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Studies on the Bioluminescence of *Renilla reniformis*. VII. Conversion of Luciferin into Luciferyl Sulfate by Luciferin Sulfokinase*

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

ABSTRACT: An enzyme preparation has been obtained from *Renilla reniformis*, essentially free of luciferase, which catalyzes the 3′,5′-diphosphoadenosine-linked conversion of luciferyl sulfate into luciferin. In the presence of luciferin and [^{35}S]-adenylyl sulfate 3′-phosphate this enzyme catalyzes the formation of [^{35}S]luciferyl sulfate. The enzymatically produced [^{35}S]luciferyl sulfate has the same chromatographic and chemical properties as does the naturally isolated luciferyl sulfate. We propose the name luciferin sulfokinase (3′-phosphoadenylyl sulfate:luciferin sulfotransferase) for this enzyme.

The enzyme is apparently specific for luciferin since it does not exhibit any phenol sulfokinase activity. Luciferin is stored in *Renilla* as luciferyl sulfate. Thus luciferin sulfokinase may play an important role in regulating the levels of luciferin available for bioluminescence in this animal.

It was previously reported (Hori and Cormier, 1965; Cormier, 1962; Hori and Cormier, 1966) that the overall reaction leading to light emission in the sea pansy, *Renilla reniformis*, proceeds as follows:

$$\text{luciferyl-X} \xrightarrow[\text{3′5′-diphosphoadenosine}]{\text{enzyme; } Ca^{2+}} \text{luciferin} + \text{X} \qquad (1)$$

$$\text{luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{light} + \text{oxidized products} \qquad (2)$$

In reference to eq 1 it was not known whether X was transferred to 3′,5′-diphosphoadenosine (DPA).[1] It is known that X is an acid-labile component (Cormier and Hori, 1964) and previous data has shown that X is sulfate. This was based on the fact that acid treatment of luciferyl-X liberates a compound that reacts as and cochromatographs with inorganic sulfate (Cormier *et al.*, 1966).

In the past it was impossible to study the mechanism of reaction 1 owing to the facts that the enzymes catalyzing reactions 1 and 2 were not separated from one another and that sufficient amounts of pure luciferyl-X and luciferin were unavailable. These obstacles have now been overcome and it is the purpose of this report to present data on the mecha-

* From the Department of Biochemistry, University of Georgia, Athens, Georgia 30601. *Received November 21, 1969*. This work was supported in part by the National Science Foundation and the U. S. Atomic Energy Commission. M. J. C. is Career Development Awardee No. 1-K3-6M-3331-05 of the U. S. Public Health Service. Contribution No. 191 from the University of Georgia Marine Institute, Sapelo Island, Ga.

[1] The following abbreviations are used: DPA, 3′,5′-diphosphoadenosine; PAPS, 3′-phosphoadenylyl sulfate; APS, adenylyl sulfate.

TABLE I: Summary of Fractionation Procedures Used to Separate Luciferase from Luciferin Sulfokinase.[a]

Fraction	Vol (ml)	Protein (mg)	Activity (Quanta $sec^{-1} \times 10^{-11}$) Luciferyl Sulfate	Luciferin
Crude extract	1700	9600	397	6630
DEAE eluate	1920	7700	358	6550
First $(NH_4)_2SO_4$ precipitate (49% w/v)	200	5000	67	420
$(NH_4)_2SO_4$ supernatant	2010	2000	20	5628
Dialysis *vs.* 0.01 M phosphate buffer, pH 7.5	220	1800	33	85
Second $(NH_4)_2SO_4$ precipitate (49% w/v)	90	1650	11	21

[a] Conditions: assay conditions are as earlier described (Cormier, 1962; Cormier and Hori, 1964). Maximum levels of luminescence were used as a measure of activity for each substrate. The activity values listed were adjusted to account for the total volume.

