Arch. Mikrobiol. 88, 319—330 (1973)

Studies on a Gram-Positive Hydrogen Bacterium, *Nocardia opaca* Strain *1b*

II. Enzyme Formation and Regulation under the Influence of Hydrogen or Fructose as Growth Substrates

Irmelin Probst and H. G. Schlegel

Institut für Mikrobiologie der Gesellschaft für Strahlen- und Umweltforschung mbH, München, in Göttingen, Federal Republic of Germany

Received October 10, 1972

Summary. In *Nocardia opaca* strain *1b* the key enzymes of the Entner-Doudoroff pathway, 6-phosphogluconate dehydrase and 2-keto-3-deoxy-6-phosphogluconate aldolase, are inducible, while glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are constitutively formed. In autotrophically grown cells the fructose diphosphate aldolase has six-fold specific activity compared to fructose- or gluconate-grown cells. Molecular hydrogen represses the adaptation to fructose, glucose, or gluconate and inhibits the utilization of glucose or fructose.

Glucose-6-phosphate dehydrogenase has been purified 22fold. It utilizes either NADP of NAD as coenzyme; it requires magnesium ions for maximal activity. The dehydrogenation reaction is competitively inhibited by rather high concentrations (2—6 mM) of ATP, and in a similar fashion by phosphoenolpyruvate. Homotropic or heterotropic interactions between substrate molecules were not detected. The effect of ATP is relieved by equimolar concentrations of magnesium.

In *Hydrogenomonas eutropha* strain *H 16* the enzymes of fructose degradation are inducible and are subject to repression by hydrogen (Gottschalk, 1965; Schlegel and Trüper, 1966; Blackkolb and Schlegel, 1968a, b). Furthermore, fructose degradation by fully induced cells is inhibited by hydrogen (Blackkolb and Schlegel, 1968b) presumably due to allosteric inhibition of glucose-6-phosphate dehydrogenase by adenosine triphosphate and reduced pyridine nucleotides. The hydrogenase activity of heterotrophically grown cells is less than that of autotrophically grown cells and varies with the growth substrates. Regulatory phenomena observed in hydrogen bacteria have been recently reviewed (Schlegel and Eberhardt, 1972).

While all obligate autotrophs and the majority of the hydrogen bacteria are Gram-negative a few hydrogen bacteria are Gram-positive. A strain of *Nocardia opaca* isolated recently (Siebert, 1969) and characterized by Aggag and Schlegel (1973) grows very well as a hydrogen bacterium and lent itself to a comparative study of the mutual influences of hydrogen and fructose on the formation of the Entner-Doudoroff enzymes as well as the action of possible effectors on glucose-6-phosphate dehydrogenase.

Materials and Methods

Culture of Organism. Nocardia opaca strain *1b* (Aggag and Schlegel, 1972) was grown in the basal medium described by Schlegel *et al.* (1961), which was supplemented with 0.5% organic substrate for heterotrophic growth and 0.5% $NaHCO_3$ for chemolithotrophic growth. The gas atmosphere consisted of 80% H_2, 10% O_2, and 10% CO_2.

Small quantities of liquid cultures (3 l) for enzymatic investigations were grown in 6 l culture vessels with magnetic stirring at 30° C. Large quantities of cells needed for enzyme purification were grown in 10 l fermenters (Braun-Biostat, Melsungen).

Growth was determined turbidimetrically at 436 nm in a Zeiss PL 4 spectrophotometer. The cells were harvested at the end of the logarithmic phase by centrifugation at 5000 × g for 20 min at 10° C.

Preparation of Cell Extracts. Harvested cells were washed twice with 50 mM TEA-buffer pH 7.6, resuspended in the same buffer at an extinction of 270—300 at 436 nm, and stored at −20° C. The cells were disrupted by ultrasonic treatment. A sonicator of Schoeller u. Co., Frankfurt a. M., (20 kH, 600 W), was used in combination with a cooled (Ultrakryomat Tk 30 D, Meßgerätewerk, Lauda) vessel of 10 ml capacity. The sonication time was 2 min/ml cell-suspension, the temperature was maintained between + 4° C and + 10° C.

The crude cell extracts were centrifuged at 120000 × g for 40 min at 4° C (Omikron-Zentrifuge, Heraeus-Christ, Osterode) in order to remove the particulate NADH-Oxidase or at 80000 × g for 30 min for the purification of the glucose-6-phosphate dehydrogenase.

