COMPLEXATION OF AMMONIUM IONS BY THE POLYETHER MONOCARBOXYLIC ACID IONOPHORE, A23187

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1. Introduction

Ionophores such as the crown polyethers and the macrocyclic antibiotic, nonactin, have been reported to complex with ammonium ion, NH_4^+ [1]. The complex is held together by hydrogen bonds between the protons of the NH_4^+ ion and the ligands atoms such as the carbonyl oxygen atoms of the ionophore in a tetrahedral configuration. However, the ionophore–NH_4^+ complexes have not been demonstrated to mediate the transport of NH_4^+ in biological membranes. In a recent study [2], polyether monocarboxylic acid ionophores such as monensin and narasin are found to be better ionophores of NH_4^+ than of alkali metal cations in mitochondria. In here, the complexation of A23187 with NH_4^+ is detected by the enhancement of fluorescence in A23187 by NH_4^+. NH_4^+ can displace the K^+ but not Ca^{2+} from their complexes with A23187.

2. Materials and methods

A23187 free acid was kindly provided by Dr R. L. Hamill of our laboratories and was dissolved in absolute ethanol. Fluorescence measurements were made by an Aminco SPF 125 spectrophotofluorometer equipped with a xenon lamp.

3. Results

In ethanoic solution, A23187 at 6.4 X 10^{-6} M emitted fluorescence at 430 nm upon excitation at 380 nm (fig.1). The addition of KCl reduced fluorescence intensity by 50% indicative of complex formation between K^+ and the ionophore [5]. Upon the introduction of NH_4Cl, fluorescence intensity was increased beyond the original level of A23187 free acid alone. The final addition of $CaCl_2$, however, reduced the fluorescence of A23187 below the level reduced by the initial addition of KCl.

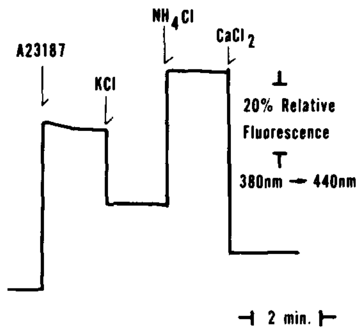

Fig.1. The fluorescence of A23187 changes upon the addition of KCl, NH_4Cl and $CaCl_2$, respectively. A23187 of 19.1 nmol was introduced into 3 ml of absolute ethanol. KCl, NH_4Cl and $CaCl_2$ each were added at 1 mM as indicated.

The uncorrected fluorescence spectra of excitation and emission for A23187 in the presence of various cations are shown in fig.2 and 3. A23187 itself had a fluorescence excitation spectrum with two maximum peaks near 280 nm and 380 nm (solid line). The addition of 1 mM NH_4Cl increased both of these peaks (open circle). The subsequent addition of 1 mM KCl did not significantly alter the spectrum (open triangles). KCl itself did lower the fluorescence spectrum of A23187 (filled squares) but the subsequent addition of NH_4Cl (filled triangles) regenerated the fluorescence

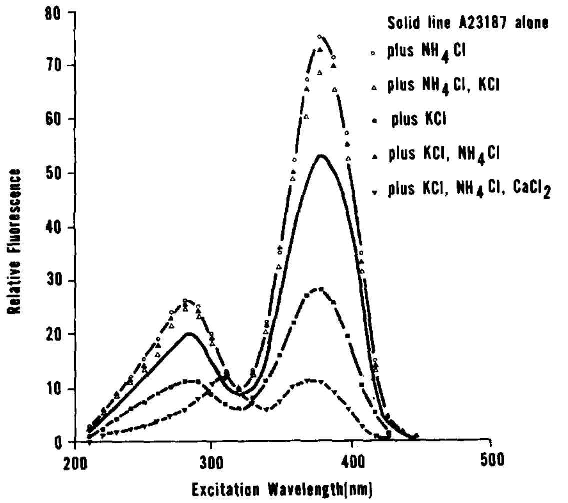

Fig.2. Fluorescence excitation spectra of A23187 in the presence of various cations. Experimental conditions were identical to those of fig.1. KCl, NH_4Cl and $CaCl_2$ were added in the order shown. Fluorescence emission of A23187 was measured at 440 nm.

spectrum to the same level as NH_4Cl was present alone. The final addition of $CaCl_2$ (inverted triangles) drastically lowered the fluorescence excitation spectrum with the maximum fluorescence peaks shifted from 280–310 nm and from 380–370 nm.

