EFFECT OF o-PHENANTHROLINE ON THE INDUCTION OF GLUCOSE DEHYDROGENASE IN ASPERGILLUS ORYZAE

Hyogo Sinohara

Department of Biochemistry, Iwate Medical College
School of Dentistry, Morioka, Japan

Received August 26, 1968

A soluble glucose dehydrogenase, which catalyzes the oxidation of glucose to glucono-δ-lactone by certain dyes and quinones, has been shown to be present in Aspergillus oryzae (Ogura, 1939). This enzyme differs from glucose oxidase (EC 1.1.3.4) or NAD(P)-linked glucose dehydrogenase (EC 1.1.1.47), since neither molecular oxygen nor pyridine nucleotides nor flavins were able to act as electron acceptors to any significant extent (Bak, 1967). Recently Bak and Sato (1967) have shown that this enzyme is specifically induced by hydroquinone or p-benzoquinone, and that the inductive synthesis is further stimulated by the simultaneous presence of some chelating agents such as o-phenanthroline or 8-hydroxyquinoline. However, they determined the enzyme activity only at one time period, i.e., 18 hours after the addition of inducer, and hence the kinetics of enzyme induction or the mechanism by which chelating agents stimulate the induction remains unclear. The present experiments have been initiated to clarify these problems. The results reported in this paper indicate that both glucose and hydroquinone are necessary for the induction of glucose dehydrogenase; and that o-phenanthroline has no direct effect on the rate of enzyme formation, but it modifies a factor(s) which determines the limit of enzyme formation, thus resulting in a twofold increase of the intracellular level of enzyme.

Aspergillus oryzae strain U, obtained from Kojiya-Sanzaemon Co., Kyoto, was grown in liquid medium containing the following components in 1000 ml of water: glucose, 20 g; NH_4NO_3, 3 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; K_2HPO_4, 1.7 g; $NaH_2PO_4 \cdot 2H_2O$, 1.6 g; peptone, 1 g. The flask was stirred magnetically at 30° for 18 hours. The mycelium was harvested, washed repeatedly with deionized water, and the excess water squeezed out. Then the mycelium (5 g, wet weight) was suspended in 500 ml of induction medium, and stirred at 30°. Aliquots were taken at varying time periods, and glucose dehydrogenase was estimated by the method of Bak and Sato (1967). One unit of enzyme activity was defined as the μmoles of dichlorophenolindophenol reduced per minute at 22-25°. Specific activity was defined as the units of enzyme per mg of protein.

As shown in Fig. 1, glucose was necessary, in addition to hydroquinone, for the induction of glucose dehydrogenase. No enzyme was induced by either glucose alone or hydroquinone alone; nor was it induced when a mycelium was incubated with glucose for 30 minutes, removed by filtration, and then reincubated with a new medium containing hydroquinone alone. The same was true under the other condition, i.e., incubation with hydroquinone followed by reincubation with glucose. It was expected that a compound derived from both glucose and hydroquinone is a real inducer. However, hydroquinone-β-glucoside, which is relatively widely distributed in nature (Bauman and Pigman, 1957), was found to be inactive as inducer. These results are inconsistent with the previous observation that the enzyme was induced by hydroquinone alone (Bak and Sato, 1967). The reason for this discrepancy is not clear at present, but it is possible that their mycelium contained enough amounts of endogeneous glucose.

Bak (1967) has shown that xylose, fructose, and mannose are oxidized by this enzyme at much slower rate than glucose. Hence, the possibility was studied that these sugars may act as inducers, and the

Table I. Relative activities of various sugars as inducer and as substrate for glucose dehydrogenase.

Sugars	Relative activities		Sugars	Relative activities	
	Inducer	Substrate*		Inducer	Substrate*
Glucose	100	100	Arabinose	100	0
Mannose	100	4	Xylose	100	12
Galactose	95	0	Sucrose	100	0
Fructose	100	5	Lactose	50	0
Glucosamine	3	29	Glycerol	36	0
N-Acetyl-glucosamine	40	0	Gluconolactone	66	0

* These results are in general agreement with the previous report (Bak, 1967) and extended to other sugars.

results are shown in Table I. No correlation has been found between the activity as inducer and that as substrate. Although the possibility that these sugars act as direct inducers can not be ruled out, it seems likely that they are converted to glucose prior to acting as inducer. If this system really requires two unrelated compounds as inducers, it may be a unique phenomenon in the enzyme induction in which a single substance usually acts as inducer.

