EVIDENCE THAT RENILLA LUCIFERASE IS NOT A CALCIUM-TRIGGERED PHOTOPROTEIN*

Milton J. Cormier, Yashwant D. Karkhanis
and Kazuo Hori

Department of Biochem., Univ. of Ga., Athens, Ga. 30601

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Summary

When crude extracts of Renilla luciferase are prepared in the presence of a chelator such as EDTA, a flash of light is observed upon the addition of calcium ions to such extracts. Although this behavior is reminiscent of calcium triggered photoproteins, we have shown that calcium ions have no effect on the activity of highly purified Renilla luciferase.

We have recently isolated and studied the properties of two enzymes linked to bioluminescence in Renilla reniformis.[1] Luciferase[1] and, more recently, luciferin sulfokinase (Karkhanis, Y. D. and M. J. Cormier, unpublished data) have been purified to homogeneity. They catalyze the following reactions:

$$\text{Luciferyl sulfate} + 3'5' - \text{diphosphoadenosine} \underset{\text{Ca}^{++}}{\overset{\text{Luciferin Sulfokinase}}{\rightleftharpoons}} \text{Luciferin} + \text{PAPS}$$

$$\text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{light} + \text{products} \qquad (2)$$

A flash of light is observed when calcium ions are added to crude extracts of Renilla prepared in the presence of chelators, such as EDTA. (Cormier, M.J. and C.B. Eckroade, unpublished data). Recently it has been suggested[2] that this identifies Renilla luciferase as a calcium triggered photoprotein analagous to those previously isolated [3,4,5]. These photoproteins emit visible light when they are mixed with calcium ions.

Because any possible effect of calcium ions on luciferase would be of importance relative to the mechanism of the light reaction we reexamined the effect of calcium on that enzyme and report the results of those investigations here.

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Materials and Methods-Luciferase and luciferin sulfokinase were prepared as previously described[1]. Similarly pure luciferyl sulfate and luciferin were prepared as described earlier[1,6]. 3¦ 5'-diphosphoadenosine was prepared by the phosphorylation of adenosine with dibenzylphosphoryl chloride[7], as modified by Moffatt and Khorana[8].

Results and Discussion-Table I shows that calcium ion is without effect on the activity of luciferase in the presence or absence of an excess of EDTA. Furthermore all buffers used during the purification of luciferase contained 2.5×10^{-4}M EDTA. As previously indicated[9] the only enzyme in this bioluminescence system that is inhibited by EDTA and stimulated by calcium is luciferin sulfokinase.

It is not possible to simulate a calcium-triggered photoprotein effect by incubating luciferase and luciferin sulfokinase together with luciferyl sulfate, 3¦ 5'-diphosphoadenosine, and EDTA. The size of the active complex recently reported[2] appears to be large since the activity sediments in 20 minutes at 30,000 x g. The molecular weight of luciferase is approximately 14,000 while that of luciferin sulfokinase is about the same. These results suggest that some component(s) in the crude extract in addition to luciferase, is responsible for the apparent photoprotein behavior of EDTA treated material.

TABLE I

Effect of EDTA and Calcium on the Activity of Renilla Luciferase

Test System	Maximal Light Intensity Quanta $Sec^{-1} \times 10^{-10}$
A. Luciferase	20
B. Luciferase + Calcium	21
C. Luciferase + EDTA; incubated 5 minutes	21

Conditions: The assay system consisted of final concentrations of the following: potassium phosphate (pH 7.5), 50 mM; luciferin, 4×10^{-3} mM; luciferase, 0.1mg in a total vol. of 1.1 ml. B contained calcium chloride, 2mM and C contained disodium EDTA, 5 mM. Temperature was maintained at 25°C.

It is clear, however, that the purified enzymes do not exhibit the photoprotein effect in the presence of EDTA.

It has been suggested[10,11] that photoprotein systems of the types mentioned above may be explained by assuming the existence of an EDTA-inhibited complex of some type that contains luciferin and luciferase and which is triggered to react upon the addition of calcium ions. The Renilla system provides a model for this type of explanation.

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