Purification and Properties of NAD-Dependent D-Glucose Dehydrogenase Produced by Alkalophilic *Corynebacterium* sp. No. 93-1

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NAD-dependent D-glucose dehydrogenase (NAD-GlcDH) was purified from the cell free extract of alkalophilic *Corynebacterium* sp. No. 93-1. The preparation obtained was homogeneous on disc gel and SDS-disc gel. The molecular weight of NAD-GlcDH was 51,000 by SDS-disc electrophoresis and 55,000 by gel filtration. The isoelectric point of the enzyme was pH 4.05. The optimal pH was 9.8, and it was stable at pH 6 to 8. NAD-GlcDH oxidized D-glucose, D-xylose, cellobiose, cellotriose, gentiobiose and laminaribiose. The K_m values for D-glucose, D-xylose, cellobiose and gentiobiose were 51 mM, 125 mM, 11 mM and 3.2 mM, respectively. The K_m value for NAD was 0.44 mM. The enzyme was inhibited completely by PCMB, Ag^+ and Hg^{2+} . NADH had an inhibitory effect on NAD-GlcDH.

Many papers have been published on D-glucose dehydrogenase (GlcDH) [E.C. 1.1.1.47] in microorganism and animal tissues. Especially, the properties of GlcDH of the genus *Bacillus* have been studied in detail in relation to spore formation.^{1~15)} Okamoto¹⁶⁾ described the purification and properties of GlcDH of *Acetobacter suboxydans*. Pritchard *et al.*¹⁷⁾ reported on GlcDH of *Chlorogloeopsis fritschii*. But up to now, as far as we know, there are no report on GlcDH of coryneform bacteria.

In a previous study,¹⁸⁾ we isolated an alkalophilic *Corynebacterium* sp. No. 93-1 which produced NAD-dependent maltose dehydrogenase (NAD-MalDH), NADP-dependent maltose dehydrogenase (NADP-MalDH) and NAD-dependent D-glucose dehydrogenase (NAD-GlcDH). The purification procedures and properties of NAD-MalDH

were reported.¹⁹⁾ The characteristics of NAD-GlcDH of this strain was interesting from a standpoint of comparison with already reported GlcDHs. This paper deals with the purification and properties of NAD-GlcDH.

MATERIALS AND METHODS

Culture. An alkalophilic *Corynebacterium* sp. No. 93-1 was cultured in 600 ml of liquid medium in 2-liters Erlenmeyer flask at 30°C for 24 hr on a rotary shaker. The composition of the medium used was as follows: 1% maltose, 1% soybean casein, 0.1% yeast extract, 0.1% K_2HPO_4 , 0.02% $MgSO_4 \cdot 7H_2O$ and 0.2% Na_2CO_3 (sterilized separately).

Enzyme assay. NAD-GlcDH activity was assayed by the method described previously.¹⁸⁾

Disc electrophoresis. Disc electrophoresis was performed using 7.5% polyacrylamide gel at running pH 8.0. Electrophoresis was carried out at 3 mA/tube at 5°C. Protein was stained with Coomassie Brilliant Blue R250. NAD-GlcDH activity was detected by incubation of the gel at room temperature in 100 mM Tris-HCl buffer (pH 9.0) with 100 mM D-glucose, 0.05 mM phenazinemetosulfate and 0.3 mM 3-(4,5-dimethyl-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT).

SDS-disc electrophoresis. SDS-disc electrophoresis was

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Abbreviations: GlcDH, NAD and/or NADP-dependent D-glucose dehydrogenase; NAD-GlcDH, NAD-dependent D-glucose dehydrogenase of *Corynebacterium* sp. No. 93-1; NAD-MalDH, NAD-dependent maltose dehydrogenase; NADP-MalDH, NADP-dependent maltose dehydrogenase.

performed according to Weber and Osborn.²⁰⁾ Electrophoresis was carried out at 8 mA/tube using 10% acrylamide gel, containing 0.1% SDS. Protein was stained with Coomassie Brilliant Blue R250.