Protein-Measurement. Protein was determined either by the method of Beisenherz *et al.* (1953) or Warburg and Christian (1941).

Enzyme Assays. The enzyme activities were determined by optical tests and the reduction or oxidation of NAD or NADP, measured at 365 nm in a Zeiss PL 4 recording spectrophotometer, equipped with a temperature controlled sample-holder at 25° C. One unit of enzyme catalyzes the conversion of 1.0 μmol of substrate per minute. Enzyme assays were carried out as described in detail elsewhere: Hexokinase (EC 2.7.1.1) and Phosphoglucoisomerase (EC 5.3.1.9); Boehringer Informations 1961, as modified by Blackkolb and Schlegel (1968a): Glucose-6-phosphate dehydrogenase (EC 1.1.1.49); Boehringer Informations 1964, as modified by Bowien (1970); the assay for the NAD-linked reaction contained NAD at 1.0 mM: Entner-Doudoroff-pathway (6-Phosphogluconate dehydrase and KDPG Aldolase); Gottschalk *et al.* (1964): 6-Phosphogluconate dehydrase (EC 4.2.1.12) and KDPG Aldolase (EC 4.1.2.14); Blackkolb and Schlegel (1968a); the assay for KDPG aldolase contained KDPG at 0.8 mM concentration: Gluconate dehydrase (EC 4.2.1.—) and KDG Kinase (EC 2.7.1.45); Bender (1972): 6-Phosphogluconate dehydrogenase (EC 1.1.1.44); Boehringer Informations 1966, as modified by Bowien (1970): Phosphofructokinase (EC 2.7.1.11); Ling *et al.* (1955): Fructose-1.6-diphosphatealdolase (EC 4.1.2.7); Boehringer Informations 1964, as modified by Bowien (1970): Fructose-1.6-diphosphatase (EC 3.1.3.11); Racker and Schroeder (1958).

Substrate Utilization under Air and Knallgas. To measure the inhibition of substrate utilization by hydrogen, cells were used which were adapted to autotrophic conditions as well as to organic substrates. For this purpose chemolithoautotrophically grown cells were shaken for 5 h in the presence of 0.5% organic substrate under air. Afterwards the cells were washed, resuspended in mineral medium and transferred into 200 ml Erlenmeyer flasks. The experiments were carried out as described by Blackkolb and Schlegel (1968a). Growth was determined turbidimetrically or by measuring the decrease in glucose concentration. The glucose

Table 1. Purification procedure of glucose-6-phosphate dehydrogenase from *Nocardia 1b*

Fraction	Volume ml	Protein mg/ml	Total Units	Units per g protein	Recovery %	Purification
Crude extract	40	21.2	31.2	36.8	100	1
CTAB supernatant	48	5.4	27.2	104.0	88	2.8
Ammonium sulfate precipitate	53	24.2	18.2	141.0	58	3.9
DEAE-Cellulose eluate	27	0.3	7.14	880.0	23	24.0

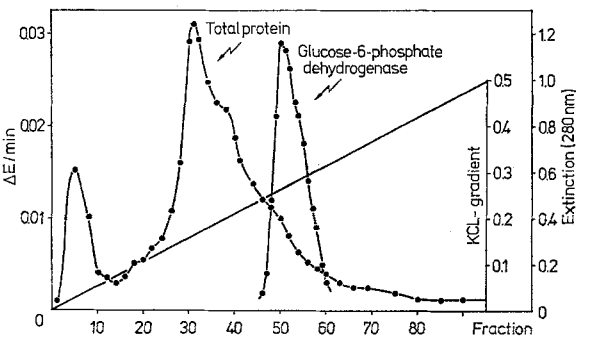

Fig. 1. Purification of glucose-6-phosphate dehydrogenase by DEAE-Cellulose column chromatography, employing a 0.0—0.5 M KCl gradient for elution

concentration was determined by the glucose oxidase method (Blood-Sugar test combination; Boehringer, Mannheim).

