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STUDIES ON GLUCOSE DEHYDROGENASE OF *ASPERGILLUS ORYZAE*

III. GENERAL ENZYMATIC PROPERTIES

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SUMMARY

Glucose dehydrogenase, highly purified, from *Aspergillus oryzae* catalyzed the oxidation of D-glucose, 2-deoxy-D-glucose, D-xylose, D-fructose and D-mannose by certain redox dyes such as 2,6-dichlorophenolindophenol (DCIP) and quinones such as β-naphthoquinone. Molecular oxygen could also be utilized as acceptor, though at an extremely slow rate. The oxidation product of D-glucose was identified as D-glucono-δ-lactone. The oxidation of glucose by DCIP was maximal at pH 6.5 and 45°. The apparent K_m values for D-glucose and DCIP were 0.025 M and 0.1 mM, respectively. Kinetic studies ruled out the involvement of a ternary complex in the enzyme catalysis.

Spectrophotometric titration of the enzyme with glucose did not give any indication of the formation of flavin semiquinone during the catalysis. The dehydrogenation of glucose by the enzyme was shown to be irreversible, since glucono-δ-lactone failed to reoxidize the reduced flavin of the enzyme.

INTRODUCTION

In a previous paper[1] we reported that the synthesis of glucose dehydrogenase in *Aspergillus oryzae* can be induced specifically by hydroquinone or *p*-benzoquinone and that this inductive synthesis is further stimulated by the simultaneous presence of EDTA. The discovery of this induction phenomenon has enabled us to isolate and purify this glucose dehydrogenase to give a homogeneous product[2]. We have also shown, contrary to the previous view[3–5], that the purified enzyme is a glycoflavoprotein containing 1 mole of FAD per mole of enzyme[2].

The general enzymatic properties, as well as the kinetic behaviour of *A. oryzae* glucose dehydrogenase, have been studied by OGURA[6,7] and by KURASAWA AND IGAUE[8]. They have demonstrated that this enzyme catalyzes the oxidation of D-glucose rapidly, and some other sugars at slower rates, in the presence of suitable

Abbreviation: DCIP, 2,6-dichlorophenolindophenol.

hydrogen acceptors such as DCIP, thionine and quinones, but not by molecular oxygen. They have also shown that the glucose oxidation catalyzed by this enzyme is practically irreversible and proceeds as follows:

D-Glucose + acceptor → D-gluconate + reduced acceptor

The enzyme preparation employed by these workers was, however, very crude; in the light of our present knowledge their preparation seems to have had a purity of only a few percent. It seemed desirable, therefore, to reinvestigate these results, using the homogeneous preparation of glucose dehydrogenase that was available to us. The role of flavin in the enzymatic catalysis had not been studied by the previous workers who did not recognize the flavoprotein nature of this enzyme.

This paper describes the results of a study with the pure enzyme on the general enzymatic properties of *A. oryzae* glucose dehydrogenase. The data obtained not only confirm most of the previous findings but also adds new information to the nature of the catalysis by this enzyme.

MATERIALS AND METHODS

Enzyme preparations and chemicals

The homogeneous preparation of glucose dehydrogenase purified from mycelia of *A. oryzae* grown in the presence of hydroquinone and EDTA[2] was used in most experiments. The partialy purified enzyme, from culture filtrate[2], was also used in a few experiments. A highly purfied preparation of glucose oxidase (specific activity, 1000) isolated from *Penicillium amagasakiense*[5] was a generous gift from Dr. I. SEKUZU. Vitamin K_2, coenzyme Q_6 and Q_7 were generous gifts from Dr. E. ITAGAKI. Coenzyme Q_0 and 2-deoxy-D-glucose were kindly supplied from Dr. K. TANAKA of the Takeda Pharmaceutical Industries, Ltd., Osaka, and Dr. T. IKENAKA, respectively. All the other chemicals were obtained from commercial sources.