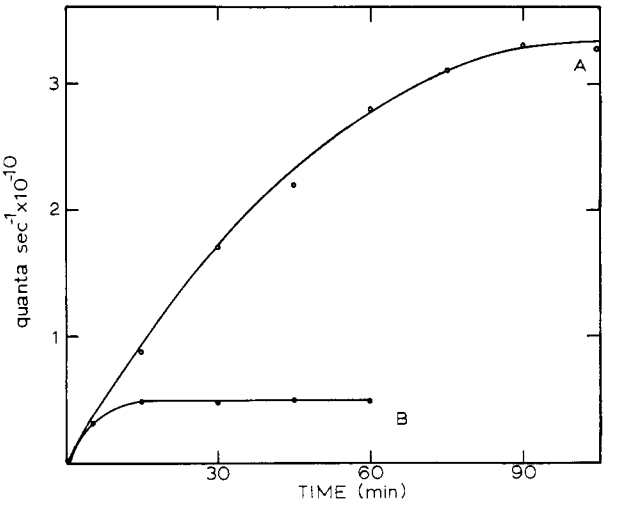

FIGURE 1: Time course of conversion of luciferyl sulfate into luciferin by luciferin sulfokinase. Final concentration of reactants were: potassium phosphate buffer, pH 7.5, 45 mM; $CaCl_2$, 5 mM; luciferyl sulfate, 0.5 μM; DPA, 2 μM; 2-mercaptoethanol, 1 mM; and protein, 1 mg/ml. Total volume was 4 ml and the solution was incubated at 30°. At points indicated on curve A, 0.5-ml aliquotes were removed and 2 μg of luciferase was added and the maximum light intensity was observed. In curve B, 6 μg of luciferase was added at zero time and the luminescence was observed as a function of time.

nism of reaction 1 and to provide additional evidence that X is indeed sulfate. Thus luciferyl-X will henceforth be referred to as luciferyl sulfate and, for reasons presented in the text, the enzyme catalyzing reaction 1 will be referred to as luciferin sulfokinase.

Materials and Methods

Preparation of Luciferin Sulfokinase. The following gives a simple procedure for obtaining luciferase-free preparations of the enzyme catalyzing reaction 1. Crude extracts of the enzyme catalyzing reaction 1 were prepared from acetone powders as described by Kreiss (1967). This extract was put through a DEAE column as described by Kreiss (1967) except that the buffer used was 0.1 M potassium phosphate buffer (pH 7.5) which contained 2.5 mM disodium ethylenediaminetetraacetic acid and 1 mM mercaptoethanol. Both enzymes involved in bioluminescence are not adsorbed to DEAE under these conditions and are eluted off the column. All steps were carried out at 2–4°. Solid ammonium sulfate was added to the pooled enzyme fractions to 49% saturation (w/v). This mixture was allowed to equilibrate for 1 hr and centrifuged at 24,000*g* for 15 min. The precipitate was dissolved in the above described buffer to a final protein concentration of 20 mg/ml and dialyzed overnight against 0.01 M potassium phosphate buffer, pH 7.5. During the dialysis a large inactive precipitate forms which represents about two-thirds of the total protein. Such preparations are usually devoid of most of the luciferase activity. If necessary the ammonium sulfate precipitation and dialysis steps may be repeated to remove the last trace of luciferase from the preparation. The ammonium sulfate step results in the precipitation of the enzyme catalyzing reaction 1 while most of the luciferase remains in solution. Table I represents a summary of the fractionation steps outlined above. As shown in Table I two substrates were used in activity measurements of the fractions. Luminescence upon the addition of luciferyl sulfate reflects the presence of both luciferin sulfokinase and luciferase in the fraction. Alternatively luminescence observed with luciferin is a measure of luciferase activity only. As noted in Table I the first ammonium sulfate precipitate results in the removal of more than 90% of the luciferase activity from that fraction. This results in a large decrease in the luminescence activity when assayed with luciferyl sulfate. After repeated ammonium sulfate fractionation followed by dialysis such activity becomes negligible. This is due to the removal of luciferase from these fractions. Whereas such fractions contain little or no luciferase it can be demonstrated that they contain significant amounts of luciferin sulfokinase. For example in an assay system consisting of luciferin sulfokinase, luciferyl sulfate, DPA, and Ca^{2+} no luminescence is observed after 1-hr incubation at 30°. If 10 μg of luciferase is then added to such an incubated mixture a flash of light is observed (about 10^{12} quanta sec^{-1} at the peak) whose decay is first order. The kinetics of the flash are identical with those observed when luciferin is added to luciferase (Cormier and Hori, 1964). In addition, if luciferase replaces luciferin sulfokinase in the above incubation mixture, again no luminescence is observed. When luciferin sulfokinase is added to this system a slow rise in luminescence intensity is observed which resembles that of crude extracts to which luciferyl sulfate has been added. Figure 1 (curve A) illustrates the time course of production of luciferin from luciferyl sulfate in the presence of luciferin sulfokinase (see text). Curve B illustrates the effect of having a small amount of luciferase in the preparation.