Enzyme Purification. All fractionations were carried out at 4° C. The purification procedure of glucose-6-phosphate dehydrogenase involved the following steps (Table 1): Gluconate-grown cells were disrupted by sonication, and after removal of cell debris by centrifugation nucleic acids and part of inactive protein was precipitated by cetyltrimethylammonium bromide. The supernatant was fractionated by ammonium sulfate precipitation; the 50—65% saturation fraction was dissolved in 0.1 M phosphate buffer, pH 7.5, desalted by treatment with a sephadex G-25 column and further purified by fractionating on a DEAE-cellulose column employing a 0.0—0.5 M KCL-gradient for elution (Fig. 1). The active fractions were pooled and concentrated by means of a Diaflo-device. The final preparation had a 22fold increased specific activity compared with the crude extract and was used for kinetic studies.

Chemicals. All reagents were obtained from Merck, Darmstadt. The enzymes and biochemicals were purchased from C. F. Boehringer, Mannheim, Fructose-1.6-diphosphate was obtained from The British Drug Houses, Ltd., Poole.

KDPG and KDPG aldolase were kindly donated by Dr. K. H. Robra, Department of Microbiology of the University of Göttingen.

Results

Induction of the Fructose Degrading Enzymes

The specific activities of the key enzymes of the Entner-Doudoroff (ED) and the Embden-Meyerhof (EM) pathways were determined in cells grown either autotrophically or on gluconate or fructose salt medium. The results summarized in Table 2 indicate that the enzymes of the ED-pathway are present in gluconate—as well as in fructose-grown cells while in autotrophically grown cells 6-phosphogluconate dehydrase is completely absent and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase is present at a low activity. The ED-enzymes are apparently inducible and are formed in cells grown on fructose or gluconate. The induction by both these substrates suggests that not only gluconate is degraded via the ED-pathway, but fructose is as well. The regulation is, therefore, different from that in *Escherichia coli*, in which hexoses induce the EM glycolytic pathway while gluconate induces the ED-pathway (Eisenberg and Dobrogosz, 1967). The conclusion that hexoses are preferentially degraded via the ED-pathway is supported by the measurements of fructose diphosphate aldolase which has a high activity in autotrophically grown cells but a low activity in fructose-grown cells.

Table 2. Specific activities of important enzymes of carbohydrate metabolism in *Nocardia* strain *1b*

Enzyme	Specific activity (μmoles/g protein × min) after growth on		
	$H_2 + CO_2 + O_2$ (autotrophically)	Fructose 0.5%	Gluconate 0.5%
Hexokinase	31	29	29
Phosphoglucose isomerase	252	372	427
G-6-P dehydrogenase			
NADP	26	34	29
NAD	10	15	12
ED-system	0	81	40
6-PG dehydrase	0	82	43
KDPG aldolase	5	226	125
Gluconate dehydrase	0	0	0
KDG kinase	0	0	0
6-PG-dehydrogenase			
NADP	68	66	71
NAD	0	0	0
Phosphofructokinase	2	3	<1
FDP aldolase	195	29	38
+ 3.3 mM EDTA	131		
Fructose diphosphatase	38	33	45

The extremely low activity of phosphofructokinase lends further support to this conclusion.

Hexokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are present in the three extracts at rather low levels, each activity being independent on the growth substrate. These enzymes are, therefore, constitutive; this behaviour is different from the other autotrophic bacteria studied so far, and the constitutive enzyme formation indicates the importance of the pentose phosphate pathway for *Nocardia 1b*. In *H. eutropha H 16* glucose-6-phosphate dehydrogenase is inducible and 6-PG dehydrogenase is absent; in *P. facilis* both enzymes are inducible; however, in both organisms the oxidative pentose phosphate pathway does not function and does not contribute to the formation of RNA pentose (Bowien and Schlegel, 1972). The enzyme pattern found in *Nocardia 1b* raised the possibility that the classical ED-pathway may not be the only pathway of fructose degradation and may be supplemented by a second route. Gluconate dehydrogenase and 2-keto-3-deoxygluconate kinase could not be detected.

The fructose diphosphate aldolase of strain *1b* is strongly inhibited by EDTA and apparently belongs to the type II aldolase and requires magnesium for maximal activity. The enzyme is strongly repressed by fructose and gluconate and seems to function mainly within the reductive pentose phosphate cycle of CO_2-fixation.