Electrofocusing. Electrofocusing was performed using 1% (v/v) Carrier Ampholine (pH 3.5~6) and sucrose gradient in a 110 ml column (LKB Produkters) at 1000 V for 15 hr at 2°C. After fractionation (2 ml/tube), NAD-GlcDH activity was assayed.

Molecular weight determination by gel filtration on Ultrogel AcA44. The molecular weight of NAD-GlcDH was determined according to Andrews,²¹⁾ using a column (1.6 × 100 cm) of Ultrogel AcA44 which had been equilibrated with 50 mM phosphate buffer (pH 7.5), containing 10% (v/v) glycerol and 1 mM 2-mercaptoethanol.

Ultracentrifugation. Analytical ultracentrifugation was carried out to estimate the sedimentation velocity of NAD-GlcDH by a Spinco Model E ultracentrifuge (Beckman Instrument) equipped with a schlieren optic. Protein concentration used was 3.0 mg/ml in 20 mM phosphate buffer (pH 7.2).

Protein determination. Protein was measured from absorbance at 260 nm and 280 nm according to Layne.²²⁾

Chemicals. NAD, NADP, NADH and NADPH were purchased from Kyowa Hakko Kogyo Co. Maltotriose was a gift from Dr. N. Nakamura of Nihon Shokuhin Kako Co. Laminaribiose was a gift from Dr. K. Watanabe of our laboratory. All other chemicals were reagent grade.

RESULTS

Purification

All purification procedures were carried out at 5°C. The enzyme solution was concentrated by a Diaflow membrane PM 10 throughout the purification.

Step 1. Extraction. Wet cells (80 g) were suspended in 100 ml of 20 mM phosphate buffer (pH 7.3), containing 5 mM 2-mercaptoethanol, 1 mM EDTA and phenyl-methylsulfonyl-fluoride (PMSF) (2 µg/ml) and disrupted by sonication at 20 kHz for 15 min. The sonicated suspension was centrifuged at 20,000 × *g* for 20 min, and the cell debris was removed. And unbroken cells were subjected to sonic treatment in the same manner for another three cycles. The cell free extracts

obtained were combined (300 ml).

Step 2. Streptomycin-sulfate treatment. The cell free extract was diluted with water to 900 ml, and 120 ml of 5% streptomycin-sulfate solution was added in dropwise. After adjusting pH to 7.0 with 0.2 N NaOH solution, the suspension was allowed to stand for 30 min. The precipitate was removed by centrifugation, and the supernatant solution (970 ml) was obtained.

Step 3. Ammonium sulfate fractionation. Ammonium sulfate fractionation was carried out at 30~70% saturation. After standing for an hour, the precipitate was collected by centrifugation and dissolved in a minimal volume of 50 mM phosphate buffer (pH 7.3), containing 10% (v/v) glycerol, 1 mM 2-mercaptoethanol, 1 mM EDTA and 1 mM PMSF (Buffer A), and dialyzed overnight against the same buffer. After dialysis, 212 ml of the enzyme solution was obtained.

Step 4. DEAE cellulose column chromatography. The enzyme solution was loaded onto a DEAE cellulose column (9 × 40 cm) equilibrated with Buffer A. After washing the column with the same buffer, the enzyme was eluted with a linear gradient of NaCl (0~0.8 M). Twenty milliliters of the fraction was collected and the enzyme activity was assayed. As described in the previous paper,¹⁹⁾ NAD-MalDH, NADP-MalDH and NAD-GlcDH activities were separated by this chromatography method. The active fractions of NAD-GlcDH were pooled and concentrated.

Step 5. First gel filtration on Ultrogel AcA44. Concentrated NAD-GlcDH solution was applied to a column (2.5 × 100 cm) of Ultrogel AcA44 equilibrated with Buffer A, and it was eluted with the same buffer. Figure 1 shows the elution pattern of NAD-GlcDH in this step. The active fractions (fraction No. 59~63) were combined.