A reliable assay, based on luminescence, for luciferin

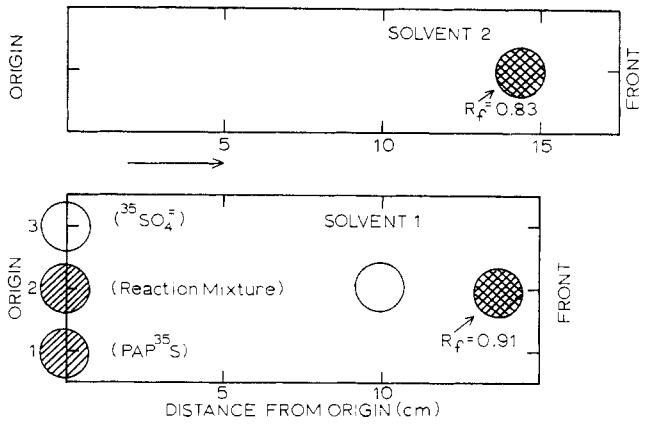

FIGURE 2: Formation of [^{35}S]luciferyl sulfate from [^{35}S]PAPS and luciferin. The conditions and experimental procedure were as described under Table I. Chromatography was performed first with solvent I and then with solvent II as described. Open areas refer to positions of radioactivity, single thatched areas to those of radioactivity and ultraviolet quenching, and double thatched areas to those of radioactivity, ultraviolet quenching, and luciferyl sulfate activity. Luciferyl sulfate remaining on the paper was eluted off with solvent I and rechromatographed on paper using solvent II.

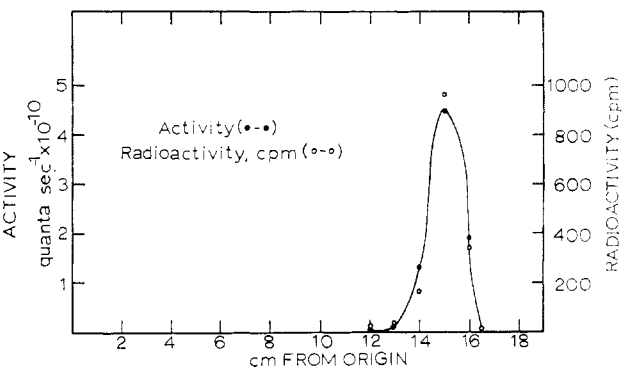

FIGURE 3: Correspondence between radioactivity and luciferyl sulfate activity on paper chromatograms. Material chromatographed on solvent II, as shown in Figure 5, was assayed as described under Methods.

sulfokinase can be obtained only after essentially all of the luciferase has been removed from the preparation. Thus it is not possible to calculate the degree of purification of luciferin sulfokinase over that of the crude extract from the data in Table I. For the sake of the experiments reported here, however, it is important that the sulfokinase preparation be essentially luciferase free so that reaction 1 can be studied independently of reaction 2. As indicated above this has been accomplished.