Suppression of Substrate Utilization by Hydrogen

The suppression of the utilization of organic substrates by the presence of hydrogen seems to be a general phenomenon (Wilde, 1962; Gottschalk, 1965; Schlegel and Trüper, 1966). Many enzymes involved in the utilization of organic substrates are inducible, and their formation is repressed by hydrogen. All 58 strains tested responded in a similar fashion (Blackkolb and Schlegel, 1968a).

Experiments with *Nocardia 1b* revealed an almost identical response to hydrogen. Autotrophically grown cells were incubated in nutrient media containing either fructose, glucose, or gluconate as substrates and were shaken either under air or under a hydrogen-oxygen atmosphere (Fig. 2). Under air after a 2 to 5 h lag period the turbidity of the suspensions increased exponentially indicating synthesis of the catabolic enzymes and growth. In the presence of hydrogen no growth occurred within 10 h. These results were confirmed by measuring the consumption of glucose. Under air glucose was utilized exponentially, whereas hydrogen completely suppressed the utilization of glucose. From these experiments it can be concluded that *Nocardia 1b* belongs to those hydrogen bacteria in which hexose utilization is suppressed by hydrogen.

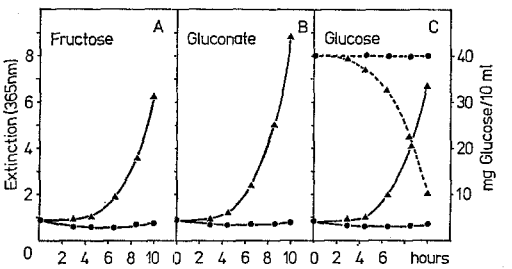

Fig. 2 A—C. Adaptation of autotrophically grown cells of *Nocardia* strain *1b* to organic substrates in the presence and absence of gaseous hydrogen in the atmosphere. Symbols: 80% hydrogen + 20% oxygen (•); air (▲); turbidity measurement ——; glucose concentration – – – –

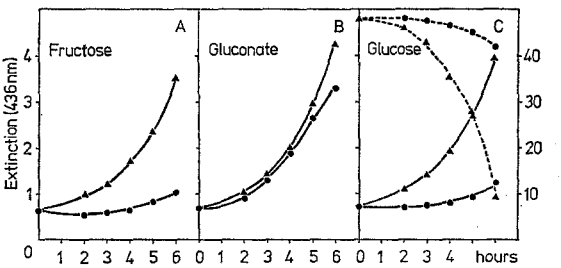

Fig. 3 A—C. Utilization of organic substrates by fully induced cells of *Nocardia* strain *1b* in the presence and absence of gaseous hydrogen in the atmosphere. Following autotrophic growth the cells had been incubated for 5 h in the presence of the corresponding organic substrates and were then exposed to a CO_2-free knallgas air-atmosphere. Symbols as in Fig. 2

Inhibition of Substrate Utilization by Hydrogen

Hydrogen not only represses the formation of the fructose degrading enzymes, but suppresses fructose utilization even in fully induced cells of *H. eutropha H 16* (Schlegel and Blackkolb, 1967; Blackkolb and Schlegel, 1968b). The inhibition of the rate of PHB-synthesis amounts to 85% compared with the control under air.

Similar experiments were carried out with cells of *Nocardia 1b* which after autotrophic growth had been incubated for 5 h in the presence of the corresponding substrate (glucose, fructose, or gluconate) under air for enzyme induction. After this pretreatment the cells were exposed to the hydrogen-oxygen atmosphere or to air. As shown in Fig. 3 A—C the cells grow exponentially under air; however, under the knallgas mixture growth on glucose and fructose is strictly inhibited and glucose utilization

is severely impaired (Fig. 3C). Growth on gluconate is not initially influenced by hydrogen; the decrease of the growth rate in the fifth and sixth h is due to the repression of enzyme formation by hydrogen.

Since the utilization of glucose is inhibited by hydrogen and that of gluconate is not, the inhibition should be localized in one of the enzymes catalysing the degradation of the hexose to 6-phosphogluconate, i.e. hexokinase of glucose-6-phosphate dehydrogenase. Furthermore, the inhibition is exerted solely by hydrogen, which is the source of energy and reducing power, and does not involve carbon dioxide. One could, therefore, assume that, analogous to the mechanism described for *H. eutropha H 16* (Blackkolb and Schlegel, 1968b), the inhibition exerted by hydrogen is due to an allosteric inhibition of glucose-6-phosphate dehydrogenase by nucleoside triphosphates or reduced pyridine nucleotides.