Step 6. DE 52 Cellulose column chromatog-

FIG. 1. First Gel Filtration of NAD-GlcDH on Ultrogel AcA44.

NAD-GlcDH activity was assayed by the standard assay method. NAD-GlcDH activity (○); protein (●).

raphy. The enzyme solution was loaded onto a DE 52 cellulose column (1.2×20 cm), which had been equilibrated with Buffer A. After washing the column with the same buffer, the enzyme was eluted with a linear gradient of NaCl (0~0.8 M). The enzyme fractions (fraction No. 20~26) were combined.

Step 7. Hydroxyl apatite column chromatography. After dialysis of the enzyme solution against 10 mM phosphate buffer (pH 7.3), containing 10% (v/v) glycerol, 1 mM 2-mercaptoethanol and 1 mM EDTA (Buffer B), the enzyme placed on a Hydroxyl apatite column (2.5×20 cm) equilibrated with Buffer B. The column was washed with the same

FIG. 2. Second Gel Filtration of NAD-GlcDH on Ultrogel AcA44.

NAD-GlcDH activity was assayed by the standard assay method. NAD-GlcDH activity (○); protein (●).

buffer and the enzyme was eluted with a linear gradient of 10~100 mM phosphate buffer (pH 7.3), containing the same contents as Buffer B. The active fractions (fraction No. 40~50) were pooled and concentrated.

Step 8. Second gel filtration on Ultrogel AcA44. The enzyme solution was passed through an Ultrogel AcA44 column (2.5×100 cm) equilibrated with Buffer C (excepted PMSF from Buffer A). As shown in Fig. 2, NAD-GlcDH was highly purified. The enzyme fractions (fraction No. 55~59) were combined and concentrated. This preparation was used for the following experiments. A summary of the purification is shown in Table I. In this purification of NAD-GlcDH, we used Blue

TABLE I. SUMMARY OF THE PURIFICATION OF NAD-GlcDH

	Total volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (u/mg prot.)	Yield (%)	Purification (-fold)
Crude extracts	300	3714	7560	0.491	100	1
Streptomycin-sulfate treatment	970	3264	3735	0.874	87.9	1.78
Ammonium sulfate fractionation	212	3029	2078	1.46	81.6	2.97
DEAE cellulose	754	2348	1039	2.26	63.2	4.60
Ultrogel AcA44, 1st	33	1913	51.8	36.9	51.5	75.2
DE 52 cellulose	41	1861	34.0	54.7	50.1	111
Hydroxyl apatite	63	1525	15.0	102	41.1	208
Ultrogel AcA44, 2nd	23	924	8.70	106	24.9	216

Sephacrose CL-6B and 2',5'-ADP Sepharose for affinity chromatography, but the enzyme showed no affinity for these materials.

Homogeneity

The homogeneity of the purified enzyme was checked by disc electrophoresis and SDS-disc electrophoresis. Figure 3 shows that the preparation was composed of a single component. Activity staining of NAD-GlcDH on the disc gel gave a single band at a location corresponding to the protein band observed. From these results, it was clear the preparation was homogeneous.

Physical properties

Molecular weight. The molecular weight of NAD-GlcDH was estimated using gel filtration and SDS-disc electrophoresis. Gel filtration on Ultrogel AcA44 column was performed, and the enzyme was eluted at the position of molecular weight 55,000 (Fig. 4). The enzyme was also located at molecular weight 51,000 by SDS-disc gel (Fig. 4). On the basis of these results, NAD-GlcDH was composed of a single peptide chain and no subunit structure was observed.

Isoelectric point. The isoelectric point was determined by the isoelectrofocusing method.

FIG. 3. Disc Electrophoresis and SDS-Disc Electrophoresis.