Preparation of Substrates. Pure luciferyl sulfate was prepared by a previously described procedure (Hori and Cormier, 1965). Pure luciferin was prepared by treating 1–3 mg of luciferyl sulfate with 0.5 ml of 0.2 N HCl, under H_2, at 100° for 2 min. The initially colorless solution turns to deep yellow during this procedure. The solution was allowed to stand at 0° for 30 min during which time luciferin precipitates as an amorphous yellow material. The stoppered vessel was centrifuged at 700*g* for 3 min to collect luciferin. The yellow precipitate was washed twice with 0.4 ml of 0.1 N HCl to remove traces of inorganic salts. The precipitate was dried overnight, *in vacuo*, over solid KOH and then suspended in 10 ml of benzene overnight which solubilizes any oxidized products of luciferin. The benzene was removed by decantation and luciferin then dried overnight, *in vacuo*, over solid KOH and stored in the dark until used.

[^{35}S]Adenylyl sulfate 3′-phosphate and the unlabeled compound PAPS[1] were prepared by the method of Robbins and Lipmann (1958) except that the yeast was crumbled by hand and dropped into liquid nitrogen (Robbins, 1962). The [^{35}S]PAPS used had a specific activity of 6.7×10^6 cpm/μmole.

The authenticity of the [^{35}S]PAPS used was determined from absorption spectra, paper chromatography, and enzymatic assay. We found that the *Renilla* bioluminescence system provides a very rapid and sensitive assay for PAPS. The reaction is very specific for DPA and shows no activity with PAPS (Cormier, 1962). However, PAPS can be converted into DPA + SO_4^{2-} by treating with 0.1 N HCl at 100° for 2 min. By comparison with a standard DPA curve the expected amount of DPA was liberated from PAPS by the above treatment. Since the K_m for DPA is 7.3×10^{-8} M (Cormier, 1962) it follows that the bioluminescence system provides an excellent and very sensitive assay for PAPS.

[^{35}S]Sulfate was purchased from New England Nuclear with a specific activity of 448 mCi/mmole.

Physical Measurements. Luminescence measurements were made as previously described (Cormier, 1962). Relative units were converted into quanta sec^{-1} by the use of light standards as described by Hastings and Weber (1963). Luminescence assays for luciferyl sulfate were conducted as earlier described (Cormier, 1962).

Absorbancy measurements were made with a Cary Model 14 recording spectrophotometer. Synthetic DPA was a generous gift of Dr. E. A. Davidson, Duke University.

Radioactivity from paper chromatograms was determined by cutting 1-cm sections and placing them in vials containing 7 ml of a solution of 2,5-bis[2-15-*t*-butylbenzoxazalyl]thiophene in toluene (4 g/l.) and recording cpm with a Packard Tri-Carb scintillation spectrometer. Corresponding 1-cm sections were used to measure luciferyl sulfate activity on such chromatograms.

Chromatographic Procedures. Paper chromatography was done on Whatman No. 3MM paper that had been prewashed by descending chromatography using 90% ethanol which contained 0.5 mM potassium phosphate buffer, pH 7.5 (solvent I). The paper was air dried and stored until used. *n*-Propyl alcohol–concentrated NH_4OH–water (6:3:1), referred to as solvent II, was used as a second chromatographic solvent (Cherniak and Davidson, 1964).

Column chromatography of luciferyl sulfate was done on a 1.5×30 cm column of LH-20 (Sigma) that had been equilibrated with 80% methanol which contained 10 mM potassium phosphate buffer, pH 7.5. After introducing the sample the material was eluted with the above methanol–buffer mixture and 2-ml fractions were collected. On such columns oxidized products of luciferin precede the elution of luciferyl sulfate.

[2] Solvent I was composed of 90% ethanol containing 0.5 mM potassium phosphate buffer, pH 7.5; solvent II, *n*-propyl alcohol–concentrated NH_4OH–water (6:3:1).