Preliminary experiments employing a crude extract of fructose-grown cells of *Nocardia 1b* as enzyme source indicated ATP to be an effective inhibitor of glucose-6-phosphate dehydrogenase. Furthermore, the enzyme turned out to be magnesium-dependent, and magnesium ions antagonized the action of ATP. For kinetic studies a 22fold purified enzyme preparation has been used.

The reaction catalyzed by glucose-6-phosphate dehydrogenase of *Nocardia 1b* is dependent of NADP or NAD. The ratio of the NADP-linked to the NAD-linked specific activity is 2.3 to 2.4 (Table 2). Since in the presence of both coenzymes the rate is decreased and since there is no additive effect of both single activities, one has to conclude that the preparation contains only a single glucose-6-phosphate dehydrogenase (Fig. 4). The enzyme is magnesium-dependent; the concentration for half maximal activity is $K_m = 2.7 \times 10^{-3}$ M magnesium chloride. The K_m value for glucose-6-phosphate is dependent on the magnesium ion concentration. In the absence of magnesium ions $K_m = 3.7 \times 10^{-3}$ M and in the presence of 4 mM $MgCl_2$ $K_m = 4.2 \times 10^{-4}$ M (Fig. 5). Both substrate saturation curves are hyperbolic and do not exhibit any indication of sigmoidity; in the Lineweaver-Burk plot straight lines are obtained.

The inhibition of glucose-6-phosphate dehydrogenase by ATP is less pronounced that in *H. eutropha H 16*. Therefore, substrate (G-6-P) saturation curves have been measured at rather high ATP concentrations (2 and 6 mM ATP). ATP does not affect the hyperbolic shape of the curve even at a 6 mM concentration (Fig. 6). In the Lineweaver-Burk double reciprocal plot straight lines are obtained with an intersection on the ordinate. This behaviour is indicative of a competitive inhibition, i.e. the inhibitor and the substrate compete with the active site of the enzyme. ATP does not alter the maximum velocity; only the K_m-value is increased: up to 6.7×10^{-4} M by 2 mM ATP and up to 5×10^{-3} M by 6 mM ATP. As expected from these data the Hill plot and the n-values

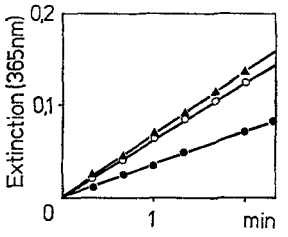

Fig. 4

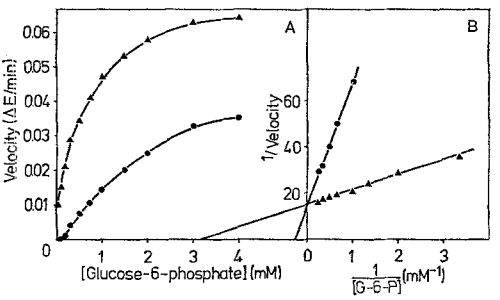

Fig. 5 A and B

Fig. 4. Coenzyme specificity of glucose-6-phosphate dehydrogenase. The assay mixture contained 3 mM glucose-6-phosphate, 4 mM $MgCl_2$ and 98 µg enzyme protein in 45 mM TEA-buffer, pH 7.6; 0.5 mM NADP (▲), 1.6 mM NAD (●), 0.5 mM NADP, and 1.6 mM NAD (○)

Fig. 5 A and B. Substrate (glucose-6-phosphate)-saturation curves for glucose-6-phosphate dehydrogenase from *Nocardia 1b* in the absence and presence of magnesium chloride. A Velocity-versus-substrate-concentration plot. B Lineweaver-Burk plot. The assay mixture had a final volume of 3.00 ml and contained in 45 mM TEA-buffer (pH 7.6) 0.5 mM NADP, 98 µg enzyme protein and G-6-P as indicated, without magnesium chloride (●), with 4 mM magnesium chloride (▲)

