Vol. 99, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1981 Pages 913-919

TERBIUM FLUORESCENCE STUDIES OF THE METAL-ANGIOTENSIN II COMPLEX

Robert G. Canada

Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Md. 20205

Received February 17,1981

SUMMARY

The fluorescent properties of Tb^{3+} are used to monitor the metal-peptide interactions of angiotensin II. The fluorescence emission of Tb^{3+} is enhanced with the binding of angiotensin II, and the excitation maximum of the metal shifted to resemble that of the peptides fluorescence. The fluorescence enhancement of Tb^{3+} is decreased with increasing concentrations of Na^{+} or Ca^{2+}. The apparent dissociation constant of Na^{+} and Ca^{2+} for angiotensin II is estimated to be about 27.3 mM and 15.5 mM, respectively. The data suggests that under physiological conditions angiotensin II may exist in a complex with Na^{+} but not Ca^{2+}.

INTRODUCTION

Certain alkali metal ions have been shown to enhance the biological activity and the receptor binding of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) (1-5). Schaechtelin et al (1,2) have demonstrated, in the rat pressor and rat uterotonic assay systems, that Na^{+} and Ca^{2+} enhances the activity of angiotenisn II. Blanc et al (3) and Gunther et al (4) have shown that Ca^{2+}, Mg^{2+} and Mn^{2+} can increase the binding of ^{125}I-angiotensin II to isolated rat renal glomeruli and to a particulate fraction from rat mesenteric arteries, respectively. Mann et al (5) have demonstrated that the blood pressure and water intake responses to intracerebroventricular injections of angiotensin II, were lower in rats fed a low-sodium diet compared to rats fed a control diet. It has been suggested that these cations somehow induce conformational changes in angiotensin II, to result in a more biologically active structure (1).

Terbium fluorescence has been widely used to investigate the Ca^{2+} binding sites of proteins and biological membranes (6-16). The fluorescence

0006-291X/81/070913-07$01.00/0

intensity of Tb^{3+} undergoes a tremendous enhancement when it binds to proteins. The fluorescence emission of the protein bound Tb^{3+} can be excited by energy transfer from nearby aromatic residues which are directly excited by ultraviolet radiation (6-9, 11-16). In the present study, the fluorescent properties of Tb^{3+} are employed to obtain direct experimental evidence that metal ions can bind to angiotensin II.

MATERIALS AND METHODS

Angiotensin II was purchased from Vega Biochemical Co. Terbium chloride was obtained from Aldrich Chemical Co.; terbium chloride stock solutions were made with double-distilled water. All other solutions were made with 0.01 M hepes (N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid) (Sigma Chemical Co.) buffer; the pH was adjusted to 7.4 with 1.0 N KOH.

The fluorescence excitation and emission spectra (uncorrected) were made on an Aminco-Bowman Spectrophotofluorometer with Ellipsoidal Condensing System, at room temperature, using 2.0 ml samples in 1x1-cm quartz cuvettes. The excitation and emission wavelengths were 280 nm and 543 nm, respectively. In order to eliminate error due to light scattering and interferences due to the native fluorescence of the hormone, the Tb^{3+} fluorescence was followed in conjunction with a 455 nm long wavelength pass filter.

In the Na^{+} and Ca^{2+} titration experiments, 5 µl aliquots of 1.05 M $CaCl_2$ or 10 µl aliquots of 2.1 M NaCl were added to a cuvette containing $1x10^{-3}$ M $TbCl_3$ and $1x10^{-5}$ M angiotensin II in 0.01 M hepes buffer pH 7.4. Control solutions did not contain angiotensin II. After each aliquot, the solution was mixed for 20 sec. before recording its fluorescence intensity at 543 nm. The final volume change for the entire titration was less than 5 percent.

