Metabolic Pathways in Microorganisms

By Vernon H. Cheldelin

CHEMISTRY OF MICROBIAL PRODUCTS

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PREFACE

I am happy to have the opportunity to present this work on pathways of carbohydrate metabolism, which has been carried out, for the most part, in our laboratories (Science Research Institute, Oregon State University) during the last eight years. This work emphasizes heavily the peculiarities of metabolism that characterize the acetic acid bacteria, as well as emphasizing observations on microbial systems, which lend themselves especially to radiorespirometric studies. Finally, it presents a general discussion on various aspects of metabolism, which seems appropriate in this series of lectures. Because of the personal nature of the experiences described, this will not represent an attempt to review exhaustively the literature in the field, although it is hoped that this work may be of some aid in that direction.

Being selected as a lecturer to continue the series ably begun by the other speakers in the Squibb series produces in one mixed feelings—diffidence, yet at the same time the need for confidence to provide some useful thoughts about

the subject chosen for discussion. The first reaction is spontaneous, and is so well recognized by everyone as to require no further elaboration. In marshaling my confidences to present this material, I am reinforced by some of the thoughts laid before me in my graduate study by Professor Roger J. Williams, to whom these lectures are dedicated, plus the sound, carefully fabricated experimental work by many of my colleagues over a period of fifteen years. Among these are Drs. Tsoo E. King, Chih H. Wang, and R. W. Newburgh, to mention only three, and also many former graduate students who have contributed to the over-all knowledge about the problem, especially Drs. Jens G. Hauge, Paul A. Kitos, and Joseph T. Cummins. To these persons, and others of our group, I am deeply grateful.

V. H. CHELDELIN

August, 1961

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CHAPTER

1

THE ACETIC ACID BACTERIA

These bacteria, like several other species of microorganisms, have been utilized by man since antiquity; wild cultures invade cider, for example, to promote the acetic acid fermentation, and thus have been responsible for commercial vinegar production.

Acetobacter suboxydans, one of the better known members of this group, was discovered in 1924 by Kluyver and de Leeuw (1). Its discovery did much to broaden the industrial uses for the acetic acid bacteria, for in addition to the vinegar fermentation it carried out several others, mostly one- or two-step oxidations of polyols (2):

Mannitol → fructose		(1)
Sorbitol → sorbose		(2)
Glycerol → dihydroxyacetone		(3)
Erytheritol → erythrulose		(4)
Glucose → gluconic acid → ketogluconate		(5)
2,3-Butanediol → acetoin		(6)

as well as several others. The organism was at first regarded as a most versatile one, being adaptable to so many substrates; but, it may be more nearly correct to consider it as a metabolic "cripple," not particularly better able to attack substrates than many other organisms, and unable to oxidize these beyond the first one or two steps. Perhaps the chief reason for this is the fact, which will be discussed later, that the organism has no Krebs cycle, and thus lacks the "prairie fire" of terminal oxidation that most other organisms, whether men or mice, enjoy.

Our interest in this organism began, however, not with the oxidations that it carried out, but with finding a reason for its unusual pantothenic acid requirement. An earlier lecturer in this series, Dr. Frank M. Strong (3), has described much of the literature dealing with coenzyme A, so I will dwell on it only for a moment. Suffice it to say here that this organism is ten to twenty times as sensitive to bound forms of pantothenic acid [coenzyme A, pantetheine (*L. bulgaricus* factor, LBF), pantothenyl cysteine] as it is to the free vitamin (4). This fact was discovered in our laboratory, where it gave rise to the description of a pantothenic acid conjugate which we abbreviated PAC (5). This conjugate was not fully characterized, but it is now regarded as a fragment of the coenzyme A molecule. The enhanced activity of conjugates of pantothenic acid toward *A. suboxydans* has been of aid to various investigators in their studies of derivatives of pantoic acid that lead to coenzyme A (3, 6, 7).