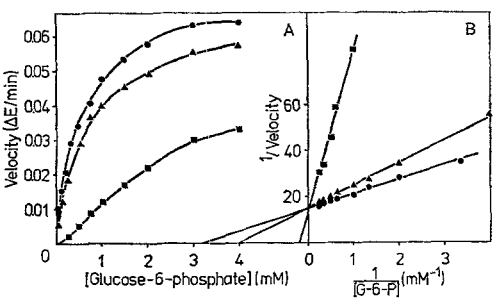

Fig. 6 A and B. Inhibitory effect of ATP on glucose-6-phosphate dehydrogenase from *Nocardia 1b*. A Velocity-versus-substrate-concentration plot. B Lineweaver-Burk plot. The assay mixture had a final volume of 3.00 ml and contained in 45 mM TEA-buffer (pH 7.6) 0.5 mM NADP, 4 mM $MgCl_2$, 98 µg enzyme protein and G-6-P as indicated, without ATP (●), in the presence of 2 mM ATP (▲) and 6 mM ATP (■)

do not reveal any cooperative binding sites. Obviously the enzyme exhibits neither homotropic nor heterotropic interactions. From the kinetic data the conclusion has to be drawn that the inhibition by ATP is due to competition with the substrate G-6-P at the same binding site.

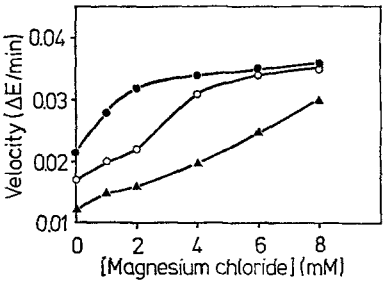

Fig. 7

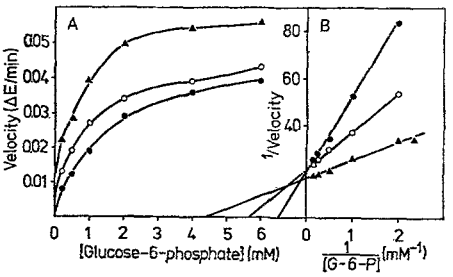

Fig. 8 A and B

Fig. 7. Effect of magnesium chloride on the activity of glucose-6-phosphate dehydrogenase from *Nocardia 1b* in the absence and in the presence of ATP. The assay mixture had a final volume of 3.00 ml and contained in 45 mM TEA-buffer (pH 7.6) 3 mM G-6-P, 0.5 mM NADP and 68 μg enzyme protein, without ATP (•), in the presence of 2 mM ATP (○) and 6 mM ATP (▲)

Fig. 8 A and B. Inhibitory effect of phosphoenolpyruvate on the activity of glucose-6-phosphate dehydrogenase from *Nocardia 1b*. A Velocity-versus-substrate-concentration plot. B Lineweaver-Burk plot. The assay mixture had a final volume of 3.00 ml and contained in 45 mM TEA-buffer (pH 7.6) 0.5 mM NADP, 4 mM $MgCl_2$, 68 μg enzyme protein and G-6-P as indicated, without PEP (▲), in the presence of 4.8 mM PEP (○) and 6 mM PEP (•)

By varying the concentrations of ATP and Mg^{++}-ions strong interactions between both ions became evident. With increasing concentrations of magnesium the inhibition exerted by ATP is relieved (Fig. 7). If $MgCl_2$ and ATP are present in equimolar concentrations the reaction is equal to that of the Mg-free control; this proportion is obtained with a 2 mM as well as a 6 mM concentration of ATP. Evidently a Mg-ATP-complex is formed at a molar ratio of 1:1, and this complex does not act as an inhibitor.

The recent finding of a PEP-sensitive glucose-6-phosphate dehydrogenase in an arthrobacter-like hydrogen bacterium strain *7C* (N. Tunail, unpublished results) gave rise to a set of experiments on the action of PEP on the *Nocardia* enzyme (Fig. 8). The inhibitory effect of PEP was, however, rather small even at high PEP concentrations (6 mM) and was not further pursued.