RESULTS AND DISCUSSION

Figure 1 shows the excitation and emission spectra of Tb^{3+} in the presence and absence of angiotensin II. The emission spectra revealed the characteristic Tb^{3+} fluorescence quartet at approximately 488, 543, 584 and 619 nm. The fluorescence of the quartet is enhanced in the presence of angiotensin II; the magnitude of the fluorescence enhancement is approximately $3x10^{2}$. (Enhancement = ratio of emission intensities of peptide bound vs. free Tb^{3+}, on a molar basis). The excitation maximum of free Tb^{3+} at 267 nm shifted to 284 nm in the presence of angiotensin II, resembling the excitation peak of the hormone (at 280 nm). The 285nm/265nm intensity ratio of the Tb^{3+} excitation spectrum is 70 percent greater in the presence of angiotenisn II than in the absence of angiotensin II. The data indicates that

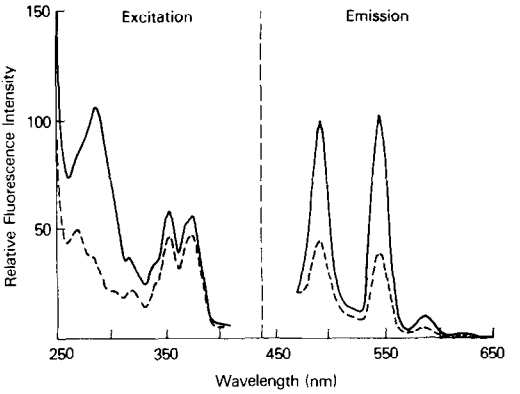


Figure 1 Fluorescence excitation and emission spectra of $1x10^{-3}$ M $TbCl_3$ with and without $1x10^{-5}$ M angiotensin II in 0.01 M hepes buffer pH 7.4. (——), Tb^{3+} plus angiotensin II. (----), Tb^{3+} alone.

energy may be transferred from the tyrosine residue of the hormone to the metal ion; especially, since the natural fluorescence emission of angiotensin II at 315 nm is quenched by 20 percent in the presence of Tb^{3+}, figure 2. The partial suppression of the energy donors fluorescence emission

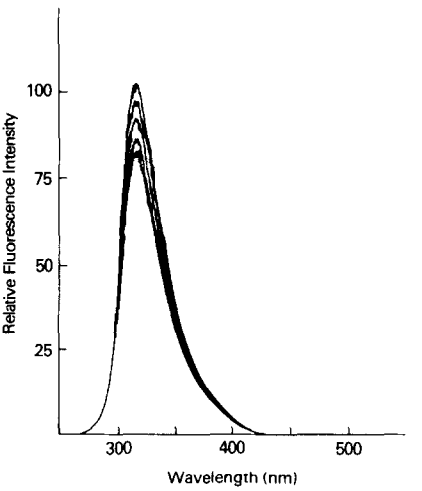


Figure 2 Native fluorescence emission spectra of $1x10^{-5}$ M angiotensin II in the presence of various concentrations of $TbCl_3$. The top trace represents angiotensin II alone in 0.01 M hepes buffer pH 7.4. The other traces in descending order show the effects of having 0.5, 1.0, 1.5 or 2.0 mM $TbCl_3$ in solution.

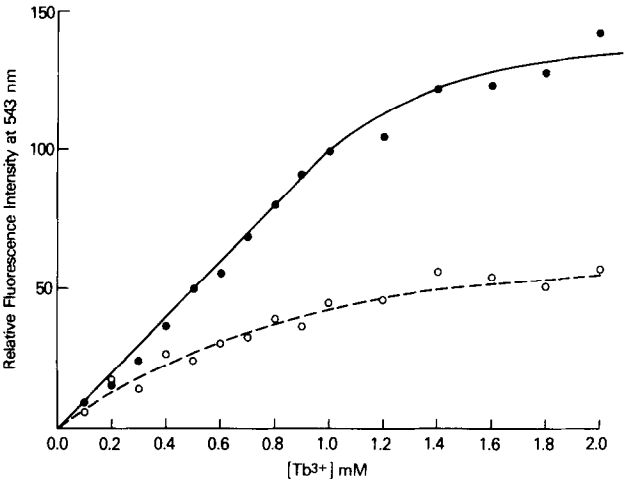
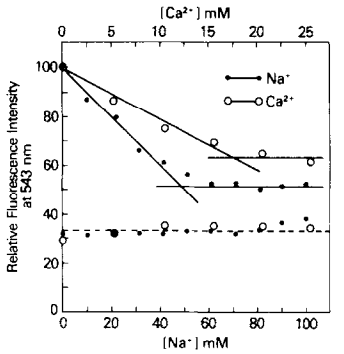


