irrespectively of whether the ascending or the descending technique is used. The use of an ordinary alarm clock makes the apparatus inexpensive but if the equilibration time must exceed eleven hours another type of timer is required.

This work has been supported by grants from Statens Medicinska Forskningsråd.


Received June 16, 1960.

A Note on the Action of Firefly-Extracts on ATP

B. O. HANSEN* and JOHN GRAAE

Institute of Biological Chemistry, University of Copenhagen, Denmark

Use of the enzyme system from Photinus pyralis for the determination of minor amounts of ATP has been suggested and further a method implying it has been described by Strehler and Totter 1. In order to study the extent of the postulated linear dependence of light intensity on ATP concentration experiments were carried out with different amounts of firefly-extracts and of ATP.

The experiments were carried out in small test tubes closed with a rubber stopper. ATP solution, enzyme extract and either arsenate buffer or water was mixed by rapid shaking. Light intensity was then determined by a photometer. If we suppose that light intensity is a direct measure of the reaction velocity and accept that the phototube (type RCA 1-P-21) is linear in the spectral area in question 2, then the photometer readings directly express reaction velocity in arbitrary units. It is known that oxygen is required for the reaction but this is supposed to be present in a concentration that is sufficient for the reaction, for two reasons: First it is demonstrated by Hastings et al. 3 that only decreasing oxygen pressure to a very minute fraction of the normal has any influence on the reaction and secondly, we have demonstrated that bubbling of atmospheric air through the test tubes did not restore activity when the light intensity had fallen to approximately zero. Yet activity could be restored by the addition of either ATP or firefly extract de-

* Present address: Centrallaboratoriet, Kommunehospitalet, Copenhagen, Denmark.
SHORT COMMUNICATIONS

$$\text{LH}_2 + \text{Mg}^{++} + \text{ATP} + E \downarrow \uparrow$$

"active intermediate"

Mg$$^{++}$$, protein

\( \rightarrow \)

"inactive complex"

light

\( X_1 + S \rightarrow X_2 \quad (\pm 1) \)

\( X_2 \rightarrow X_1 + P \quad (+2) \)

\( X_2 \rightarrow X_3 \quad (\pm 3) \)

Fig. 3. A. Reaction scheme suggested by McElroy et al.\(^5\) for the luciferase reaction, \((E = \text{enzyme}, \text{LH}_2 = \text{reduced luciferin})\). B. Reaction scheme suggested by Christiansen \(^4\) to be the general scheme for a reaction following the Henri-Michaelis chronometric integral. \((X_1, X_2\) and \(X_3\) are different forms of the enzyme or combinations of enzyme and substrate.) C. The same as B, written as ordinary equations. \((S = \text{substrate and } P = \text{products})\)

Depending on the initial ratio between these two components.

The effect of varying the amount of firefly extract is shown in Fig. 1. It is seen that light intensity measured 30 sec. after mixing the reagents is proportional to the square of the concentration of firefly extract. This is in good agreement with the commonly accepted fact that the extract contains two components that are important for the reaction, the enzyme luciferase and another component, luciferin \(^{3,4}\).

The dependence of reaction velocity on ATP concentration was studied too. It is seen from Fig. 2 that the reaction kinetics is in accordance with the Henri-Michaelis scheme, when initial velocity is measured 30 sec. after mixing. Frequent readings during initial few minutes do, however, not show reaction kinetics in accordance with a normal Henri-Michaelis reaction, as the extremely rapid initial fall in light intensity is not caused by a decrease in the concentrations of the reactants \(^4\). To explain this it has been suggested by McElroy et al.\(^5\) that part of the luciferase in one way or another is reversibly inactivated (Fig. 3). The scheme suggested for this particular reaction is then identical with the one proposed by Christiansen to be the general scheme for the simplest enzymatic reaction. It is reasonable to suggest that the luciferase reaction differs from the simple enzymatic reactions by approaching steady state with respect to reaction \(\pm 3\) (Fig. 3) after a few minutes instead of immediately.

A similar reaction mechanism is described by Andersen \(^7\) in the case with arylsulphatase A, only in this case steady state with respect to the inactivating reaction was reached about 60 min. after the initiation.


Received July 7, 1960.