tyr residue, since this is the only aromatic amino acid in ω-CTXs and the numbers are different among them (Fig. 1). To estimate the contribution of Tyr residues to the CD spectrum of ω-CTX GVIA, we analyzed the CD spectra of three analogs, **Y13A-GVIA, Y22A-GVIA,** and **Y27A-GVIA,** in which Tyr^{13}, Tyr^{22}, and Tyr^{27} were replaced by alanine, respectively. Synthesis and activity of these analogs have been reported previously (5).

MATERIALS AND METHODS

CD measurements. All the CD spectra were measured on a JASCO J-600 spectropolarimeter in H_2O solution (0.01 M sodium phosphate, pH 7.0) at 20°C at the concentrations of 0.05 mM for 190-250 nm and 1 mM for 240-360 nm by using a quartz cell of 1-mm path length. The spectra were obtained as an average of 4–8 scans at a scan speed of 10-20 nm/min, with a sensitivity range of 20 mdeg/FS, using an instrumental time constant of 1 sec. The spectra are expressed as molecular ellipticity [θ] in degree cm^2 $dmol^{-1}$.

RESULTS AND DISCUSSION

CD spectra of ω-CTX GVIA, MVIIA, and MVIIC are shown together in Fig. 2. The overall spectra of ω-CTX MVIIA and MVIIC are very similar to each other with the minima at around 200 nm and 280 nm, in good agreement with the results of NMR studies that revealed their similarity in three dimensional structures (12-15). In contrast, CD spectrum of ω-CTX GVIA shows a positive Cotton Effect at around 200 nm, suggesting no obvious correlation with the three-dimensional structure similarity. It also shows a characteristic positive band between 275 and 285 nm that is directly related to the UV-absorption of a tyrosine side chain. It should be also noted that the subtype specific-

[1] To whom correspondence should be addressed. FAX (+81)427-24-6317. E-mail: kazuki@libra.ls.m-kagaku.co.jp.

Abbreviations: CD, circular dichroism; CTX, conotoxin; NMR, nuclear magnetic resonance; UV, ultraviolet.

0006-291X/97 $25.00

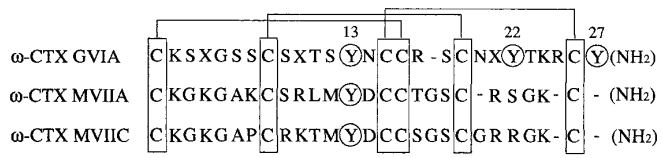

FIG. 1. Amino acid sequences and disulfide bonds of ω-CTX GVIA, MVIIA, and MVIIC (X = Hyp). Tyr residues are in circles and numbered according to the sequence of ω-CTX GVIA.

ities of ω-CTXs are not directly related to their CD spectral profiles.

CD spectra of three analogs (**Y13A-GVIA, Y22A-GVIA,** and **Y27A-GVIA**) are shown in Fig. 3 together with those of native ω-CTX GVIA and its linear analog in which all six Cys residues are replaced by Ala. **Y13A-GVIA** exhibited the spectral curve almost superimposable on that of native ω-CTX GVIA, indicating that the side chain of Tyr^{13} has a minor contribution to its CD spectrum. This result agreed well with our previous observations that the overall CD patterns of ω-CTX MVIIA and MVIIC were not affected by alanine substitutions at the same position (6, 7). **Y22A-GVIA** also yielded a CD spectrum very similar to that of native ω-CTX GVIA.

In contrast, a significant change was observed in the CD spectrum of **Y27A-GVIA,** in which the spectrum was modified in both the shape and intensity with minima at around 205 nm and 270 nm. It is interesting that the overall CD pattern of **Y27A-GVIA** is considerably resemble to those of ω-CTX MVIIA and MVIIC, rather than that of linear ω-CTX GVIA. Although the linear analog retains the Tyr^{27} residue, its CD spectrum displayed no Cotton Effects at the wavelength of the absorption by aromatic chromophore but a large negative

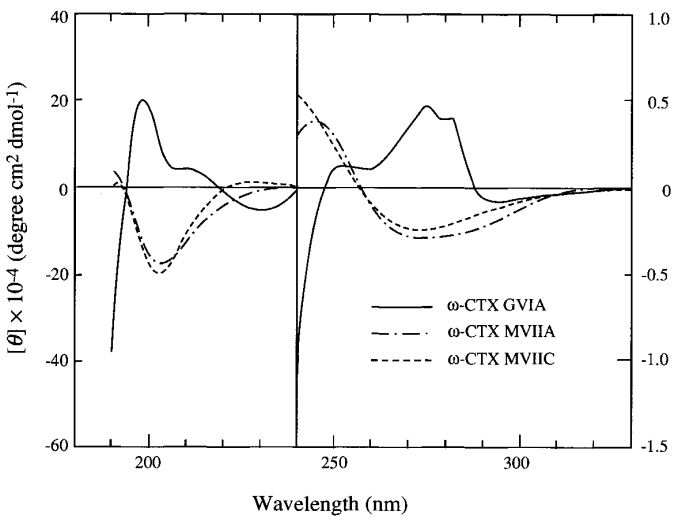

FIG. 2. CD spectra of ω-CTX GVIA, MVIIA, and MVIIC in H_2O solution (0.01 M sodium phosphate, pH 7.0) at 20°C.