Figure 3 Fluorescence intensity of Tb^{3+} as a function of the $TbCl_3$ concentration in 0.01 M hepes buffer pH 7.4, with (●——●) and without (0----0) $1x10^{-5}$ M angiotensin II.

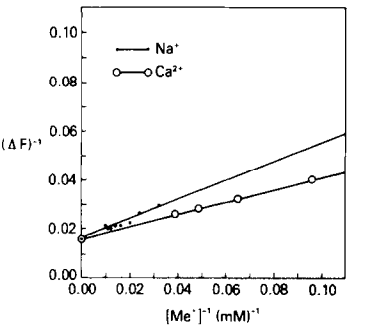
is generally considered as the consequence of energy transfer (6,7,9,12-16). Therefore, the Tb^{3+} fluorescence enhancement in the presence of angiotensin II is due to the metal-peptide complexation.

The magnitude of the fluorescence enhancement for the Tb^{3+}-angiotensin II complex is considerably lower than that reported for other systems. For example, the fluorescence enhancement of the Tb^{3+}-elastase and Tb^{3+}-trypsin systems are on the order of 10^4 (6,14). Since fluorescence intensity varies as r^{-6}, where r is the distance between the energy donor and acceptor pair, the lower fluorescence enhancement for the Tb^{3+}-angiotensin II complex presumably reflects a longer distance between the bound Tb^{3+} and the aromatic side chain (6).

The Tb^{3+} fluorescence intensity at 543 nm increased with increasing Tb^{3+} concentration, figure 3. In the presence of angiotensin II, the relative fluorescence intensities increased linearly between 0.0 mM and 1.0 mM Tb^{3+}, but above 1.0 mM Tb^{3+} the intensities gradually tapered into a plateau. The fact that the Tb^{3+} fluorescence intensities reached a limit with



4



5

Figure 4 Effects of Na^+ and Ca^{2+} on the fluorescence intensity of the Tb^{3+}-angiotensin II complex. $1x10^{-3}$ M $TbCl_3$ in the presence (——) and absence (---) of $1x10^{-5}$ M angiotensin II were titrated with NaCl or $CaCl_2$, in 0.01 M hepes buffer pH 7.4. Fluorescence intensities are expressed relative to the value in the absence of Na^+ or Ca^{2+}.

Figure 5 Double reciprocal Lineweaver-Burk type plots of the fluorescence intensity of the Tb^{3+}-angiotensin II complex against the metal ion concentration (Me^+). The change in fluorescence intensity is expressed as the value of $(I_o - I_m)$, where I_o and I_m are the intensities of Tb^{3+}-angiotensin II complexes before and after Me^+ titration, respectively. The data is from Figure 4.

increasing Tb^{3+} concentration, is indicative of binding rather than just random collisional processes. The relative fluorescence intensities of the free Tb^{3+} increased nonlinearly, as the Tb^{3+} concentration increases. The apparent dissociation constant for the Tb^{3+}-angiotensin II complex is calculated to be about 2.4 mM. The dissociation constant (K_d) of the Tb^{3+}-angiotensin II complex is determined from a double-reciprocal Lineweaver-Burk type plot of fluorescence intensity (I) against Tb^{3+} concentration, where the slope of the line is equal to K_d/I_{max} and the y-intercept is equal to $1/I_{max}$.