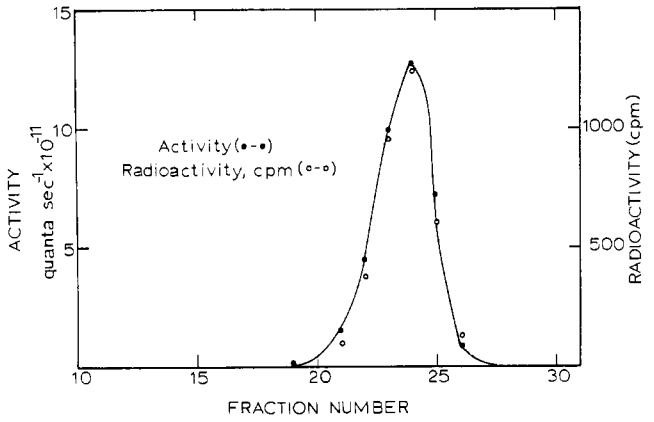

FIGURE 4: Correspondence between radioactivity and luciferyl sulfate activity on column chromatograms. [^{35}S]Luciferyl sulfate that had been chromatographed twice on paper, as shown in Figure 5, was eluted from the paper with solvent I and chromatographed on LH-20 as described under Methods. Fractions (2 ml) were collected at a flow rate of 20 ml/hr.

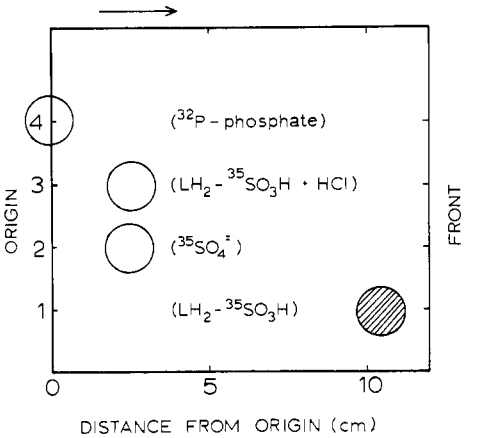

FIGURE 5: Liberation of acid-labile [^{35}S]sulfate from ^{35}S-labeled luciferyl sulfate. Open areas refer to positions of radioactivity while the single thatched area refers to a position of radioactivity, ultraviolet quenching, and luciferyl sulfate activity. No. 1 represents untreated [^{35}S]luciferyl sulfate while no. 3 represents [^{35}S]luciferyl sulfate that had been treated with 0.1 N HCl at 100° for 2 min as previously described (Cormier and Hori, 1964).

Results

Enzymatic Synthesis of [^{35}S]Luciferyl Sulfate. When luciferin and [^{35}S]PAPS are incubated together with the enzyme that catalyzes reaction 1 radioactivity appears on paper chromatograms in the area occupied by luciferyl sulfate. If the labeled luciferyl sulfate is eluted from the paper and rechromatographed on paper using a different solvent, the luciferyl sulfate area is still labeled. These results are illustrated in Figure 2. Note that solvent I retains counts attributable to [^{35}S]PAPS and [^{35}S]SO_4^{2-} at the origin while the R_f of luciferyl sulfate is 0.91. Figure 3 shows that there is close correspondence between luciferyl sulfate activity in the luminescence assay and radioactivity found on paper chromatograms during rechromatography using solvent II. Further confirmation of the correspondence between activity and radioactivity was provided by chromatographing labeled luciferyl sulfate for the third time on an LH-20 column. These results are illustrated in Figure 4.

It had previously been shown (Hori and Cormier, 1966; Cormier and Hori, 1964) that luciferyl sulfate can be converted into luciferin and inorganic sulfate by treatment with 0.1 N HCl at 100° for 2 min. Thus the sulfate attached to luciferin is acid-labile sulfate. The fact that the label in luciferyl sulfate, produced from a reaction between [^{35}S]PAPS and luciferin, is also acid-labile sulfate can be shown by an experiment the results of which are illustrated in Figure 5. When labeled luciferyl sulfate is treated with HCl at 100° as indicated above [^{35}S]SO_4^{2-} is liberated. [^{32}P]Phosphate remains at the origin.