About 30 μ g of the enzyme was used, and protein was stained with Coomassie Brilliant Blue R250. A, Disc electrophoresis; B, SDS-disc electrophoresis.

FIG. 4. Molecular Weight Determination of NAD-GlcDH by Gel Filtration and SDS-Disc Electrophoresis. The upper line is from the results of SDS-disc electrophoresis, and the lower line is from gel filtration. Standard proteins used were: A, cytochrome *c* (12,500); B, chymotrypsinogen A (25,000); C, ovalbumin (45,000); and D, bovine serum albumin (68,000).

FIG. 5. Determination of Isoelectric Point of NAD-GlcDH.

About 300 units of the enzyme was used, and NAD-GlcDH activity was assayed by the standard assay method. NAD-GlcDH activity (O); pH (●).

As shown in Fig. 5, the enzyme activity was found as a single peak, and it was located at pH 4.05. But almost all activity was lost by this procedure.

Ultraviolet spectrum. NAD-GlcDH showed the absorption spectrum of a typical protein, and a prosthetic group was not detected. The maximum absorption existed at 280 nm and the minimum at 253 nm. The absorption ratio 280 nm/260 nm was 1.83 at pH 7.2.

Apparent *S* value. On ultracentrifugal analysis, a symmetrical peak was observed, and the apparent *S* value was calculated as 5.0.

Effect of pH

The optimal pH of NAD-GlcDH was 9.8 in 50 mM glycine-NaOH buffer (Fig. 6). The stability of the enzyme at various pH values was investigated. The enzyme preparation was treated in each buffers for 30 min at 30°C, and residual activities were measured. As shown in Fig. 7, NAD-GlcDH was stable at a pH range from 6 to 8.

Effect of temperature

The optimal temperature of NAD-GlcDH was investigated and found around 30° to 35°C (Fig. 8). The thermal stability of the enzyme was examined in the presence or absence of 1 mM NAD, 1 mM 2-mercaptoethanol, 1 mM D-glucose and 5% (w/w) glycerol. The results obtained are shown in Fig. 9. NAD and glycerol provided some protection against heat inactivation. A decrease in enzyme activity was not detected for at least three months in 20 mM phosphate buffer (pH 7.3), containing 1 mM 2-mercaptoethanol and 50% (v/v) glycerol at -20°C.

FIG. 6. Optimal pH of NAD-GlcDH.

The optimal pH of NAD-GlcDH activity was investigated using the following buffer systems: pH 7~8, 100 mM phosphate buffer (Δ); pH 8~9, 100 mM Tris-HCl buffer ($\square$); and pH 9~11, 100 mM glycine-NaOH buffer ($\circ$).

Substrate specificity

The substrate specificity of NAD-GlcDH was investigated using 100 mM of 25 kinds of carbohydrates with NAD or NADP as a cofactor. As summarized in Table II, the enzyme showed oxidation activity on D-glucose, D-xylose, cellobiose and gentiobiose. 2-Deoxy-D-glucose was not effective as a substrate. NAD-GlcDH oxidized cellobiose and gentiobiose, but could not oxidize mal-

FIG. 7. pH Stability of NAD-GlcDH.

NAD-GlcDH was treated in each buffer for 30 min at 30°C. After adjusting pH to 7.0 with 200 mM phosphate buffer (pH 7.0), the residual activity was measured by the standard assay condition. The buffer systems used were: pH 5.5~6, 100 mM succinate buffer ($\bullet$); pH 6~8, 100 mM phosphate buffer (Δ); pH 8~9, 100 mM Tris-HCl buffer ($\square$); and pH 9~10.5, 100 mM glycine-NaOH buffer ($\circ$).

FIG. 8. Optimal Temperature of NAD-GlcDH.

NAD-GlcDH activity was assayed by the standard assay method at various temperatures.

FIG. 9. Thermal Stability of NAD-GlcDH.