Discussion

From the pattern of enzyme activities after growth of *Nocardia opaca* strain *1b* on fructose, gluconate or after autotrophic growth, the following conclusions can be drawn. Fructose as well as gluconate is degraded via the oxidative pentose phosphate as well as the Entner-Doudoroff

pathway. While glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are present even in autotrophically grown cells and are apparently constitutive enzymes, the Entner-Doudoroff enzymes are inducible. The oxidative pentose phosphate pathway may, therefore, be involved in the formation of pentoses for nucleic acid synthesis. The rather low level of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrase were suggestive of the involvement of a modified Entner-Doudoroff pathway observed in a mutant strain of *Rhodopseudomonas spheroides* (Szymona and Doudoroff, 1960) and in *Clostridium aceticum* (Andreesen and Gottschalk, 1969); D-gluconate is dehydrated to 2-keto-3-deoxygluconate (KDG), afterwards phosphorylated to form 2-keto-3-deoxy-6-phosphogluconate (KDPG) and cleaved by KDPG aldolase. The key enzymes of this pathway, D-gluconate dehydrase and KDG kinase, have, however, not been detected in *Nocardia 1b*. The classical ED-pathway, is, therefore, in *Nocardia 1b* the only inducible catabolic way of fructose and gluconate utilization.

When hexose, fructose or glucose, as well as hydrogen are concomitantly available to fully induced cells growth is severely impaired and the degradation of glucose is inhibited. Since growth on gluconate is not influenced by hydrogen, the inhibition may be due to an effector-controlled action of glucose-6-phosphate dehydrogenase as previously shown for the enzymes from *Hydrogenomonas eutropha H 16* (Blackkolb and Schlegel, 1968b) and *Pseudomonas aeruginosa* (Lessie and Neidhardt, 1967), and *Rhodopseudomonas spheroides* (Ohmann *et al.*, 1969). Glucose-6-phsopahte dehydrogenase of *Nocardia 1b* is linked to NADP as well as to NAD. Since in the presence of both coenzymes no additive effect is observed, glucose-6-phosphate dehydrogenase appears to be a single enzyme. Parallel enzymes, one active with NADP or NAD and being markedly inhibited by ATP, and the other being specific for NADP and not affected by ATP, have been recently found in *Pseudomonas multivorans* (Lessie and van der Wyk, 1972).

Glucose-6-phosphate dehydrogenase of *Nocardia 1b* is inhibited by ATP. However, the substrate saturation curves exhibit no sigmoidal behaviour, either in the absence of ATP or in its presence. ATP acts as a competitive inhibitor increasing the K_m. Phosphoenolpyruvate affects the enzyme at similar high concentrations and in a similar fashion as ATP. The sensitivity of the enzyme to both metabolites is rather low, and the effective concentrations of ATP or PEP appear to be unphysiologically high. Whether the inhibitory effect of fructose degradation exerted by hydrogen in vivo is due to the inhibition of glucose-6-phosphate dehydrogenase, remains doubtful.

Magnesium ions relieve the inhibitory effect of ATP. The results are in accordance with the assumption that the Mg-ATP-complex does not

exert an effector-like function and the metabolic control is solely due to ATP concentrations in excess of the Mg-bound triphosphate (Blackkolb and Schlegel, 1968b).

References

Aggag, M., Schlegel, H. G.: Studies on a Gram-positive hydrogen bacterium, *Nocardia opaca* strain *1b*. Arch. Mikrobiol. **88**, 299–318 (1973).

Andreesen, J. R., Gottschalk, G.: The occurrence of a modified Entner-Doudoroff pathway in *Clostridium aceticum*. Arch. Mikrobiol. **69**, 160–170 (1969).

Beisenherz, G., Bolze, H. J., Bücher, Th., Czok, R., Garbade, K. H., Meyer-Arendt, E., Pfleiderer, G.: Diphosphofructose-Aldolase, Phosphoglyceraldehyd-Dehydrogenase, Milchsäure-Dehydrogenase, Glycerophosphat-Dehydrogenase und Pyruvat-Kinase aus Kaninchenmuskulatur in einem Arbeitsgang. Z. Naturforsch. 8b, 555–577 (1953).

Bender, R.: Anreicherung und Charakterisierung der D-Gluconat-Dehydratase von *Clostridium pasteurianum*. Diss., Göttingen 1972.

Blackkolb F., Schlegel, H. G.: Katabolische Repression und Enzymhemmung durch molekularen Wasserstoff bei *Hydrogenomonas*. Arch. Mikrobiol. **62**, 129–143 (1968a).

Blackkolb, F., Schlegel, H. G.: Regulation der Glucose-6-phosphat-Dehydrogenase aus *Hydrogenomonas H 16* durch ATP und $NADH_2$. Arch. Mikrobiol. **63**, 177–196 (1968b).