Requirements for the formation of [^{35}S]luciferyl sulfate are shown in Table II. It is evident from the results that, in addition to [^{35}S]PAPS, both luciferin and the enzyme are required. It is also possible from these results to calculate the per cent conversion of luciferin into [^{35}S]luciferyl sulfate. In this calculation the specific activity of the [^{35}S]luciferyl sulfate is assumed to be the same as that of [^{35}S]PAPS. Since the specific activity of the [^{35}S]PAPS used was 6.7×10^6 cpm/μmole then 0.01 μmole of luciferin would represent a maximum expected number of counts of 67,000 cpm. Thus, approximately 60% conversion into [^{35}S]luciferyl sulfate was observed.

Since [^{35}S]PAPS usually contains [^{35}S]SO_4^{2-} as a contaminant efforts were made to observe an exchange between [^{35}S]SO_4^{2-} and luciferyl sulfate. This was done in an effort to look for the incorporation of SO_4^{2-} into the enzyme resulting in the formation of high group potential enzyme-bound SO_4^{2-}. An analogous situation has been found in the incorporation of inorganic phosphate by alkaline phosphatase (Schwartz and Lipmann, 1961; Schwartz, 1963). Conditions

TABLE II: Requirements for the Formation of [^{35}S]Luciferyl Sulfate.[a]

Additions	Radioactivity in Luciferyl Sulfate (cpm)
Complete system	38,580
Complete system minus luciferin	372
Complete system minus enzyme	25

[a] Conditions: the complete system consists of final concentrations of the following components: [^{35}S]PAPS, 0.3 mM; potassium phosphate buffer, pH 7.5, 50 mM; 2-mercaptoethanol, 2 mM; $CaCl_2$, 5 mM; luciferin, 0.01 mM; protein (7.6 mg/ml), 0.3 ml/ml of solution. The final volume was 1 ml. The reaction was carried out under a H_2 atmosphere at 30° for 60 min. Luciferin was placed in a side arm and tipped in to start the reaction. The reaction was stopped by adding four volumes of methanol, 0.1 μmole of carrier luciferyl sulfate was added, and the denatured protein was removed by centrifugation. The supernatant was concentrated to about 0.5 ml by flash evaporation at 38° and chromatographed on paper using solvent I, and the radioactivity was determined as described under Methods.

employed in Table I were used except that 0.03 mM luciferyl sulfate replaced luciferin and 0.1 mCi of $[^{35}S]SO_4^{2-}$ replaced [^{35}S]PAPS. Chromatography on paper using solvent I resulted in the usual luciferyl sulfate spot at R_F 0.91 but without significant radioactive labeling. Thus, the exchange mentioned above does not occur.

Involvement of ^{32}P contaminants could conceivably result in the production of a labeled compound that cochromatographs with luciferyl sulfate. However, pulse height analysis has ruled this out. The possibility also existed that these results might be accounted for by our enzyme being contaminated with a 3′-phosphatase for PAPS thus converting it into adenylyl sulfate (APS). Thus labeled APS might cochromatograph with luciferyl sulfate. However, they do not cochromatograph with the solvent systems used.

It is evident then that the enzyme catalyzing reaction 1 can be given the name luciferin sulfokinase (3′-phosphoadenylyl sulfate:luciferin sulfotransferase). The enzyme normally functions in the direction indicated during bioluminescence but is named out of precedent for the back-reaction (see reaction 3).