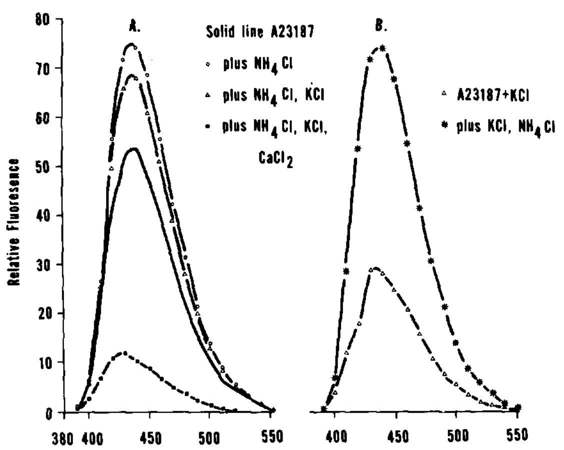

Fig.3. Fluorescence emission spectra of A23187 in the presence of various cations. Fluorescence of A23187 was excited at 380 nm for the same samples used for fig.2.

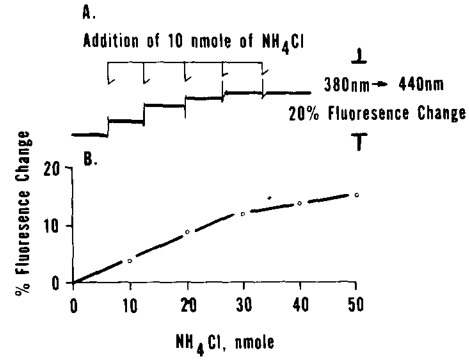

Fig.4. Increments of A23187 fluorescence upon repeated additions of NH_4Cl. NH_4Cl was added in 10 nmol portions to the quartz cuvette containing 19 nmol of A23187 in 3 ml absolute ethanol: A. tracing from chart paper; B. % fluorescence changes were plotted against amount of NH_4Cl added.

The corresponding emission spectra under each of the above conditions were taken as shown in fig.3A and 3B.

The increment of A23187 fluorescence could be accomplished by a stepwise addition of NH_4Cl until it reached a saturation level when 30 nmol of NH_4Cl had been added (fig.4A and 4B). The repeated additions of 2 nmol $CaCl_2$ to the sample reduced the fluorescence in almost equal portions until saturation in which the complexation of A23187 and Ca^{2+} achieved a 2:1 ratio (fig.5B). For comparison, the

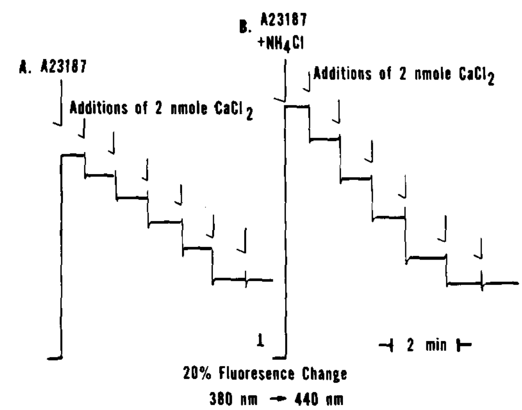

Fig.5. The decrease of A23187 fluorescence by $CaCl_2$ before (A) and after (B) the addition of NH_4Cl. $CaCl_2$ in an amount of 2 nmol was added as indicated, otherwise experimental conditions were identical to those in fig.4.

