AEQUORIN: ITS IONIC SPECIFICITY

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Received October 3,1972

SUMMARY:

The specificity of the cation-aequorin luminescent reaction was investigated Contrary to present belief, this reaction was not specific for Ca^{2+} and Sr^{2+}. Over a dozen cations were capable of eliciting light emission from aequorin. Although these cations are not normally present in significant amounts in biological fluids, the present observations may be of importance in interpreting the luminescence obtained after the intracellular injection of aequorin.

INTRODUCTION:

Aequorin is a protein that is isolated from the luminescent jellyfish Aequorea aequorea. It is unique in that it emits light in the presence of Ca^{2+}, and the rate of light emission is dependent on the Ca^{2+} concentration (1,2). These properties have led to the use of aequorin as a Ca^{2+} indicator in biological preparations (3-9). The validity of this use of aequorin depends in part on a previous finding that only Ca^{2+} (and to a lesser degree, Sr^{2} can elicit light emission from aequorin (1,2,10,11). While developing a serum Ca^{2+} assay with aequorin as a reagent, we discovered that a large number of cations can react with aequorin and produce this luminescence. We report here on qualitative features of this observation and how they would affect the interpretation of aequorin luminescence in biological applications.

METHODS:

The aequorin was extracted from the jellyfish by the method of Shimomura et al (1) and purified by multiple gel filtration on Sephadex (G-150, G-100, G-50 and G-25) as described earlier (12). Desalting was done on a wide bed

Sephadex G-10 column (3.5 cm by 40 cm, sample volume 5 mls). Elution was done with triple distilled water and separate peaks for aequorin and EDTA were obtained spectrophotometrically (280 mμ). The aequorin prepared in this way was similar to that prepared by Blinks (13) in that it ran as three bands on polyacrylamide gel electrophoresis (7.5%, pH 8.9).

The cation-aequorin reaction was performed in a test tube suspended over the photo-cathode of a photomultiplier tube (E.M.I. 9524B, -950V) equipped with a shutter to facilitate exchange of the test tubes. The reaction was initiated by injecting 0.5 ml of the appropriate salt solution (at a concentration of either 1mM or 10μM)[1] with an automatic pipette into a test tube containing 0.2 ml of an aequorin solution (usually about 0.1 mgm protein/ml). This concentration of aequorin resulted in luminescent reactions that were within the response time of our equipment. The current from the photomultiplier tube was read on an ammeter (Keithley, model 602) and also photographed on a Hewlett-Packard storage oscilloscope (Model 181A). The time constant for the system was 3 msecs. The light emission from the aequorin reaction usually reached a maximum at about 500 msecs after mixing. Thus no correction for the unintentional integration of the current signal was necessary. The pH values at which the reactions were performed are indicated in Table I. All results were corrected for Ca^{2+} contamination which was measured by atomic absorption using a Perkin-Elmer model 107 spectrophotometer.

The aequorin emission spectra (uncorrected) for different cations were compared using a Perkin Elmer MPF 2A spectrofluorimeter with a 20 mμ slit width.

RESULTS AND DISCUSSION:

The results are listed in Table I. The main finding is that numerous other cations are capable of activating aequorin in addition to the two ions (Ca^{2+} and Sr^{2+}) that were previously recognized. In addition, the ability of these cations to elicit aequorin light emission (relative to Ca^{2+}) varied with their

[1] These values were chosen to approximate the Ca^{2+} concentrations in extracellular and intracellular fluids.

Table 1. Relative Reaction Rates of Cations with Aequorin [1,2]