Figure 1 shows that after addition of inducers, a period of about 15 minutes elapsed before a measurable amount of enzyme was present in the mycelium. During the next 60 minutes glucose dehydrogenase activity was increased and then gradually levelled off (curve C). When o-phenanthroline was added to this mycelial suspension, the increase in enzyme activity was resumed after a lag of about 40 minutes (curve B). This resumption process as well as the initial increase was dependent on concomitant protein synthesis, since no increase occurred in the presence of cycloheximide (5 μg/ml). To determine what compounds are necessary for the resumption of enzyme synthesis, a mycelium was remov-

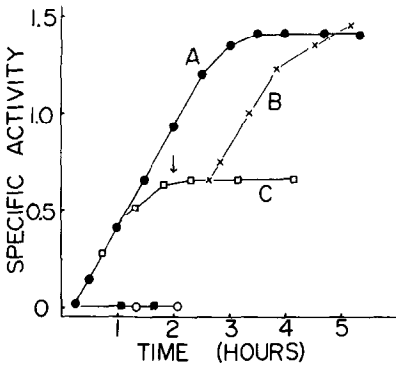

Fig. 1. Induction of glucose dehydrogenase in Asp. oryzae.
Mycelia were suspended in 0.01 M phosphate buffer, pH 7.0, and samples were withdrawn at varying time periods for the measurement of glucose dehydrogenase. (○) glucose; (■) hydroquinone; (□) glucose + hydroquinone; (●) glucose + hydroquinone + o-phenanthroline; (×) glucose + hydroquinone were added at the start of experiment and 2 hours later (arrow) o-phenanthroline was added. The final concentration of glucose, hydroquinone, and o-phenanthroline was 50 mM, 10 mM, and 0.1 mM, respectively.

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ed after the cessation of enzyme synthesis, and divided into several portions each of which was resuspended in fresh medium containing various combinations of glucose, hydroquinone, and o-phenanthroline. The formation of glucose dehydrogenase was resumed only in the simultaneous presence of all the three compounds, but not in any combinations of two compounds. When o-phenanthroline was added at the same time as glucose and hydroquinone, the enzyme was formed at a constant rate for 3 hours without interruption (curve A). Essentially the same results were obtained with another strain of Asp. oryzae, i.e. NRRL 695, and Aspergillus parasiticus QM 884, although the final enzyme levels were approximately 1/20 and 1/5, respectively, of Asp. oryzae U. These results clearly indicate that o-phenanthroline had no direct effect on the rate of enzyme synthesis, but that it modified factors which determine the limit of enzyme formation.

Since mere addition of o-phenanthroline caused the resumption of enzyme synthesis, the possibility seems to be eliminated that the enzyme

formation ceased because of the exhaustion of glucose, hydroquinone, or amino acids, while o-phenanthroline inhibited the catabolism of these compounds, thus making them available for longer period. This was further substantiated by the fact that no change in the induction kinetics was observed when additional amino acids or inducers were added to the induction medium after the cessation of enzyme formation.

Bak and Sato (1967) have shown that glucose dehydrogenase was excreted into the medium. It is possible that in the absence of o-phenanthroline an equilibrium reached between the synthesis and excretion of enzyme, while o-phenanthroline inhibited the excretion, thus resulting in the intracellular accumulation of enzyme. Under these conditions, however, less than 3 % of the total enzyme formed was excreted into the medium whether the presence or absence of o-phenanthroline.

Enzyme synthesis might be stopped by an intracellular accumulation of catabolites (Magasanik, 1961). As shown in Table I, however, gluconolactone had no inhibitory effect, but it rather stimulated the enzyme formation.

These results would suggest that the level of glucose dehydrogenase is controlled by factors other than variations in concentration of inducers, amino acids, or catabolites. Although a number of hypotheses have been proposed to account for various aspects of enzyme induction (Jacob and Monod, 1961; Ralph and Baguley, 1966; Cline and Bock, 1966; Vogel and Vogel, 1967), relatively little attention has been given to the mechanisms controlling intracellular levels of enzymes. In bacterial systems a few models have been suggested: e.g., enzyme level may be controlled by a specific gene called promoter (Scaife and Beckwith, 1966) or a "firmator" which stabilizes messenger ribonucleic acid (Csanyi et al., 1967). It is not clear, however, whether these models can be extended to eucaryotes which are known to differ from procaryotes in mechanisms of protein synthesis and its regulation

(Ris and Chandler, 1963).

At present little is known about the regulation of glucose dehydrogenase synthesis in Asp. oryzae, and a number of mechanisms can be proposed to explain the effect of o-phenanthroline. The present data, however, suggest that whatever the detailed mechanisms, o-phenanthroline may bind ions which interact with regulatory substance, resulting in its altered affinity for gene or protein-synthesizing machinery in such a way that more enzyme is produced. Experiments are in progress to elucidate these mechanisms.

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