Measurements of enzyme activities

Unless otherwise stated, the activity of glucose dehydrogenase was measured at pH 6.5 as described previously[2], using 0.2 M D-glucose as hydrogen donor and 0.1 mM DCIP as acceptor. One unit of the enzyme was defined as described previously[2]. The activity measured under these conditions was proportional to the enzyme concentration at least up to 1 μg of enzyme per vessel. The rate of DCIP reduction was not altered when the reaction was carried out under anaerobic conditions. For the study of the specificity for hydrogen donors, D-glucose in the reaction mixture was replaced by various donors (0.2 M). The activities of the enzyme with thionine (0.1 mM), ferricyanide (1 mM), pyridine nucleotide (0.4 mM), flavins (0.1 mM), and cytochrome *c* (50 μM) as acceptors were measured by following the changes in absorbance at 600, 420, 340, 460 and 550 mμ, respectively. The reaction mixture used was the same as was used for the glucose–DCIP assay, except that DCIP was replaced by the respective acceptors. The molar extinction coefficients used for thionine and ferricyanide were $14.1 \cdot 10^6$ cm$^2 \cdot$mole^{-1} and $1.02 \cdot 10^6$ cm$^2 \cdot$mole^{-1} (ref. 9), respectively. Since reduced phenazine methosulfate is rapidly autoxidizable, the reduction of this dye by glucose was followed by measuring oxygen uptake in a conventional Warburg manometer; the composition of the reaction mixture was the same as that for DCIP

reduction, except that DCIP was replaced by 0.02% phenazine methosulfate and the volume was scaled up to 2.0 ml. The reduction of quinones by glucose was measured indirectly by following the reduction of cytochrome *c* by the quinols formed, because as will be reported below, cytochrome *c* was not reduced directly by glucose *plus A. oryzae* glucose dehydrogenase, but could be reduced quantitatively by many quinols. The reaction mixture (1.5 ml) contained 0.1 M potassium phosphate buffer (pH 6.5), 0.2 M D-glucose, 0.1 mM quinone, 50 μM cytochrome *c* and glucose dehydrogenase, and the reaction was followed spectrophotometrically at 550 mμ, using molar extinction coefficients of $27.7 \cdot 10^6$ and $9.0 \cdot 10^6$ $cm^2 \cdot mole^{-1}$ for reduced and oxidized cytochrome *c*, respectively[10]. The other methods employed are given in the legends for Figs. 2, 3 and 10.

Analytical procedures

Protein was determined by the method of LOWRY *et al.*[11], using bovine serum albumin as standard. Glucose and gluconolactone were determined by the anthrone method[12] and by the hydroxamate method[13], respectively. The amount of DCIP reduced was estimated from the decrease in absorbance at 600 mμ, using the molar extinction coefficient given in a previous communication[2]. For identification of the lactone formed from D-glucose, the reaction mixture (15 ml) was passed through a column of Dowex 50, H^+ form (0.8 cm $\times$ 7 cm) and the substances not retained by the column were concentrated *in vacuo*. The concentrated material was subjected to ascending papar chromagraphy, using butanol–methanol–water (4:1:1, by vol.) as solvent. The lactone spot was detected by the alkaline hydroxylamine reagent[14] consisting of 1 M methanolic $NH_2OH \cdot HCl$ and 1.1 M methanolic KOH.

Anaerobic titration of enzyme with glucose

1.5 ml of a solution containing 10.6 mμmoles of purified glucose dehydrogenase in 0.05 M potassium phosphate buffer (pH 6.5) was placed in a spectrophotometer cuvette (optical path, 1.0 cm) equipped with a microsyringe and a stopper having a cocked inlet. The gas phase was replaced by oxygen-free nitrogen by at least four cycles of evacuation and flushing. After recording the absorption spectrum of the oxidized enzyme in a Cary 14 spectrophotometer, 0.03 ml portions of 1 M glucose were added stepwise to the cuvette by means of the microsyringe. After each addition, the spectrum was recorded.

RESULTS

Hydrogen donor specificity

The hydrogen donor specificity of *A. oryzae* glucose dehydrogenase was studied with DCIP as acceptor. As shown in Table I, D-glucose was the best donor for the enzyme preparations purified from both induced mycelia and culture filtrates, though 2-deoxy-D-glucose, D-xylose, D-fructose and D-mannose were also oxidized at slower rates. The other sugars and related compounds tested, such as D-galactose, D-ribose, D- and L-arabinose, D-fucose, D-gluconic acid, D-glucuronolactone, maltose, sucrose, rhamnose, raffinose, melibiose, and glucose-6 phosphate did not react to measurable extents. The oxidation of NADH and NADPH by DCIP was not detected in the purified enzyme preparation. It has been reported that the β-form of D-glucose is

TABLE I

HYDROGEN DONOR SPECIFICITY OF PURIFIED GLUCOSE DEHYDROGENASE

The activity was measured with DCIP as acceptor as described in the text. The final concentration of hydrogen donor was 0.2 M.