ion	concentration 1mM	concentration 10μM
Group Ia		
Li^+	N.E.[3,4]	
Na^+	N.E.[4]	
K^+	N.E.[4]	
Group Ib		
Cu^{2+}	.03[5,9]	.80
Group IIa		
Sr^{2+}	.06[4]	.80
Ba^{2+}	.02[4,10]	.20
Mg^{2+}	N.E.[4]	
Group IIb		
Zn^{2+}	.02[6]	0
Cd^{2+}	.50[4]	.16
Hg^{2+}	N.E.[4,11]	
Group IIIb		
Y^{3+}	3.0[6]	1.0
La^{3+}	2.0[7]	0.50
Ce^{3+}	1.0[5]	0.25
Ce^{4+}	N.E.[7]	
Pr^{3+}	0.50[4]	0.50
Sm^{3+}	3.0[7]	0.50
Eu^{3+}	3.0[6]	0.67
Dy^{3+}	3.0[4]	0.25
Tm^{3+}	2.0[4]	0.50
Yb^{3+}	2.0[5]	5.0
Th^{3+}	0.05[4,9]	0.25
U^{3+}	N.E.[6]	
Group IVa		
Pb	1.0[6]	1.0
Group VIb		
Mo^{+6}	N.E.[8,11]	
Group VIIb		
Mn^{2+}	N.E.[4]	
Group VIIIb		
Fe^{3+}	N.E.[4,11]	
Co^{2+}	0.01[6]	1.0
Ni^{2+}	0.10[6]	0.20

1. Expressed relative to the reaction rate of Ca^{2+} (at the same concentration) as 1.0. All measurements were at pH 6.0 except as noted.
2. These values are the means of 3 readings. There was considerable spread in the results and a range of $\pm$ 10% about the mean was not unusual.
3. N.E. not effective.
4. chloride
5. sulfate
6. acetate
7. nitrate
8. trioxide
9. pH 5.0
10. pH 11.0
11. pH 3.0

concentration. At a concentration of 1mM, nine cations had maximum reaction rates that were equal to or greater than that for Ca^{2+}. At a concentration of 10μM, Cu^{2+}, Sr^{2+}, Pb^{2+}, and Co^{2+}, and some of the lanthanides had reaction rates that were comparable to that for Ca^{2+}. In addition, the aequorin light emission spectrum had a maximum at approximately 460 mμ independent of whether Ca^{2+}, Pb^{2+} or Tm^{3+} was used as the activating cation. These results may be of importance in view of the low value of intracellular Ca^{2+} concentration.

It is also of interest that the valence of the interacting cations does not seem to be critical for aequorin activation. Both Ca^{2+} and Sr^{2+} are dipositive while all of the lanthanides listed in Table I are tripositive. Cerium was tested in the tripositive (cerous) and tetrapositive (ceric) states, and the cerous ion (1.07 angstroms) was capable of aequorin activation while the ceric ion (0.94 angstroms) was not.

These results are not consistent with the earlier studies on the specificity of the aequorin reaction. Pb^{2+} and Cd^{2+} in particular were previously found to be ineffective (1,2). However, these earlier measurements were done in the presence of EDTA (ethylenediamine-tetraacetic acid) while ours were not, and therein was the difference. Because the binding constant betwen Pb^{2+} and EDTA is 6 orders of magnitude greater than that between Ca^{2+} and EDTA, a greater suppresion of the Pb^{2+} induced luminescence (relative to the Ca^{2+} induced luminescence) would be expected in the presence of EDTA. This is what was found (Fig. I). Two other cations (Y^{3+} and Sr^{2+}) whose binding constants are greater than and less than that of Ca^{2+} were also tested. In both instances the differential effect could be predicted from a consideration of the relative binding constants.

We conclude that there are may ions in addition to Ca^{2+} and Sr^{2+} which are capable of eliciting light emission from aequorin, and that the current belief in a high ionic specificity for the aequorin luminescence reaction is based on an artifact caused by the inclusion of EDTA in the assay tubes. We appreciate that none of the above cations are normally present in biological