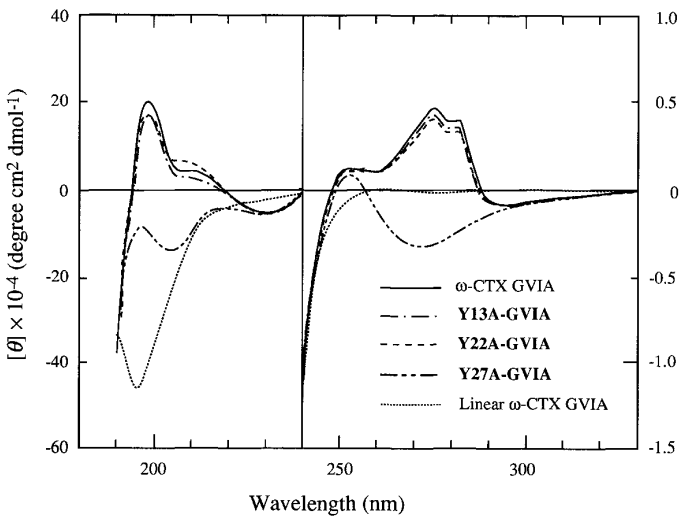

FIG. 3. CD spectra of ω-CTX-GVIA and its analogs in H_2O solution (0.01 M sodium phosphate, pH 7.0) at 20°C.

Cotton Effect near 195 nm, that is typical of a random conformation. In a competitive assay, the linear analog showed no binding potency to the N-type calcium channels, whereas **Y27A-GVIA** showed the same activity as native ω-CTX GVIA (5). These results indicate that a remarkable spectral change observed for **Y27A-GVIA** was attributed to the loss of the Tyr^{27} chromophore, but not a structural disruption, and thus lead to good agreement between CD and NMR structural analyses.

The contribution of Tyr^{27} chromophore on the CD spectrum may be explained from the overall shape of ω-CTX GVIA molecule. In the three dimensional structure, Tyr^{13}, Tyr^{22}, and Tyr^{27} are located in the long loop between the first and second β-strands, the fourth turn (hairpin) connecting the second and third β-strands, and the third β-strand, respectively, and are relatively far away from one another (8-11). Pallaghy *et al.* reported that Tyr side-chains, all three of which are on the surface of the molecule, are defined within a cone structure of approximately 45°, except for that of Tyr^{27}, which has more limited orientation (10). This structural inspection suggests that the phenolic ring of Tyr^{27} is in more asymmetric environment than those of other two Tyr residues, and seems to give a comprehensive explanation for the dominant contribution of Tyr^{27} chromophore to the unique CD spectrum of ω-CTX GVIA. This is further supported by the fact that Tyr^{27} in ω-CTX GVIA is an extra residue attached to its C-terminus, and both ω-CTX MVIIA and MVIIC lack this residue (Fig. 1).

The far-UV CD spectrum of **Y27A-GVIA** still leaves some differences when compared to those of ω-CTX MVIIA and MVIIC. All three ω-CTXs have four reverse β-turns. Among them, the conformations of the first, second and third turns are almost conserved in all

three ω-CTXs, but there is a significant difference in the fourth turn that connects the second and third β-strands. For this hairpin turn, ω-CTX GVIA takes on a type I β-turn, whereas both ω-CTX MVIIA and MVIIC adopt its mirror image (type I′) (8-15). Since the geometric difference of hairpin turn leads to a twist between adjacent β-strands (16), this may be one of the reason for the discrepancy of the backbone CD spectrum between **Y27A-GVIA** and ω-CTX MVIIA and MVIIC.

Different from the CD spectra of globular proteins or linear peptides (17, 18), those of cyclic peptides with multiple disulfide bonds are difficult to be related to their three dimensional structures. To address the origin of their CD spectra, it will be useful to examine the spectra of the analogs with amino acid substitution. Here we compared the CD spectra of three ω-CTXs in terms of three-dimensional structure similarity, and subsequently estimated the degree of contribution of individual Tyr residues to the unique CD spectrum of ω-CTX GVIA.