Hydrogen donor	*Relative activity*	
	Enzyme from mycelia	*Enzyme from culture filtrate*
D-Glucose	100	100
β-D-Glucose	100	—
2-Deoxy-D-glucose	30	—
D-Xylose	13	14
D-Fructose	8	7
D-Mannose	7	5

the true substrate of liver glucose dehydrogenase[15] and Penicillium glucose oxidase[16]. The specificity for the β-form has also been observed with galactose dehydrogenase of *Pseudomonas saccharophila*[17]. Table I shows, however, that β-D-glucose, prepared immediately before use, was oxidized at the same rate as α-D-glucose by *A. oryzae* glucose dehydrogenase, indicating that this enzyme is not specific for the β-form as in the case of glucose dehydrogenase from *Bacterium anitratum*[18]. Although liver glucose dehydrogenase has been shown to be inhibited by adenosine, ATP, and glucose-6-phosphate[19], glucose oxidation by the *A. oryzae* enzyme was affected neither by these compounds nor by cyclic 3',5'-AMP, ADP, D-galactose, D- and L-arabinose, D-ribose, D-fucose, sorbitol, and rhamnose. Oxidizable sugars, however, such as D-fructose, D-xylose and D-mannose seemed to inhibit the oxidation of D-glucose, since a mixture of D-glucose and either one of the three sugars reduced DCIP more slowly than D-glucose alone.

Hydrogen acceptor specificity

When 0.2 M D-glucose was used as hydrogen donor, the purified enzyme catalyzed the reduction of redox dyes and quinones, in confirmation of previous findings with crude enzyme preparations[3]. Table II shows the relative rates of reduction of various acceptors. As can be seen, DCIP was the best acceptor under

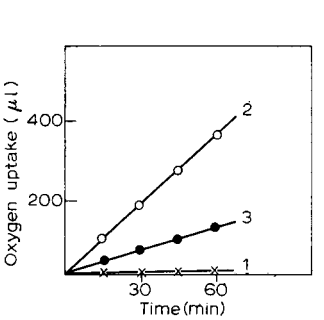
TABLE II

HYDROGEN ACCEPTOR SPECIFICITY OF PURIFIED GLUCOSE DEHYDROGENASE

Hydrogen acceptor	*Concn. (mM)*	*Relative activity*
DCIP	0.1	100
β-Naphthoquinone	0.1	90
Vitamin K_3	0.1	6
Phenazine methosulfate	0.02%	3.5
Coenzyme Q_0	0.1	1.3
α-Naphthoquinone	0.1	1.3
Ferricyanide	0.1	1.2
Thionine	0.1	2
Benzoquinone	0.1	0.9

these conditions and the efficiency of β-naphthoquinone was about 90% that of DCIP. The other acceptors listed reacted at much slower rates. The compounds which failed to act as acceptors included NAD^+, $NADP^+$, FAD, FMN, riboflavin, cytochrome *c*, toluidine blue, methylene blue, toluylene blue, Nile blue, Janus green, triphenyltetrazolium chloride, vitamins K_1 and K_2, and coenzymes Q_2, Q_6 and Q_7. The reduction of DCIP by glucose was not stimulated by the addition of NAD^+, $NADP^+$, FAD, FMN or riboflavin.

One of the major characteristics of *A. oryzae* glucose dehydrogenase, distinguishing it from glucose oxidase of other fungi, has been the inability of the *A. oryzae* enzyme to react directly with molecular oxygen[7]. Using the purified enzyme, this property was apparently confirmed. As shown in Fig. 1, practically no oxygen uptake was observed monometrically when the enzyme was shaken with glucose under aerobic conditions. The addition of an autoxidizable acceptor such as phenazine methosulfate, however, induced an active oxygen uptake. Glucose oxidase from *P. amagasakiense*[5] could of course consume oxygen with glucose as substrate.