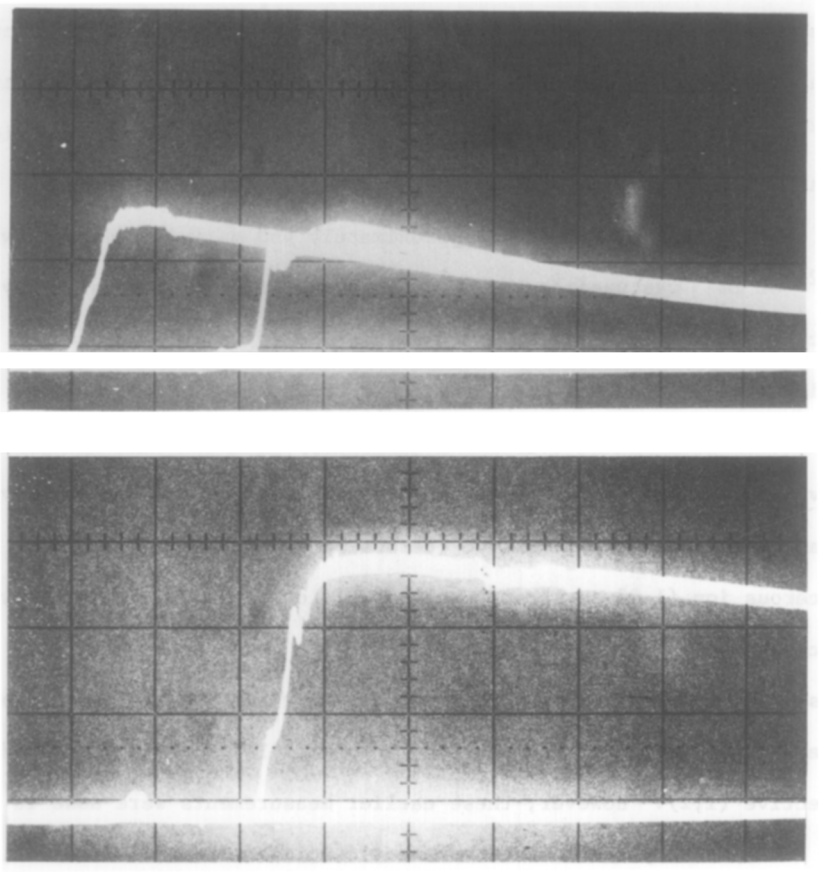

Fig. 1 (above) Two superimposed oscilloscope traces of current from the photomultiplier tube used to monitor the light emitted from the aequorin reaction. The first upward current trace to the left resulted from light emission from a Pb^{2+}(1mM)-aequorin reaction. The second upward current trace resulted from a Ca^{2+}(1mM)-aequorin reaction. No EDTA was present in either test tube. (below) Same as above except in the presence of EDTA (1mM) which markedly reduced the Pb^{2+}-aequorin reaction relative to the Ca^{2+}-aequorin reaction.

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fluids in significant concentrations as compared to the Ca^{2+} concentration. However, the above findings should be borne in mind when interpreting the aequorin luminescence in intracellular injection studies where the value of

the Ca^{2+} concentration is low and the concentrations and physiology of the trace metals have not yet been determined.

ACKNOWLEDGEMENTS:

We thank Dr. J.E. Heavner for much useful discussion of the rare earths, Larry Sieker for supplying us certain rare earths, and Dr. B.R. Fink for logistical and moral support of this project. We gratefully acknowledge the use of the facilities of the University of Washington research laboratory at Friday Harbor. This work was done with the technical assistance of J. McGough and J. Crawford. This research was supported in part by N.I.H. grants DE 02600 and GM 01160 and by the Washington State Sea Grant Program under grant number NG-1-72 from the N.O.A.A.

REFERENCES:

1. O. Shimomura, F.H. Johnson, Y. Saiga, J. Cell. Comp. Physiol. 59, 223 (1962).

2. O. Shimomura, F.H. Johnson, Y. Saiga, J. Cell. Comp. Physiol. 62, 1 (1963).

3. E.B. Ridgway, C.C. Ashley, Biochem. Biophys. Res. Comm. 29, 229 (1967).

4. O. Shimomura, F.H. Johnson, Y. Saiga, Science 140, 1339 (1963).

5. A. Azzi, B. Chance, Biochim. Biophys. Acta 189, 141 (1969).

6. P.F. Baker, A.L. Hodgkin, E.B. Ridgway, J. Physiol. (London) 218, 709 (1971).

7. R. Llinas, J.R. Blinks, C. Nicholson, Science, 176, 1127 (1972).

8. B. Kaminer, J. Kimura, Science, 176, 406 (1972).

9. P.F. Baker, A.E. Warner, J. Cell Biol. 53, 579 (1972).

10. G. Loschen, B. Chance, Nature 233, 273 (1971).

11. F.H. Johnson, O. Shimomura, Nature 237, 287 (1972).

12. K.T. Izutsu, S.P. Felton, Clin. Chem. 18, 77 (1971).

13. J.R. Blinks, Proc. Int. Union Physiol. Sci. 9, 68 (1971).