The binding of other metal ions to angiotensin II can be estimated from their ability to displace Tb^{3+} from its binding site on the hormone. The Tb^{3+} fluorescence enhancement is dependent upon the concentration of Na^+ or Ca^{2+} in solution, figure 4. At low cation concentraions, the fluores-

cence intensity of the Tb^{3+}-angiotenisn II complex is inversely proportional to the ion concentration. As the cation concentration increases, the decline in fluorescence falls off and reaches a limit. The cations did not completely displace Tb^{3+} from its metal binding site on angiotensin II. The affinities of Na^{+} and Ca^{2+} for angiotensin II are less than that of Tb^{3+} because of the more electropositive character of Tb^{3+}. In the absence of angiotensin II, the fluorescence intensity of free Tb^{3+} is essentially constant and independent of the Na^{+} or Ca^{2+} concentration. Double-reciprocal plots of the data revealed a linear relationship with a common intercept on the y-axis, figure 5. The apparent dissociation constants for Na^{+} and Ca^{2+} are estimated to be about 27.3 mM and 15.5 mM, respectively. The value of the dissociation constant for Na^{+} is low enough to indicate that under physiological conditions, Na^{+} can exist in a complex with angiotensin II. On the otherhand, this is not true for Ca^{2+}. Eventhough, the affinity of the divalent cation is greater than the affinity of the monovalent cation, Ca^{2+} is not likely to form a complex with angiotenisn II under physiological conditions, because the value of the dissociation constant for Ca^{2+} is considerably greater than its physiological concentration.

The integrity of the metal-angiotensin II complex can be ascertained by the magnitude of the Tb^{3+} fluorescence enhancement. The fluorescent properties of Tb^{3+} may be employed to study the effects of degrading enzymes, chelating agents, receptor binding, etc. on the conformation of angiotensin II. It is implied that the formation of the metal-peptide complex may effect the biological activity of angiotensin II. The above results suggests that the concentration and type of cation in solution must be considered when interpreting the molecular behavior of angiotensin II.

References

1. Schaechtelin, G., Walter, R., Salomon, H., Jelinek, J., Karen, P. and Cort, J.H. (1974) Mol. Pharm. 10, 57-67.
2. Schaechtelin, G., Surovec, D. and Walter, R. (1975) Experimentia 31, 346-347.

3. Blanc, E., Sraer, J., Sraer, J-D., Baud, L. and Ardaillou, R. (1978) Biochem. Pharm. 27, 517-524.
4. Gunther, S., Gimbrone, M.A. and Alexander, R.W. (1980) Circulation Research 47, 278-286.
5. Mann, J.F.E., Schiffrin, E.L., Schiller, P.W., Rascher, W., Boucher, R. and Genest, J. (1980) Hypertension 2, 437-443.
6. Jersey, J. and Martin, R.B. (1980) Biochem. 19, 1127-1132.
7. Kilhoffer, M-C., Demaille, J.G. and Gerard, D. (1980) FEBS Letters 116, 269-272.
8. Pjura, W.J., Kleinfeld, A.M., Karnovsky, M.J., Klausner, R.D. and Dragsten, P. (1980) Fed. Proc. 39, 1627.
9. Ohyashiki, T., Chiba, K. and Mohri, T. (1979) J. Biochem. 86, 1479-1485.
10. Garrett, K.M. and Ross, D.H. (1978) Pharmacolog. 20, 272.
11. Stephens, E.M. and Grisham, C.M. (1978) Fed. Proc. 36, 1483.
12. Morris, S.J. and Schober, R. (1977) Eur. J. Biochem. 75, 1-12.
13. Berner, V.G., Darnall, D.W. and Birnbaum, E.R. (1975) Biochem. Biophys. Res. Commun. 66, 763-768.
14. Epstein, M., Levitzki, A. and Reuben, J. (1974) Biochem. 13, 1777-1782.
15. Mikkelsen, R.B. and Wallach, D.F.H. (1974) Biochem. Biophys. Acta 363, 211-218.
16. Sherry, A.D. and Cottam, G.L. (1973) Arch. Biochem. Biophys. 156, 665-672.