NAD-GlcDH was treated at each temperature point for 10 min in 50 mM phosphate buffer (pH 7.0) and the residual activity was measured by the standard assay method. The stabilizing effect of several kinds of reagents on NAD-GlcDH was investigated. None (○); 1 mM NAD (△); 1 mM 2-mercaptoethanol (●); 1 mM D-glucose (▲); 5% (w/w) glycerol (□).

tose, lactose and melibiose. Cellotriose and laminaribiose were also suitable substrates. Therefore, the velocities at 20 mM concentration of D-glucose, cellobiose, cellotriose and laminaribiose were compared. Cellotriose was oxidized to the same degree as cellobiose.

NAD was more effective as a cofactor than NADP on all substrates tested.

Effect of concentration of substrates and NAD

Lineweaver-Burk analysis was carried out to estimate K_m values for D-glucose, D-xylose, cellobiose and gentiobiose. Figure 10 shows that K_m values for D-glucose, D-xylose, cellobiose and gentiobiose were 51 mM, 125 mM, 11 mM and 3.2 mM, respectively. The same analysis was performed on the concentration effect of NAD, using 100 mM of D-glucose as substrate. A K_m value of 0.44 mM was obtained.

Effect of various kinds of reagents on NAD-GlcDH activity

The effect on the activity of various kinds of salts, inhibitors, metal ions, nucleotides, sugar phosphates was examined. The results obtained are summarized in Table III. Of salts tested, LiCl and $(\text{NH}_4)_2\text{SO}_4$ showed about 60~70% inhibitory activity. On SH reagents and metal ions, 0.1 mM PCMB, 1 mM Hg^{2+} and 1 mM Ag^+ showed complete inhibition. Nucleotides, such as AMP, ADP, ATP and

TABLE II. SUBSTRATE SPECIFICITY OF NAD-GlcDH

The assay was carried out by the standard assay method using 100 mM of substrate (Section A) and 20 mM of substrate (Section B). The relative reaction velocities of various kinds of substrates were related to D-glucose as 100% with NAD as a cofactor.

Section A						Section B	
Substrate	Relative rate (%)		Substrate	Relative rate (%)		Substrate	Relative rate (%)
	NAD	NADP		NAD	NADP		
D-Glucose	100	1	D-Mannitol	0	0	D-Glucose	100
D-Mannose	2	0	D-Xylitol	0	0	Cellobiose	158
D-Galactose	6	0	D-Sorbitol	1	0	Cellotriose	158
D-Fructose	9	6	Inositol	1	0	Laminaribiose	96
D-Xylose	44	1	D-Arabinitol	0	0		
D-Ribose	4	2	Maltotriose	4	0		
D-Arabinose	0	0	L-Rhamnose	0	0		
Maltose	6	0	L-Fucose	0	0		
Lactose	2	0	2-Deoxy-D-glucose	8	4		
Cellobiose	89	2	Glucose-6-P	1	0		
Gentiobiose	86	1	D-Glucosamine	0	0		
Melibiose	4	0	NAC-D-glucosamine	2	0		
Sucrose	0	0

FIG. 10. Lineweaver-Burk Analysis of the Effect of Substrate Concentration on NAD-GlcDH.

The effects of substrate concentrations on the velocities were analyzed by Lineweaver-Burk analysis at pH 9.8. NAD-GlcDH (0.017 unit) in 50 mM glycine-NaOH buffer (pH 9.8), and 3 mM NAD were used for the assay. D-Glucose (□); D-xylose (Δ); cellobiose (○); gelatinose (●).

cAMP, and intermediates of glycolysis, such as G-6-P, F-6-P, FDP and 6-PG, had no effects. NADH (0.1 mM), NADPH (0.1 mM) and NADP (3 mM) showed inhibitory activities of about 45%, 20% and 30%, respectively. The effect of 2-deoxy-D-glucose on NAD-GlcDH activity was examined, but it had no effect.