Attempts to demonstrate the production of [^{35}S]PAPS from [^{35}S]luciferyl sulfate and DPA have failed. Experiments were performed both aerobically and anaerobically. In some experiments 0.8 μmole of carrier PAPS was added at the beginning, and in others at the end, of the experiment. Further, no sulfatase for PAPS could be demonstrated in the enzyme preparation. These results are consistent with observations made on phenol sulfokinase (Gregory and Lipmann, 1957). In the case of luciferin sulfokinase we do know that the rate of the forward reaction is very low and may introduce some difficulties. Figure 1 illustrates the point. Curve A shows that approximately 90 min are required to reach a maximum response. This is a minimum time since the leveling off point at 90 min does not represent substrate depletion but rather a steady state between the rate of luciferin production and its subsequent autooxidation. Thus it appears that the rate constant for the forward reaction must be relatively small. Curve B shows the effect of adding 6 μg of highly purified luciferase (Y. D. Karkhanis and M. J. Cormier, unpublished data) at zero time. This results in a relatively low level, steady state luminescence due to utilization, by luciferase, of the luciferin being produced.

Specificity of Luciferin Sulfokinase. The enzyme was tested for phenol sulfokinase activity by using PAPS and *p*-nitrophenol as described by Gregory and Lipmann (1957). It was also tested using *p*-nitrophenyl sulfate and DPA as described by the same authors. No phenol sulfokinase activity could be demonstrated. Thus luciferin sulfokinase is not of the phenol sulfokinase type.

Discussion

From the foregoing evidence it is now appropriate to refer to luciferyl-X as luciferyl sulfate and to the enzyme that catalyzes the conversion of luciferyl sulfate into luciferin as luciferin sulfokinase. The reverse reaction, as experimentally demonstrated, may be written as follows:

$$\text{PAPS} + \text{luciferin} \xrightarrow{\text{luciferin sulfokinase; } Ca^{2+}} \text{luciferyl sulfate} + \text{DPA} \quad (3)$$

As mentioned above it is peculiar that we failed to demonstrate the synthesis of PAPS by the forward reaction. In fact if one examines the lability of luciferyl sulfate *vs.* PAPS in 0.1 N HCl one finds that the half-lives of each are about the same suggesting that the sulfate in luciferyl sulfate is of reasonably high group potential.

It is clear from previous data that sulfate must be removed from luciferyl sulfate prior to light emission. It was also found that the luciferin produced by enzymatic conversion or by treatment of luciferyl sulfate with acid is the same compound as judged by a variety of criteria (Cormier, 1962; Cormier and Hori, 1964; Cormier *et al.*, 1966). We know from data presented here that luciferin sulfokinase will convert luciferin into luciferyl sulfate *via* a PAPS-linked reaction. Further, the sulfate in the luciferyl sulfate so produced is acid-labile sulfate and thereby reacts just as does the naturally isolated compound.

Because DPA is required for the forward reaction it is tempting to write it as follows:

$$\text{luciferyl sulfate} + \text{DPA} \xrightarrow{\text{luciferin sulfokinase; } Ca^{2+}} \text{luciferin} + \text{PAPS} \quad (4)$$

The apparently low rate constant for the forward reaction makes it difficult to measure the PAPS produced (if any) although the very sensitive luminescence assay makes the detection of luciferin an easy task.

Thus it is questionable as to whether free PAPS is formed in reaction 4, *i.e.*, not bound to the enzyme. In this regard it is of interest that Gregory and Lipmann (1957) noted that phenol sulfokinase cannot be used to transfer sulfate from *p*-nitrophenylsulfate to steroids in the presence of steroid sulfokinase. This observation suggests that *p*-nitrophenyl sulfate cannot be used as a generating system for PAPS in the presence of DPA and phenol sulfokinase. This is consistent with our own data on luciferin sulfokinase in regard to the mechanism of the forward reaction.

These data, when coupled with previous observations outlined below, provide some insight into the regulation of luciferin levels in *Renilla* available for bioluminescence. During extraction of the crude material we have looked for the presence of both luciferin and luciferyl sulfate. We have never observed the presence of free luciferin but there is some luciferin bound to luciferase during the early stages of purification. Essentially all of the luciferin in *Renilla*, except that which is enzyme bound, exist as luciferyl sulfate. These observations suggest that free luciferin levels are very low in *Renilla*. The low rate of synthesis of luciferin by luciferin sulfokinase may be a significant factor, therefore, in the fatigue phenomenon which occurs on repeated stimulation of *Renilla* resulting in the propagation of a luminous wave (Nicol, 1955).