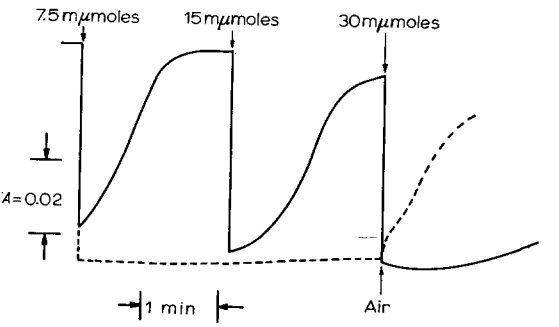


Fig. 1. Oxygen uptake by glucose dehydrogenase and glucose oxidase with glucose as hydrogen donor. The oxygen uptake was measured at 30° in a Warburg manometer using air as the gas phase. The reaction mixture (final volume, 2.0 ml) contained 0.14 M potassium phosphate buffer (pH 6.5), 0.1 M D-glucose, and other additions indicated below. The center well received 0.2 ml of 20% KOH. Curve 1, 24 μg of purified glucose dehydrogenase. Curve 2, 24 μg of purified glucose dehydrogenase and 0.05% phenazine methosulfate. Curve 3, 50 μg of Penicillium glucose oxidase.

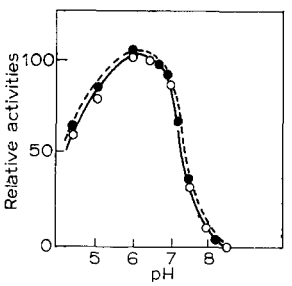
Fig. 2. Slow autoxidation of FAD bound to purified glucose dehydrogenase. 10 mμmoles of the purified enzyme was dissolved in 1.5 ml of 50 mM phosphate buffer (pH 6.5) and placed in a sample cuvette (optical path, 1.0 cm) of a Cary 14 spectrophotometer. The wavelength was set at 460 mμ. As indicated by arrows, glucose was added to the cuvette under aerobic (———) and anaerobic (- - - -) conditions and the change in absorbance was recorded.

Despite these findings apparently excluding oxygen as an effective acceptor, spectrophotometric evidence was obtained to indicate that the FAD of the enzyme was very slowly autoxidizable. When the oxidized form of glucose dehydrogenase was mixed with small amounts of glucose under aerobic conditions, the FAD of the enzyme was reduced rapidly as evidenced by a rapid decrease in absorbance at 460 mμ. As shown in Fig. 2, however, the absorbance thus decreased was found to increase again at a slow rate. The onset of such reoxidation of the flavin was increasingly delayed as the amount of glucose added was increased. Under anaerobic conditions no reoxidation occurred. It was therefore concluded that the reduced FAD of glucose dehydrogenase could react directly with molecular oxygen at a very

slow rate. The turnover number of this reoxidation was determined to be about 1 min^{-1}, ruling out any physiological significance for this process.

Effects of pH and temperature

Fig. 3 shows the effects of pH on the rates of oxidation of D-glucose and D-xylose by DCIP in the presence of glucose dehydrogenase. As may be seen the pH–activity curve was identical for both substrates showing a pH optimum of about 6.5. Although shifts in the pH optimum have been reported for *B. anitratum* glucose dehydrogenase on changing the ratio of concentration of glucose to DCIP[20], no such shifts were observed for the *A. oryzae* enzyme. The oxidation of glucose by DCIP catalyzed by the *A. oryzae* enzyme was stimulated by 70% when the concentration of phosphate buffer (pH 6.5) was increased from 0.002 M to 0.4 M. Glucose oxidation by this enzyme was dependent on temperature, the velocity increasing with temperature up to 45°. Above this temperature, the activity decreased abruptly. From an Arrhenius plot, an activation energy of about 5500 calories was obtained for the catalyzed reaction. This value was smaller than those reported for ordinary enzymes[21].



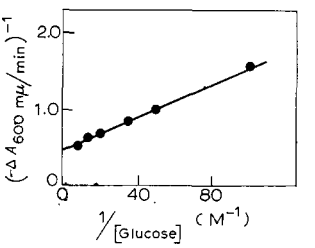


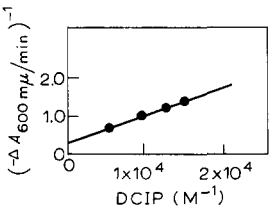
Fig. 3. Effects of pH on the oxidation of D-glucose and D-xylose by purified glucose dehydrogenase. 0.1 mM DCIP was used as acceptor. ○—○, 0.2 M D-glucose as donor; ●- - -●, 0.2 M D-xylose as donor. The buffers used were acetate (pH 4.5–5.2), phosphate (pH 6.0–7.6), and Tris–HCl (pH 7.8–9.0).