Inhibitory effect of NADH on NAD-GlcDH activity

As described above, NADH inhibited NAD-GlcDH activity. Therefore, the NADH effect was analyzed by a Lineweaver-Burk plot. Using 0.1 mM or 0.2 mM NADH, the velocities of the reaction under saturated NAD (3 mM) were compared with that of the control in which D-glucose concentration was changed from 10 mM to 200 mM. As shown in Fig. 11 (A), NADH acted as a noncompetitive inhibitor of D-glucose with a K_i value of 0.11 mM. The same analysis was carried out with saturated D-glucose (200 mM) with changes in concentration of NAD from 0.1 mM to 2 mM. Figure 11(B) shows that inhibition was competitive with K_i value of 0.048 mM.

Reversibility of reaction

The velocities of the reverse reaction of this enzyme action were measured at pH 7.0 and 8.0. The assay mixture used was as follows: 0.5 ml of 100 mM phosphate buffer (pH 7.0 or 8.0), 0.1 ml of 2 mM NADH solution, 0.1 ml of 1 M glucono- δ -lactone solution and 1.0 unit of NAD-GlcDH in 0.3 ml of 10 mM phosphate

TABLE III. EFFECT OF VARIOUS KINDS OF REAGENTS ON NAD-GlcDH ACTIVITY

Reagents	Concentration	Activity (%)	Reagents	Concentration	Activity (%)
None		100	Hg ²⁺	1 mM	0
NaCl	1 M	72	Ag ⁺	1 mM	1
KCl	1 M	56	Mn ²⁺	1 mM	84
LiCl	1 M	36	Ni ²⁺	1 mM	96
(NH ₄) ₂ SO ₄	1 M	29	Co ²⁺	1 mM	26
Tris	100 mM	100	Ba ²⁺	1 mM	93
EDTA	10 mM	100	Fe ²⁺	1 mM	59
DTT	10 mM	72	Fe ³⁺	1 mM	59
DTT	1 mM	94	AMP	1 mM	101
2-Mercaptoethanol	10 mM	90	ADP	1 mM	100
2-Mercaptoethanol	1 mM	100	ATP	1 mM	100
MIA	10 mM	97	cAMP	1 mM	99
NaF	10 mM	97	G-6-P	1 mM	101
KCN	10 mM	102	F-6-P	1 mM	100
PCMB	0.1 mM	0	FDP	1 mM	100
Zn ²⁺	1 mM	92	6-PG	1 mM	102
Mg ²⁺	1 mM	102	NADH	0.1 mM	55
Cu ²⁺	1 mM	86	NADPH	0.1 mM	81
Ca ²⁺	1 mM	86	NADP	3 mM	67

FIG. 11. Lineweaver-Burk Analysis of the Effect of NADH Concentration on NAD-GlcDH.

(A) The effect was examined with saturated D-glucose (3 mM). NADH concentrations used were 0.1 mM (●) and 0.2 mM (□). The control sample (○) was assayed by the standard assay condition. NAD-GlcDH activity used was 0.023 unit.

(B) The same analysis was carried out with saturated D-glucose (200 mM). NADH concentrations used were 0.1 mM (▲) and 0.2 mM (△). The control sample (□) was assayed by the standard assay condition. NAD-GlcDH used was 0.025 unit.

buffer (pH 7.0). A decrease of O.D. 340 nm was measured at 30°C, and the velocities observed were compared with the results obtained by oxidative reaction assayed in the same condition using D-glucose and NAD. The velocities of the reverse reaction at pH 7.0 and 8.0 were 2.2% and 5.9%, respectively, of oxidative reaction.

