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BRIEF PAPERS

THE PHOTOINACTIVATION OF ENZYMES BY RIBOFLAVIN

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Recently GALSTON and BAKER (1) reported that riboflavin could cause the photoinactivation of certain enzymes (urease, tyrosinase, α amylase) by visible light and discussed the significance of this mechanism in interpreting various responses of green plants to light (phototropism, etc.). The present study was undertaken to determine the possible relationship of this type of enzyme inactivation to the physiology of the cellulolytic fungus, *Myrothecium verrucaria*, USDA 1334.2.

Experimental

The susceptibility of invertase to photoinactivation in the presence of riboflavin was demonstrated by exposing a dilute solution of Difco invertase (1:4000) in 0.05 M KH_2PO_4 containing 50 p.p.m. of riboflavin, to the light from a north window. After a two-hour exposure at room temperature an equal volume of 10% sucrose, was added to the exposed solutions as well as to controls maintained in the dark. All of the tubes were then placed in the dark. To determine invertase activity reducing sugars were measured at intervals by the dinitrosalicylic acid method of SUMNER (2). The data (table I) parallel those reported by Galston and Baker.

TABLE I
PHOTOINACTIVATION OF DIFCO INVERTASE BY RIBOFLAVIN

ENZYME	TREATMENT		INVERTASE ACTIVITY*	RELATIVE ACTIVITY
	RIBOFLAVIN	LIGHT		
+	+	+	0.1	5
+	-	+	1.6	80
+	+	-	2.0	100
+	-	-	2.0	100
-	+	+	0	0
-	+	-	0	0

* Mg. red. sugar/ml./hr. as glucose.

A similar experiment was carried out in which a suspension of spores was substituted for the Difco invertase in the above experiment. The results (table II) show no effect of light or riboflavin on the invertase of *M. verrucaria* spores. While the concentration of spores was not determined, the suspension used was not sufficiently dense to cut out a large proportion of the incident light.

Another experiment, in which the light exposure was increased to 16 hours of artificial illumination, also failed to show inactivation. The failure to get photoinactivation of invertase extracted from *M. verrucaria*

TABLE II

EFFECT OF RIBOFLAVIN ON INVERTASE ACTIVITY OF *Myrothecium verrucaria* SPORES IN LIGHT AND DARK

TREATMENT		INVERTASE ACTIVITY*	RELATIVE ACTIVITY
RIBOFLAVIN	LIGHT		
+	+	0.26	104
-	+	0.25	100
+	-	0.25	100
-	-	0.25	100

* Mg. red. sugar/ml./hr.

spores is shown in table III. In this experiment lyophilized spores moistened with water were ground with powdered pyrex glass in a mechanical mortar for about two hours, suspended in buffer and centrifuged at high speed and the supernatant decanted through a bacterial filter. While the activity of this preparation was low similar data have been obtained with more active extracts.

TABLE III

EFFECT OF RIBOFLAVIN ON INVERTASE ACTIVITY OF EXTRACTS OF *Myrothecium verrucaria* SPORES

TREATMENT		INVERTASE ACTIVITY*	RELATIVE ACTIVITY
RIBOFLAVIN	LIGHT		
+	+	0.08	94
-	+	0.08	94
+	-	0.085	100
-	-	0.085	100

* Mg. red. sugar/ml./hr.

It was considered possible that some substances in the spores and spore extracts was protecting the invertase from being inactivated by light. If this were so then addition of spore extract to a solution of Difco invertase might protect it from inactivation. In table IV results are given of an experiment to test this hypothesis in which a portion of the same spore extract as used in the preceding experiment (table III) was added. A boiled portion of this extract was added to other tubes. While the data give no indication of any protection this may have been due to a concentration effect—the enzyme activity of the pure invertase being some 30 times that of the spore extract. It is also possible that the two enzymes are different chemically or that the protective substance is coupled with the *M. verrucaria* invertase.

The influence of riboflavin on germination of the spores was determined by inoculating Petri dishes containing agar, salts, 1% glucose and 1% yeast extract at pH 6.5; riboflavin was added at a concentration of 100 p.p.m. to half of the plates which were then placed either in the dark or in

TABLE IV

EFFECT OF SPORE EXTRACT ON PHOTOINACTIVATION OF DIFCO INVERTASE BY RIBOFLAVIN

TREATMENT			INVERTASE ACTIVITY*	RELATIVE ACTIVITY
SPORE EXTRACT	RIBOFLAVIN	LIGHT		
-	+	+	0.07	5
-	-	+	1.3	87
-	+	-	1.3	87
-	-	-	1.5	100
+	+	+	0.09	6
+	-	+	1.5	100
+	+	-	1.5	100
+	-	-	1.5	100
boiled	+	+	0.06	4
boiled	-	+	1.5	100
boiled	+	-	1.5	100
boiled	-	-	1.5	100

* Mg. red. sugar/ml./hr.

the light of a north window. The riboflavin had no significant effect on germination either in the light or in the dark, approximately 100% germination being observed in all cases after three hours.

To determine whether riboflavin could affect the rate of degradation of cellulose by *M. verrucaria* in the light strips of 8.25 oz. bleached duck (in sextuplicate) were placed on a layer of glass beads in Petri dishes containing inorganic nutrient solution (pH 6.5) to some of which riboflavin was added to give a concentration of 100 p.p.m. The strips were inoculated with *M. verrucaria* spores. One series was placed in the dark in an incubator at 30° C and 80% relative humidity. A second series was exposed to daylight from a north window at room temperature. After seven days the strips were harvested, conditioned, and their breaking strength determined on a motor driven Scott tester. Results (table V) indicate no sig-

TABLE V

EFFECT OF RIBOFLAVIN ON THE BREAKDOWN OF COTTON DUCK IN THE LIGHT AND IN THE DARK BY *M. verrucaria*

TREATMENT			BREAKING STRENGTH	RELATIVE* BREAKDOWN
INOCULATED	RIBOFLAVIN	LIGHT		
-	-	-	94 lbs.	
+	+	+	61	89
+	-	+	57	100
+	+	-	32	91
+	-	-	26	100

* As compared to corresponding treatment with riboflavin.

nificant photoinactivation due to riboflavin. The less rapid breakdown in the light is probably due to a temperature effect and to a deficiency of water due to more rapid evaporation from the cultures in the light rather than to a specific effect of light.

Discussion

The data presented here indicate that the enzymes of the fungus *M. verrucaria* are not susceptible to photoinactivation by visible light either in the presence or absence of riboflavin. The implication is that either some protective mechanism is present in this organism or that the specific groups present in certain enzymes which make them susceptible to inactivation by this mechanism are lacking. On the other hand it is possible that the enzyme preparations used by Galston and Baker (alpha-amylase, urease, tyrosinase) contained some photosensitizing substance since the purity of the preparations used was not stated. The data do not permit conclusive evaluation of these hypotheses but they do indicate that the protective mechanism is lacking, since addition of a spore extract to the yeast invertase did not prevent photoinactivation of the latter. It is possible, however, that the concentration of this hypothetical protective mechanism was too low. The difference in behavior of the invertase from yeast and *M. verrucaria* may indicate a more fundamental difference between the two enzymes. Supporting evidence for the latter hypothesis is implied by the slightly different pH optima and the differences in Michaelis' constants (3).

Summary

It was found that while riboflavin caused rapid photoinactivation of a purified invertase preparation from yeast (Difco analytical) no effect was found on the invertase activity of *M. verrucaria* spores, nor upon the invertase activity of extracts of ground spores. Riboflavin in the light had no inhibitory effect on the germination of the spores and no effect upon the breakdown of cellulose by this organism.

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