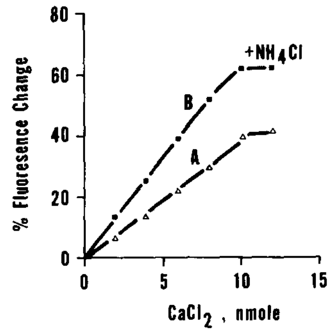

Fig.6. A23187 fluorescence is stoichiometrically reduced by $CaCl_2$ before and after the addition of NH_4Cl. The decrease of A23187 fluorescence observed in the previous experiment (fig.5) was plotted against the concentration of $CaCl_2$.

fluorescence of A23187 itself was quenched by the identical additions of 2 nmol $CaCl_2$ (fig.5A) and achieved a similar ratio of complexation (fig.6). It is significant to notice that the amount of fluorescence reduced by each addition of $CaCl_2$ in the presence of NH_4Cl was about twice the amount of fluorescence reduced by the addition of $CaCl_2$ in the absence of NH_4Cl.

4. Discussion

The fluorescent property of A23187 has provided a method to detect its complexation with metal cations [3,4]. The present study has extended the technique to detect the complexation of NH_4^+ with A23187. However, NH_4^+ enhances, whereas, both alkali earth and alkali metal cations reduce the fluorescence of A23187 [3,4]. In the presence of NH_4^+, K^+ of the same concentration (1 mM) has no effect on the enhanced fluorescence of A23187. On the contrary, NH_4^+ can reverse the quenched fluorescence of the K^+–A23187 complexes (fig.1–3). Thus, NH_4^+ can displace K^+ from its complex with A23187. This is indicative of a more stable complex of A23187 with NH_4^+ than with K^+, despite similar ionic radii for both ions. The actual ionic radius for NH_4^+ may in reality be much smaller because of hydrogen bonding between the protons of NH_4^+ and the ligands in A23187. It is known that NH_4^+ forms tetrahedral complexes in ligand coordination. The likely candidates of ligands in A23187 for hydrogen bondings are the carbonyl oxygen and nitrogen of the benzoxazole ring, one of the spiral ether oxygen and the other carbonyl oxygen of the molecule. The is mainly suggested by the structure of A23187–Ca^{2+} complex [5].

According to the increment of fluorescence by NH_4^+, A23187 forms complexes with NH_4^+ in a possible ratio of 1:1, which has been reported for other monovalent cation complexes of the ionophore with such ion as Li^+ [4]. However, the stoichiometry between the ionophore and the divalent cation, Ca^{2+}, is maintained at a 2:1 ratio even in the presence of NH_4^+. Furthermore, NH_4^+ can be quantitatively displaced by Ca^{2+}. In other words, A23187 favors the complexation with Ca^{2+} over NH_4^+.

In measuring the change in fluorescence of A23187, I have repeatedly recognized that a small increase of fluorescence persists when NH_4Cl is added beyond the apparent endpoint, whereas the decrease of fluorescence by $CaCl_2$ is complete once a 2:1 ratio is reached. This is probably due to the fact that an equilibrium exists in the NH_4^+ complexes dependent upon the forming and the breaking of hydrogen bonds; whereas Ca^{2+} is caged inside two molecules of A23187 [5].

References

[1] Eisenman, G. and Krasna, S. J. (1975) in: Biochemistry of Cell Walls and Membranes, (Fox, C. F., ed) vol. 2, p. 27, Butterworth, University Park Press, Baltimore.

[2] Wong, D. T. (1976) Sixteenth Interscience Conference on Antimicrobial Agents and Chemotherapy, in press.

[3] Caswell, A. H. and Pressman, B. C. (1972) Biochem. Biophys. Res. Commun. 49, 292.

[4] Pfeiffer, D. R., Reed, P. W. and Lardy, H. A. (1974) Biochemistry 13, 4007.

[5] Chaney, M. O., Demarco, P. V., Jones, N. D. and Occolowitz, J. L. (1976) J. Am. Chem. Soc. 96, 1932.