Although it is still difficult to explain the CD spectra of ω-CTX MVIIA, MVIIC, and **Y27A-GVIA** by their three dimensional structures, the common CD profile is considered to be originated by a conserved disulfide bond combination and a triple-stranded antiparallel β-sheet. Therefore, this CD profile would be helpful for the CD characterization of other cysteine-rich peptides that contain a similar folding motif with a triple-stranded antiparallel β-sheet, such as ω-agatoxin IVA (19), ω-agatoxin IVB (20, 21), some protease inhibitors (22), and gurmarin (23).

ACKNOWLEDGMENTS

We thank Dr. Masami Takahashi for helpful discussion. This work was supported in part by a project grant from the Japan Health Science Foundation.

REFERENCES

1. Gray, W. R., Olivera, B. M., and Cruz, L. J. (1988) *Ann. Rev. Biochem.* **57,** 665–700.
2. Olivera, B. M., Miljanich, G. P., Ramachandran, J., and Adams. M. E. (1994) *Ann. Rev. Biochem.* **63,** 823–867.
3. Tsien, R. W., Lipscombe, D., Madison, D. V., Bley, K. R., and Fox, A. P. (1988) *Trends Neurosci.* **11,** 431–438.
4. Birnbaumer, L., Campbell, K. P., Catterall, W. A., Harpold, M. M., Hofman, F., Horne, W. A., Y., M., Schwartz, A., Snutch, T. P., Tanabe, T., and Tsien, R. W. (1994) *Neuron* **13,** 505–506.
5. Kim, J.-I., Takahashi, M., Ogura, A., Kohno, T., Kudo, Y., and Sato, K. (1994) *J. Biol. Chem.* **269,** 23876–23878.
6. Kim, J.-I., Takahashi, M., Ohtake, A., Wakamiya, A., and Sato, K. (1995) *Biochem. Biophys. Res. Commun.* **206,** 449–454.
7. Kim, J.-I., Takahashi, M., Martin-Moutot, N., Seagar, M. J., Ohtake, A., and Sato, K. (1995) *Biochem. Biophys. Res. Commun.* **214,** 305–309.
8. Sevilla, P., Bruix, M., Santoro, J., Gago, F., Garcia, A. G., and Rico, M. (1993) *Biochem. Biophys. Res. Commun.* **192,** 1238–1244.
9. Davis, J. H., Bradley, E. K., Miljanich, G. P., Nadasdi, L., Ramachandran, J., and Basus, V. J. (1993) *Biochemistry* **32,** 7396–7405.
10. Pallaghy, P. K., Duggan, B. M., Pennington, M. W., and Norton, R. S. (1993) *J. Mol. Biol.* **234,** 405–420.
11. Skalicky, J. J., Metzler, W. J., Ciesla, D. J., Galdes, A., and Pardi, A. (1993) *Protein Science* **2,** 1591–1603.
12. Kohno, T., Kim, J.-I., Kobayashi, K., Kodera, Y., Maeda, T., and Sato, K. (1995) *Biochemistry* **34,** 10256–10265.
13. Basus, V. J., Nadasdi, L., Ramachandran, J., and Miljanich, G. P. (1995) *FEBS Lett.* **370,** 163–169.
14. Nemoto, N., Kubo, S., Yoshida, T., Chino, N., Kimura, T., Sakakibara, S., Kyogoku, Y., and Kobayashi, Y. (1995) *Biochem. Biophys. Res. Commun.* **207,** 695–700.
15. Farr-Jones, S., Miljanich, G. P., Nadasdi, L., Ramachandran, J., and Basus, V. J. (1995) *J. Mol. Biol.* **248,** 106–124.
16. Sibanda, B. L., and Thornton, J. M. (1985) *Nature* **316,** 170–174.
17. Perczel, A., Park, K., and Fasman, G. D. (1992) *Proteins* **13,** 57–69.
18. Greenfield, N. J. (1996) *Anal. Biochem.* **235,** 1–10.
19. Kim, J.-I., Konishi, S., Iwai, H., Kohno, T., Gouda, H., Shimada, I., Sato, K., and Arata, Y. (1995) *J. Mol. Biol.* **250,** 659–671.
20. Yu, H., Rosen, M. K., Saccomano, N. A., Phillips, D., Volkmann, R. A., and Schreiber, S. L. (1993) *Biochemistry* **32,** 13123–13129.
21. Reily, M. D., Thanabal, V., and Adams, M. E. (1995) *J. Biomol. NMR.* **5,** 122–132.
22. Pallaghy, P. K., Nielsen, K. J., Craik, D. J., and Norton, R. S. (1994) *Protein Sci.* **3,** 1833–1839.
23. Arai, K., Ishima, R., Morikawa, S., Miyasaka, A., Imoto, T., Yoshimura, S., Aimoto, S., and Akasaka, K. (1995) *J. Biomol. NMR.* **5,** 297–305.