The observed superiority of coenzyme A over the free vitamin as a growth promoting agent, may be rationalized by the fact that cells grown deficient in pantothenate (and hence coenzyme A) have a lower lipid content than normal

cells. In view of the known function of this coenzyme in fat formation (8) this seems reasonable. Other activities, such as glucose oxidation, appear not to be influenced by coenzyme A deficiency, although the oxidation of glycerol is markedly reduced (see Fig. 1.1) (9) in a manner that is not yet understood.

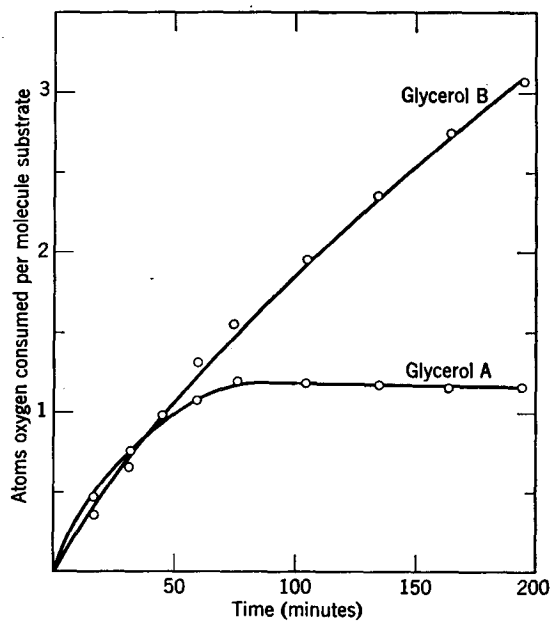

Fig. 1.1. Glycerol oxidation by pantothenate-deficient *A. suboxydans* cells. Deficient cells were grown in 0.0006 γ pantothenic acid equivalent per milliliter of medium. This concentration permitted growth of only 0.33 g. per liter, in contrast to sufficient cells which were grown in excess CoA and yielded a crop of over 1 g. per liter.

CARBOHYDRATE AND POLYOL OXIDATIONS

Testing of various carbon compounds soon revealed that several were inert, whereas others were susceptible to attack to only a limited degree. Table 1.1 indicates the extent of such oxidations. Where extensive oxidation oc-

TABLE 1.1

Oxidation of Various Substrates by *A. suboxydans*

	O_2 Consumption: μatoms/μmole	
	Dinitrophenol Addition	
Substrate	0	$1 \times 10^{-4}M$
Glycerol	3.7	1.0
Dihydroxyacetone	3.0	0.3
Ethanol	1.9	2.0
Acetaldehyde	1.0	
Sorbitol	4.0	1.2
Erythritol	1.0	
Pyruvate	0.9	
Lactate	2.0	
Acetate	0	
Ketoglutarate	0	
Malate	0	
Succinate	0	
Fumarate	0	
Citrate	0.4	

The systems contained $0.05M$ phosphate, $0.01M$ $MgCl_2$, $10^{-4}M$ DPN, and 10 mg. dry weight of washed cells. Volume = 2.8 ml., pH = 6.0, temperature = 29° C. The substrate was tipped into the main compartment containing dinitrophenol ($10^{-4}M$) after 5 minutes' preincubation. All values corrected for endogenous blanks, which were about 0.1 μatom oxygen/μmole substrate.

curred, as in glycerol or sorbitol, this could be reduced to one atom of oxygen per molecule of substrate by including dinitrophenol in the medium. The further oxidation of the one-step oxidation products (dihydroxyacetone or sorbose) was virtually completely repressed in dinitrophenol solutions, which presumably prevented coenzyme-linked phosphorylations.

The cells were therefore broken, and cell-free preparations were made. This was accomplished either by disintegrating the cells in a 10-kc. Raytheon sonic oscillator or by grinding with alumina. The broken cell suspension was mixed with phosphate buffer and centrifuged at 20,000*g* for an hour; then the residue was re-extracted and the combined extracts pooled.