DISCUSSION

In previous papers,^{18,19)} we have reported that alkalophilic *Corynebacterium* sp. No. 93-1 produced NAD-GlcDH, and the enzyme was separated from NAD(P)-MalDH activities by DEAE cellulose column chromatography. The purified NAD-GlcDH was composed of a single peptide chain (M.W. 51,000~55,000). Although several investigators reported on the relationship between enzyme activity and subunit structure of GlcDH,^{6,7,14,16)} we could not detect multiple form of NAD-GlcDH in the cell free extract. The optimal pH (9.8) of our enzyme existed more alkaline region than enzymes so far reported. Our enzyme showed oxidative activity on D-glucose, D-xylose, gentiobiose, cellobiose and laminaribiose with NAD as a cofactor. The enzyme could not oxidize maltose, lactose and melibiose. From these results, we expected that our enzyme had affinity on reducing disaccharides and trisaccharide, which contained glucose residues in the reducing end, and β -linkage in two or three sugar components. This property was very unique compared with sugar dehydrogenases already known and NAD-MalDH.¹⁹⁾ Our enzyme scarcely oxidize 2-deoxy-D-glucose and it could not oxidize D-glucosamine and NAc-D-glucosamine. GlcDH of *B. megaterium*,^{12,13)} *B. subtilis*¹⁵⁾ and *Bacillus* sp.²³⁾ oxidized D-glucose and 2-deoxy-D-glucose in the same degree. Metzger *et al.*²⁴⁾ examined the properties of GlcDH in rat liver. The enzyme did not oxidize 2-deoxy-D-glucose and NAc-D-glucosamine, and they reported that the second carbon atom of the hexose molecule must have a hydroxyl group in the glucose configuration to serve as a substrate for GlcDH. Our enzyme had similar substrate specificity to that of rat liver.

The *K_m* value for reactive substrates, for gentiobiose was the smallest, and the next smallest was cellobiose. We expect that these properties show that the enzyme plays a role in the direct oxidation system of disaccharides, such as gentiobiose and cellobiose. The *K_m* value for D-glucose (51 mM)

of our enzyme was almost similar to the value found in *B. megaterium* (47.5 mM)¹³⁾ and *B. subtilis* (55.9 mM).¹⁵⁾ But it was greater than that of *B. cereus* (7 mM),⁹⁾ *Bacillus* sp. (4.2 mM),²³⁾ *A. suboxydans* (5.3 mM).¹⁶⁾ GlcDH of *B. megaterium*,^{4,12,13)} *B. cereus*,¹⁾ *B. subtilis*,¹⁵⁾ *Bacillus* sp.,²³⁾ *C. fritschii*,¹⁷⁾ and rat liver used both NAD and NADP as cofactors. The enzyme of *A. suboxydans*¹⁶⁾ used only NADP and that of *B. cereus*⁹⁾ was NAD specific. Our enzyme was NAD specific, and it was similar to that of *B. cereus*.⁹⁾ The *K_m* value of our enzyme for NAD was 0.44 mM, and it was similar to the *K_m* value of *B. subtilis* (0.23 mM)¹⁵⁾ and *B. cereus* (0.35 mM,¹⁾ 0.2 mM⁹⁾), but it was greater than that of *B. megaterium* (0.07 mM)⁴⁾ and *Bacillus* sp. (0.082 mM).²³⁾

Sadoff¹⁾ reported that GlcDH of *B. cereus* was not inhibited by sulfhydryl group inhibitors and several kinds of metal ions. Pauly and Pfeidere¹³⁾ reported that GlcDH of *B. megaterium* was not inhibited by sulfhydryl group inhibitors, heavy metal ions and various kinds of chelating reagents. But our enzyme was inhibited completely by PCMB, Hg²⁺ and Ag⁺. This result suggested that the sulfhydryl group(s) was contributing to the activity in the same manner as NAD-MalDH.¹⁹⁾ NADH showed a non-competitive type of inhibition to D-glucose in saturated NAD. The enzyme showed a competitive type of inhibition to NAD under saturated D-glucose. These results were the same as those with galactose dehydrogenase in rat liver.²⁵⁾

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