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SHORT COMMUNICATION

PHOTOREGULATION OF β-D-GLUCOSE OXIDASE BY BLUE LIGHT

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Abstract—Glucose oxidase, a FAD-enzyme is not inhibited by blue light in contrast to some FMN-enzymes. If the natural coenzyme is substituted by FMN, the enzymatic activity is low. Blue light, however, restores the enzymatic activity of this combination to almost the activity of the native enzyme. The experiments indicate that FMN plus blue light or the excited FMN molecule can substitute for the original coenzyme FAD of glucose oxidase. It is possible to photoregulate the FMN-combination by substances such as KI or $NiSO_4$ that quench the triplet state of excited flavin molecules.

INTRODUCTION

ZELITCH developed the hypothesis that glycolate and its oxidation occupy a key position in photorespiration of leaves.[1] In our earlier papers we were able to demonstrate that glycolate oxidase, a FMN enzyme from tobacco is inhibited by blue light.[2,3] We have shown that some other FMN enzymes behave similarly.[4] Moreover we have been able to show that the blue light enhanced respiration in a colorless chlorella mutant was due to a FMN-enzyme mediated glycine oxidation.[3] We therefore wondered whether FAD-enzymes are equally influenced by blue light. In the present paper we investigated the effect of blue light on glucose oxidase. In context with our earlier work, glucose oxidase is of interest because glycolate may be produced via the pentosephosphate cycle.[1,5]

RESULTS AND DISCUSSION

Glucose oxidase catalyses the reaction between β-D-glucose and oxygen to yield hydrogen peroxide and gluconolactone. The intact enzyme shows, after 35 min of irradiation with 2100 erg sec^{-1} cm^{-2} of blue light, no change in enzyme activity in comparison to the dark control. This result does not change in the presence of exogenous FMN. Fonda and Anderson describe for D-amino acid oxidase, which is a FAD-enzyme like glucose oxidase, that addition of FMN or riboflavin inactivated the amino acid oxidase in the light.[6] This observation was confirmed by our laboratory.[4] However, the activity of the glucose oxidase preparation was not affected after 35 min of irradiation with blue light in

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TABLE 1. THE EFFECT OF BLUE LIGHT ON THE ACTIVITY OF β-D-GLUCOSE OXIDASE

Enzyme	Blue light intensity 380 nm < λ < 580 nm (erg × sec^{-1} × cm^{-2})	Specific enzyme activity (μ moles O_2 uptake/mg protein × h)
Native	Dark	251
Native + FMN	Dark	290
Native	2100	255
Native + FMN	2100	243
Dialysed + FMN	Dark	162
Dialysed + FAD	Dark	260
Dialysed + FMN	2100	248

Temperature 30°. When indicated the assay contained 0·1 μmoles FMN or FAD per 50 μg of enzyme protein. The reaction rate was determined 5 min after substrate addition.

the presence of FMN (Table 1). Dialysis of the glucose oxidase preparation yields a decreased specific enzyme activity which is due to the well known loss of coenzyme in the course of dialysis (Table 1). Addition of FMN to this FAD deficient preparation leads to an enzyme condition in which the oxidase becomes sensitive to blue light. Table 1 shows that the effect of blue light on the dialysed glucose oxidase consists of an enhancement of this decreased specific enzyme activity to the original level of the undialysed preparation. A low saturation level of O_2-uptake per unit enzyme protein in the dark assay means that more inactive protein is present than in the irradiated assay. Therefore *FMN plus blue light appear to substitute for the original coenzyme FAD.* For the first time our experiments indicate that an apoenzyme which with the 'unnatural' coenzyme (FMN) yields no appreciable activity, is activated by blue light to almost its full activity. According to the literature this activation might be caused by a conformational change of the apoenzyme induced by the excited FMN.[7–10]

EXPERIMENTAL

Enzyme

Glucose oxidase and FMN were purchased from Boehringer, Mannheim GmbH: (The enzyme used was salt-free, lyophilized, grade 1). The enzyme activity was assayed manometrically in a Warburg apparatus at 30° either in acetate buffer of pH 5·6 or in phosphate buffer of pH 8·6.

Assay for Enzyme Activity

The Warburg vessel contained in the main compartment: 1·2 ml 0·1 M acetate buffer pH 5·6; 0·1 ml (50 μg) glucose oxidase; catalase containing 200 μg protein/ml, 0·1 ml 98% ethanol. If not specified otherwise in Table 1 the mixture contained 0·1 ml 1 mM FMN and water to give a final volume of 2 ml. The center well contained 0·1 ml 30% KOH. The side arm of the vessel contained 0·3 ml 0·2% glucose. In order to remove part of the FAD from the enzyme, 5 ml of glucose oxidase (2·5 mg) were dialysed for 2 hr in 6–7 ml of water against either 0·01 M Na-acetate pH 5·6 or 0·01 M K_2HPO_4 pH 8 at 0–5°; the dialysed suspension was then made up to 10 ml. The reaction was usually measured against the complete, illuminated assay mixture without enzyme added. The illuminated assay mixture containing FMN and boiled enzyme was used as reference for all values shown in Table 1 in order to allow for nonenzymatic photoreactions. Light sources and filters were used as described in our earlier publications.[2,3]

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