Bowien, B.: Hexoseabbau und Pentosebiosynthese in *Hydrogenomonas*. Diss., Göttingen 1970.

Bowien, B., Schlegel, H. G.: Der Biosyntheseweg der RNS-Ribose in *Hydrogenomonas* Stamm *H 16* und *Pseudomonas facilis*. Arch. Mikrobiol. **85**, 95–112 (1972).

Eisenberg, R. C., Dobrogosz, W. J.: Gluconate metabolism in *Escherichia coli*. J. Bact. **93**, 941–949 (1967).

Gottschalk, G.: Die Verwertung organischer Substrate durch *Hydrogenomonas* in Gegenwart von molekularem Wasserstoff. Biochem. Z. **341**, 260–270 (1965).

Gottschalk, G., Eberhardt, U., Schlegel, H. G.: Verwertung von Fructose durch *Hydrogenomonas H 16*. Arch. Mikrobiol. **48**, 95–108 (1964).

Lessie, T., Neidhardt, F. C.: ATP-linked control of *Pseudomonas aeruginosa* glucose-6-phosphate dehydrogenase. J. Bact. **93**, 1337–1345 (1967).

Lessie, T. G., Van der Wyk, J. C.: Multiple forms of *Pseudomonas multivorans* glucose-6-phosphate and 6-phosphogluconate dehydrogenases: Differences in size, pyridine nucleotide specificity, and susceptibility to inhibition by adenosine 5′-triphosphate. J. Bact. **110**, 1107–1117 (1972).

Ling, K. H., Byme, W. L., Laroly, H.: Phosphohexokinase. In: Methods in enzymology, S. P. Colowick and N. O. Kaplan, eds., Vol. I, pp. 306–310. New York: Academic Press 1955.

Ohmann, E., Rindt, K. R., Borris, R.: Glucose-6-phosphat-Dehydrogenase in autotrophen Mikroorganismen. I. Regulation der Synthese in *Euglena gracilis* und *Rhodopseudomonas spheroides* in Abhängigkeit von den Kulturbedingungen. Z. allg. Mikrobiol. **9**, 557–564 (1969).

Racker, E., Schroeder, E. A. R.: The reductive pentosephosphate cycle. II. Specific c_1-phosphatases for fructose-1.6-diphosphate and seduheptulose-1.7-diphosphate. Arch. Biochem. **74**, 326–344 (1958).

Schlegel, H. G., Blackkolb, F.: Regulation des Hexoseabbaus über den Entner-Doudoroff-Weg durch Enzymhemmung. Nachr. Akad. Wiss. Göttingen, II. Math. Physik. Kl. **1967**, 51–60.

Schlegel, H. G., Eberhardt, U.: Regulatory phenomena in the metabolism of Knallgasbacteria. Advanc. Microbiol. Physiol. **7**, 205—242 (1972).

Schlegel, H. G., Kaltwasser, H., Gottschalk, G.: Ein Submersverfahren zur Kultur wasserstoffoxidierender Bakterien: Wachstumsphysiologische Untersuchungen. Arch. Mikrobiol. **39**, 209—222 (1961).

Schlegel, H. G., Trüper, H. G.: Repression of enzyme formation in *Hydrogenomonas* strain *H 16* G^+ by molecular H_2 and fructose. Antonie v. Leeuwenhoek **32**, 277—292 (1966).

Siebert, D.: Über propanverwertende, wasserstoffoxidierende Bakterien und die Charakterisierung eines Förderungsfaktors. Diss., Göttingen 1969.

Szymona, M., Doudoroff, M.: Carbohydrate metabolism in *Rhodopseudomonas spheroides*. J. gen. Microbiol. **22**, 167—183 (1960).

Warburg, O., Christian, W.: Isolierung und Kristallisation des Gärungsfermentes Enolase. Biochem. Z. **310**, 384—421 (1941).

Wilde, E.: Untersuchungen über Wachstum und Speicherstoff-Synthese von *Hydrogenomonas*. Arch. Mikrobiol. **43**, 109—137 (1962).

Dipl.-Biol. Irmelin Probst
Prof. Dr. H. G. Schlegel
Institut für Mikrobiologie
der Gesellschaft für Strahlen- und
Umweltforschung mbH, München
D-3400 Göttingen, Grisebachstr. 8
Federal Republik of Germany