Particulate Enzymes

These extracts revealed that the oxidizing enzymes of *A. suboxydans* varied considerably, more than had been suspected. Two, and sometimes three, systems existed side by side in the organism for the breakdown of individual polyhydroxy compounds. There were, for example, a number of particle-bound, phosphate-independent dehydrogenases (10) which oxidized mannitol, sorbitol, erythritol, glycerol, and glucose to the extent of one atom of oxygen per molecule of substrate. Two atoms of oxygen were used per molecule of ethanol or propanol. The dehydrogenases appeared different from each other, since purification of the particulate suspensions effected a ten-fold increase in the concentration of glucose dehydrogenase (oxidase), four-fold for erythritol, three-fold for glycerol, but did not affect the concentration of ethanol dehydrogenase.

δ-D-Gluconolactone has been indicated as the product of

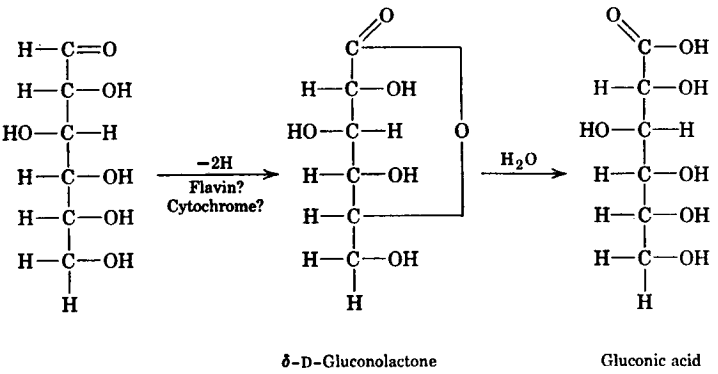

Fig. 1.2. Oxidation of glucose by particulate glucose oxidase in *A. suboxydans.*

D-glucose oxidation by a particulate enzyme (see Fig. 1.2). The optimum pH was 5.5 (11). A separate enzyme also exists in this fraction, which hydrolyzes the lactone to D-gluconic acid. Deoxycholate extracts of the particulate fraction retained activity for oxidation of glucose, but they no longer contained the hydrolyzing enzyme.

"Soluble" Enzymes

In addition, the cell-free extracts contained several *soluble* enzymes that cooperate to effect the *terminal* oxidation of glucose, other carbohydrates, and polyols. These were, for the most part, phosphate-dependent and DPN- or TPN-linked. Upon fractionation, the extracts were found to contain the entire pentose cycle complex of enzymes (Fig. 1.3).

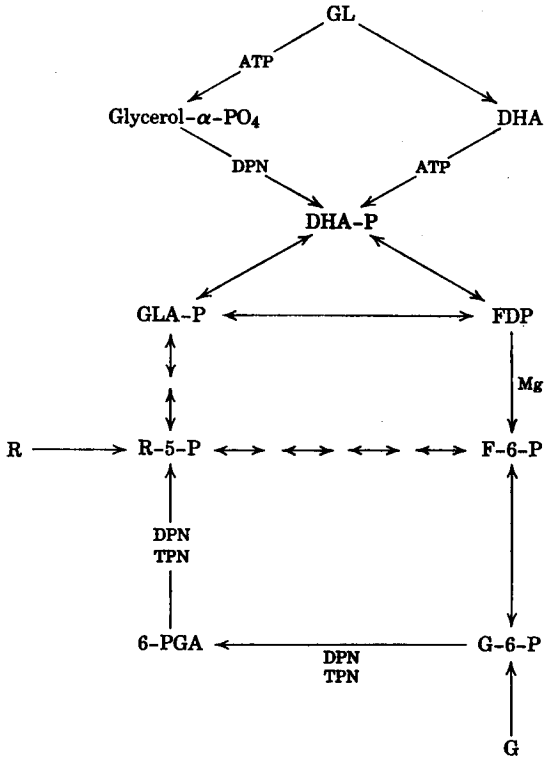

Fig. 1.3. The pentose cycle in *A. suboxydans.*

Glucose. The first example of a soluble dehydrogenase is an exception to the generalization just stated: phosphate does not participate in glucose oxidation to gluconate, though pyridine nucleotide (TPN) does. The soluble dehydrogenase catalyzing this oxidation is a separate enzyme from the particulate enzyme already discussed, since it has