Fig. 4. Double-reciprocal plot of glucose concentration *versus* activity. 0.1 mM DCIP was used as acceptor, and the concentration of glucose used varied. 1.0 unit of purified glucose dehydrogenase was used. Other conditions are described in text.

Kinetic studies

In the presence of 0.1 mM DCIP, the rate of glucose oxidation was dependent on the glucose concentration (Fig. 4). The apparent K_m value for glucose was determined to be 0.025 M. The values for D-xylose, D-mannose and D-fructose were similarly estimated to be 0.025 M, 1 M and 0.8 M, respectively. These values are considerably higher than those usually obtained for other sugar dehydrogenases[22–24]. A possible explanation for such high K_m values will be given in a later communication. The apparent K_m for DCIP in the presence of 0.2 M D-glucose, on the other hand, was determined to be 0.1 mM from Fig. 5, which shows the double reciprocal plots of the DCIP concentration *versus* the reaction velocity.

The velocity was also measured at various concentrations of both D-glucose and DCIP and the results were plotted according to the Lineweaver–Burk procedure. As shown in Fig. 6, it was thus found that the $(glucose)^{-1}$ *versus* $(velocity)^{-1}$ plots



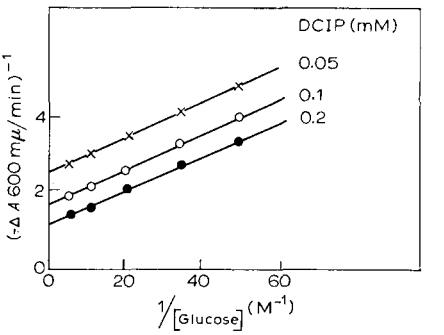


Fig. 5. Double-recipocal plot of DCIP concentration *versus* activity. 0.2 M glucose was used as donor, and the concentration of DCIP was varied. 1.0 unit of purified glucose dehydrogenase was used. Other conditions were as in Fig. 3.

Fig. 6. Lineweaver–Burk plots of glucose dehydrogenase activity with varying concentrations of glucose and DCIP. 0.9 unit of purified enzyme was used. The assay conditions were as described in text except that the concentrations of glucose and DCIP were varied as indicated.

at different DCIP concentrations were parallel to one another. As reported by ALBERTY[25], such parallelism in the Lineweaver–Burk plots is characteristic of an enzyme reaction where the enzyme reacts with the first substrate (donor) to be converted to a modified form before reacting with the second substrate (acceptor) which reconverts the enzyme to the original form, as formulated in the following equations:

$$E + A \rightleftarrows EA \rightleftarrows E' + C$$

$$E' + B \rightleftarrows EB \rightleftarrows E + D$$

Here, E, A, B, C and D represent the oxidized enzyme, D-glucose, DCIP, D-gluconoδ-lactone and reduced DCIP, respectively. In the case of glucose dehydrogenase, "the modified form of the enzyme" (E') may correspond to the reduced flavoprotein. This finding seemed to exclude the formation of an enzyme–donor–acceptor ternary complex during catalysis. Similar mechanisms have also been reported for lipoyl dehydrogenase[26] and other flavoprotein enzymes[27].

Titration of enzyme with glucose

To determine whether or not the semiquinone form of FAD participated in the reaction of glucose dehydrogenase, the enzyme was spectrophotometrically titrated with glucose at pH 6.5 under anaerobic conditions. As shown in Fig. 7, the absorption bands of the oxidized flavoprotein at 380 and 460 mμ decreased gradually on addition of glucose. The spectrum of the partially reduced enzyme was stable with time. When the molar ratio of added glucose to enzyme exceeded 1, the absorption band at 460 mμ was bleached almost completely (Curve 4 of Fig. 7). In the experiment, no absorption peak was observed in the 540–550 mμ region, where a peak of flavin semiquinone was expected[28,42]. When air or 7.0 μM vitamin K_3 was introduced into the solution of the reduced enzyme, the spectrum returned rapidly to that of the fully oxidized form. Titration of the enzyme with glucose at pH 4.1 and 7.7 gave similar results. It was therefore concluded that the FAD attached to glucose dehydro-