Acknowledgments

The authors wish to acknowledge the competent technical assistance of Mrs. S. Bleeker and Mrs. E. Billue during the course of these investigations.

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Anomalous Mutarotation of Glucose 6-Phosphate. An Example of Intramolecular Catalysis*

J. M. Bailey, P. H. Fishman, and P. G. Pentchev

ABSTRACT: Glucose 6-phosphate is metabolized by enzymes for which distinct anomeric specificities have recently been demonstrated. The mutarotation of glucose 6-phosphate was measured by utilizing the reduced triphosphopyridine nucleotide coupled rate of oxidation with glucose 6-phosphate dehydrogenase. This enzyme is specific for the β anomer. The mutarotation was 240 times faster than that of α-D-glucose measured polarimetrically under the same conditions. It was found that the higher rate was not caused by increased ring strain, since the activation energy for the mutarotation of glucose 6-phosphate (21.8 kcal/mole) was essentially the same as for D-glucose (22.2 kcal/mole). The mutarotation of glucose and a number of other sugars was shown to be accelerated by inorganic phosphate. The pH and concentration dependence of this reaction were determined. The theoretical mutarotation rate of glucose 6-phosphate, based upon a model of intramolecular catalysis, was calculated from these results to be 0.97 min^{-1}. This was in excellent agreement with the experimentally observed value of 1.1 min^{-1} at 10°. In addition, glucose 6-phosphate accelerated the mutarotation of free glucose in the expected manner. It is concluded that the explanation for the anomalous mutarotation of glucose 6-phosphate lies in an intramolecular catalysis of the mutarotation reaction at the anomeric OH by the phosphate group at C-6 of the molecule.

The compound glucose 6-phosphate (G-6-P) is of central importance in glucose metabolism. The anomeric specificities of the enzymes metabolizing glucose and glucose 6-phosphate have recently been defined [Salas *et al.* (1965); Bailey *et al.* (1968)]. Enzymes involved in the phosphorylation of glucose (hexokinase, glucokinase, and pyrophosphate phosphotransferase) display no preference for either the α or β anomer of D-glucose. The anomeric configuration of the G-6-P produced is thus the same as that of the glucose used as substrate. Similarly, it has been shown that the enzymes dephosphorylating glucose 6-phosphate produce free glucose which has the same anomeric composition as the substrate [Bailey *et al.* (1968)]. It has been shown by Salas *et al.* (1965), however, that the enzymes involved in the further metabolism of G-6-P do display marked preferences for the particular anomer used as substrate. Thus the enzyme glucose 6-phosphate dehydrogenase which channels glucose metabolism into the pentose phosphate pathway is completely specific for the β anomer. As described below, we have used this fact to measure the anomeric composition and mutarotation rate of glucose 6-phosphate under different experimental conditions.

The main glycolytic pathway for glucose metabolism is initiated *via* the α anomer of G-6-P by the enzyme phosphoglucose isomerase. This enzyme is thought to act on the open-chain form of glucose 6-phosphate as an intermediate (Salas *et al.* (1965)]. This was shown to be derived from the α anomer under the catalytic action of phoshoglucose isomerase functioning as a "mutarotase." The enzyme mutarotase itself, which occurs in many tissues, particularly kidney, liver, and intestine, is specific for the free sugar and has no action on either anomer of glucose 6-phosphate [Bailey *et al.* (1968)].

The enzyme phosphoglucomutase is also specific for the α

* From the Biochemistry Department of George Washington University School of Medicine, Washington, D. C. 20005. *Received October 28, 1969.* This paper is number VII in a series. Part VI in the series is Studies on Mutarotases. Enzyme Levels and Sugar Reabsorption in Developing Rat Kidney and Intestine: Bailey *et al.* (1970). This investigation was supported by U. S. Public Health Service Grants AM10082 and 5K3 16730.