its pH optimum at 8.6. We have purified this enzyme about 100-fold and have found it to be strictly TPN-specific. The reaction is reversible, as tested with δ-D-gluconolactone and TPNH, but the γ-lactone is less active. Among the sugars and their phosphates tested, only D-glucose and 2-deoxy-D-glucose were oxidized, whereas glucose-6-phosphate, glucose-1-phosphate, gluconolactone, and gluconic acid were not attacked. This enzyme therefore appears different from those described by de Ley and Stouthamer (12) for this organism in the oxidation of gluconate.

Glucose-6-Phosphate and 6-Phosphogluconate. These two soluble enzymes usually are closely associated in *A. suboxydans,* as they are in most organisms. They have been highly purified, to the point that they catalyze the disappearance of about 100 and 50 μmoles of substrate per minute, respectively, per milligram of enzyme. With a pH optimum at 8.0, the turnover numbers for G-6-P dehydrogenase are about equal with DPN and TPN, whereas DPN is much superior with 6-phosphogluconic dehydrogenase. The purifications are effected by combinations of protamine precipitation, ammonium sulfate fractionation, calcium phosphate gel adsorption, and column electrophoresis (13). A comparison of properties of G-6-P dehydrogenase from various sources is given in Table 1.2, whereas Table 1.3 contains a similar listing of properties of 6-PGA dehydrogenases.

For clarification, the enzymes that oxidize glucose or glucose-6-phosphate, as reported from different laboratories, are listed in Table 1.4 along with some of their distinguishing properties. It is evident from Table 1.4 that *A. suboxydans* is well endowed with separate enzymes all of which attack these substrates. The reasons for this diversity are not yet clear. It is possible that there is little or no

TABLE 1.2

Comparison of Characteristics of Glucose-6-Phosphate Dehydrogenase from Different Sources

Source	Coenzyme Specificity	Fold Purification	Highest Specific Activity *	Optimum pH	Substrate K_m	Reference
Yeast	TPN, Mg, or Mn	600–900	86	8.5	$2.0–6.9 \times 10^{-5}$	14
E. coli	TPN, Mg	11	7	7.7–8.6	3×10^{-4}	15
A. suboxydans	TPN or DPN	100	100	8.0	3.9×10^{-4} (DPN)	
Mammary gland	TPN	10,000 (crystalline)	420			16

* 1 unit = amount causing 1.0 optical density change at 340 mμ. Specific activity = units/minute/milligram protein.

TABLE 1.3

Comparison of Characteristics of 6-Phosphogluconate Dehydrogenase from Different Sources

Source	Coenzyme Specificity	Separation of 6-PGA and G-6-P Dehydrogenases	Highest Specific Activity *	Optimum pH	Substrate K_m	Reference
Yeast	TPN	30% G-6-P dehydrogenase	15.6	7.4	5×10^{-5}	17
Leuconostoc	DPN		2.0	7.8	7.8×10^{-5}	18
E. coli	TPN	4% G-6-P dehydrogenase	2.7	6.6–7.7	3×10^{-5}	15
A. suboxydans	TPN or DPN	Complete	50	8.0	2.3×10^{-4} (DPN) 1.3×10^{-3} (TPN)	

* 1 unit = amount causing 1.0 optical density change at 340 mμ. Specific activity = units/minute/milligram protein.