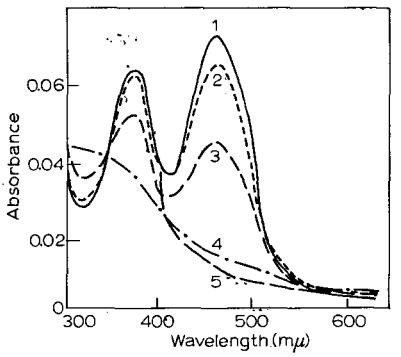


Fig. 7. Anaerobic titration of purified glucose dehydrogenase with glucose. 10.6 mμmoles of enzyme were dissolved in 1.5 ml of 50 mM phosphate buffer (pH 6.5) and the solution was placed in a cuvette under nitrogen as described in MATERIALS AND METHODS. Curve 1, oxidized enzyme. Curve 2, enzyme treated with 3.29 mμmoles of glucose (glucose/enzyme = 0.31). Curve 3, enzyme treated with 6.3 mμmoles of glucose (glucose/enzyme = 0.60). Curve 4, enzyme treated with 11.9 mμmoles of glucose (glucose/enzyme = 1.12). Curve 5, enzyme treated with $Na_2S_2O_4$.

genase functions between the fully oxidized and fully reduced forms and does not take the semiquinone form during the catalysis. This conclusion was further confirmed by a preliminary electron spin resonance experiment, kindly carried out by Dr. Y. ICHIKAWA. It has been reported by several workers[42] that no free radical formation could be detected during glucose oxidation by purified glucose oxidase.

Reaction products and stoichiometry

It has been reported that gluconate is produced from glucose in the reactio-catalyzed by *A. oryzae* glucose dehydrogenase[6]. Similarly the formation of glucono δ-lactone[29,30] or glucono-γ-lactone[30–32] as the primary product has been demonstrated with many other glucose-oxidizing enzymes. To identify the product of glucose oxidation and to establish the stoichiometry of the reaction catalyzed by the *A. oryzae* enzyme, 150 μmoles of D-glucose, 100 μmoles of DCIP, and 1900 units of purified glucose dehydrogenase were allowed to react in 20 ml (final volume) of 0.025 M potassium phosphate buffer (pH 6.5) for 20 min at 20°. It was thus found that a lactone-like substance, reacting with hydroxylamine under the conditions described by HESTRIN[13], accumulated in the reaction mixture. Assuming that this substance was a gluconolactone, the amount of accumulated lactone was estimated to be 50.5 μmoles. It was also estimated that during the same period of reaction time 47 μmoles of glucose had been consumed and 67 μmoles of DCIP had been converted to the reduced form. These results seem to indicate that a gluconolactone was the product of glucose oxidation and that the reaction catalyzed could be expressed by the following equation:

Glucose + DCIP → gluconolactone + reduced DCIP

To confirm further the nature of the accumulated lactone, the reaction mixture was passed through a Dowex 50 column (0.8 cm × 7 cm) and the effluent was concentrated under reduced pressure. When the concentrated material was subjected to paper chromatography, a spot reacting with an alkaline hydroxylamine reagent[14] was detected and its R_F value was identical with that of authentic D-glucono-δ-lactone.

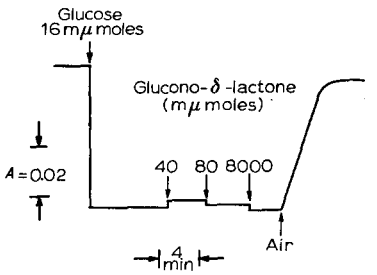


Fig. 8. Reaction of reduced FAD bound to glucose dehydrogenase with D-glucono-δ-lactone. 10.5 mμmoles of purified enzyme was dissolved in 1.6 ml of 50 mM phosphate buffer (pH 6.5) and the solution was placed in a cuvette under nitrogen. The wavelength was set at 460 mμ. D-Glucose and D-glucono-δ-lactone were introduced into the cuvette by means of a microsyringe as indicated, and the change in absorbance was recorded.

Irreversibility

OGURA[6] has shown previously that D-gluconate could not oxidize the reduced dye in the presence of *A. oryzae* glucose dehydrogenase and concluded that the oxidation of glucose by this enzyme was irreversible. However, the primary product of glucose oxidation was found to be D-glucono-δ-lactone rather than D-gluconate, as described above. It seemed desirable therefore to study the reversibility using D-glucono-δ-lactone. As shown in Fig. 8, it was found that the reduced flavin of the enzyme, which had been treated with a small amount of glucose, could not be re-oxidized under anaerobic conditions by the addition of a large excess of D-glucono-δ-lactone. The reduced flavin could, however, be reoxidized by the introduction of air. It was thus concluded that the oxidation of glucose by this enzyme is essentially irreversible.

DISCUSSION

The properties of highly purified glucose dehydrogenase from *A. oryzae* described in this paper are generally in agreement with those reported previously for crude preparations, especially in its specificity for hydrogen donors and acceptors[3] and the irreversibility of the reaction[6]. The present study has also clarified several important aspects of its properties, most of which could be studied only with the use of highly purified preparations. Since the properties of *A. oryzae* glucose dehydrogenase thus elucidated are not unexpected for a flavoprotein dehydrogenase, the discussion will be confined only to the specificity and reaction product.

While this enzyme oxidizes D-glucose rapidly and D-xylose, D-fructose, D-mannose and 2-deoxy-D-glucose at slower rates, glucose 6-phosphate does not serve as hydrogen donor. It seems, therefore, that the C-6 position of a hexose should be free to be a hydrogen donor for this enzyme. The glucose configuration at the C-4 position is essential, thus D-galactose is completely inactive. Since 2-deoxy-D-glucose, fructose and mannose are oxidized, though slowly, the configuration at the C-2 position does not seem very critical.

Judging from the substrate specificity, this enzyme is different from the many monosaccharide oxidizing enzymes so far reported, such as Gluconobactor D-fructose dehydrogenase[33], Trametes D-sorbose oxidase[34], and Bacterium glucose dehydro-

genase[35]. The fact that *A. oryzae* glucose dehydrogenase, though containing FAD as a prosthetic group, cannot effectively utilize oxygen as acceptor distinguishes this enzyme from fungal glucose oxidase[5], galactose oxidase[36] and algal carbohydrate oxidase[37]. The failure of pyridine nucleotides to act as acceptors for the *A. oryzae* enzyme is also in marked contrast to liver glucose dehydrogenase[19], Bacillus glucose dehydrogenase[23], and Pseudomonas aldose dehydrogenase[38]. Although glucose dehydrogenase resembles, in certain respects, glucose oxidase found in Aspergilli and Penicillia, the two enzymes are different from each other. Thus, the turnover number of dehydrogenase with DCIP as acceptor is higher than that of oxidase, and CoQ_0 and vitamin K_3 are not effective electron acceptors for the oxidase. An aldose dehydrogenase purified from a halophilic Pseudomonas species by MAENO[22], also oxidizes several aldoses to the corresponding aldonic acids using DCIP as the hydrogen acceptor, and requires 1 M NaCl and a halophilic factor for optimal activity. However, the glucose dehydrogenase of *A. oryzae* is inhibited by 1 M NaCl[22].

CORI AND LIPMANN[39] first discovered that the primary product of the reaction catalyzed by D-glucose-6-phosphate dehydrogenase is the δ-lactone of D-gluconic acid-6-phosphate. Since then, it has generally been assumed that glucose is oxidized to glucono-δ-lactone in biological systems. Supporting evidence usually cited is that cell-free extracts contain a lactonase which acts on this product[26,40,41,43]. On the other hand, it has been shown that the product of the enzymic oxidation of D-glucose by some enzymes is D-glucono-γ-lactone rather than the δ-lactone[15]. Based upon the difference in reaction products, WEIMBERG[31] has pointed out that there are two different glucose oxidizing enzymes; particulate enzymes oxidize glucose to glucono-γ-lactone, and soluble enzymes produce the δ-lactone. However, glucono-δ-lactone is the primary product of the reaction catalyzed by the glucose dehydrogenase of *A. oryzae*, despite the fact that this enzyme is soluble in nature.

A great deal of information concerning the microbial oxidation of aldose sugars to the corresponding aldonic acids has accumulated, and in most cases the oxidative enzyme systems have been shown to be linked to the respiratory chain of the organism[15,22,43]. However, the nature of the interaction of *A. oryzae* glucose dehydrogenase with the cytochrome system still remains unclear.

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