PENTOSE METABOLISM IN BACTERIA

B. L. Horecker

CIBA Lectures in Microbial Biochemistry

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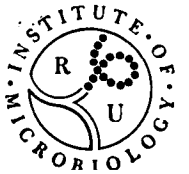

PENTOSE METABOLISM IN BACTERIA

BY B. L. HORECKER

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PREFACE

The opportunity to present this series of lectures on pentose metabolism, which was delivered at Rutgers University in the spring of 1962, came at a most appropriate time. During the past thirty years there has accumulated a large body of information relating to the role of the five-carbon sugars in the economy of microorganisms and higher forms. The impetus for these studies stemmed largely from the pioneering work of Otto Warburg and Frank Dickens on biochemical aspects, and the even earlier work of E. B. Fred and W. H. Peterson and their associates on fermentation products. It was my good fortune to find myself an active participant in the later developments in pentose metabolism and to share in the exciting explorations and discoveries which marked this period. We have now reached the point of recognizing nearly all of the intermediates and enzymes involved in pentose metabolism, although the fact that some important developments have been very recent might suggest that our work along these lines is not yet completed. In

any event, this is certainly an appropriate time for a review of these metabolic pathways and for a beginning of attempts to relate these to the problems of cell function and development. It is clear that much is yet to be learned from studies of comparative biochemistry and from efforts to correlate metabolic pathways with cell structure and function.

For the enzymologist this is definitely a period of transition. In the past enzymes have usually been regarded as tools, to be used to reproduce in vitro under controlled conditions the events occurring in the cell. But of the enzymes themselves we know very little, and in this respect our work is yet before us. Whereas the nucleic acids represent the machinery for preserving and transmitting the precious information required for protein synthesis and while the substrates provide both the fuel and the substance for this synthesis, it is the protein catalysts, the enzymes, which are the end and object of living processes. What confers upon these enzyme proteins their powerful catalytic activity? This is the great challenge for the future. We know from the classical work of Henri and Michaelis that proteins combine with their substrates but we understand little of the chemical basis of this affinity and even less of the basis for specificity and catalytic activity.

Present methods for the isolation of pure, often crystalline, enzymes have brought us to the point where the study of enzyme mechanisms at the molecular level is no longer entirely beyond our reach. The highly automated methods of amino acid analysis and sequence determination are being applied to larger and larger protein molecules. Today we find enzymologists in increasing numbers turning their attention to problems of enzyme mechanisms. In this series of lectures on the metabolism of pentoses I have attempted both to review past accomplishments and to indicate a beginning for future work. We have been fortunate in our study of the aldolases to find a way of tagging the active site of these

enzymes in a manner which leaves little doubt as to the relation of this site to the activation of the substrate molecule. It seems now only a matter of time before a picture of the structures and chemical forces involved will begin to emerge.

I am very grateful to CIBA for providing me with the opportunity to pass three very pleasant days of stimulating discussion at the Institute of Microbiology, and to the members of the Institute I would like to express my heartfelt thanks for their warm reception and kind hospitality.

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January 1963

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CHAPTER

1

OXIDATIVE PATHWAYS

Occurrence of Pentoses in Nature. The existence of five-carbon sugars in nature has been recognized for many years, since their discovery in plant materials[1] and the isolation of D-ribose from nucleic acid by Levene and Jacobs.[2] Pentoses occur in plants both as the free sugars and in the form of polysaccharides called pentosans. Most important in nature, from a quantitative point of view, are D-xylose and L-arabinose in the plant polysaccharides, and D-ribose and 2-deoxy-D-ribose in the nucleic acids. The linear formulas of these sugars are shown in Fig. 1.

Several ketoses must be added to this list of naturally occurring pentoses: most important is L-xylulose, which is excreted in large quantities in the congenital disease pentosuria. Other ketopentoses occur as phosphorylated intermediates but do not ordinarily appear in nature as the free sugars.

Two aspects of pentose metabolism are important in microbiology. In the first place, all cells must be provided with, or must be able to synthesize, D-ribose and 2-deoxy-

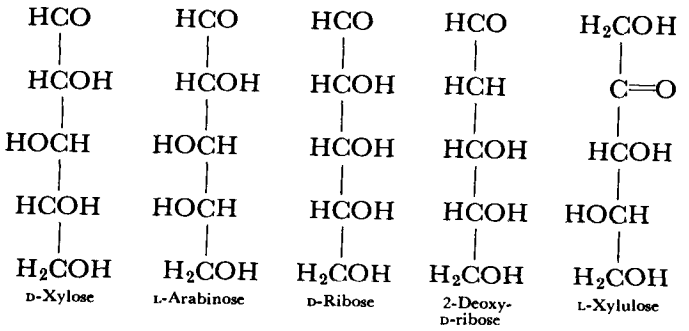

Fig. 1. Linear formulas for the more common naturally occurring pentoses.

D-ribose, which are essential components of the nucleic acids. Few, if any, living organisms are unable to synthesize these sugars, and indeed in most cells more than one metabolic pathway from hexose to pentose is present.

Commonly, catabolic pathways for these sugars are present as well, but these mechanisms are relatively unimportant since rarely do microorganisms depend on ribose or deoxyribose for carbon and energy. On the other hand, L-arabinose and D-xylose are plentiful in plant materials and thus provide an excellent source of carbon and energy for growth. It is not surprising to find that these are precisely the pentoses which are most commonly utilized by bacteria and molds. D-Arabinose, which structurally resembles D-glucose much more closely than does L-arabinose, is rarely a good growth substrate, although it can be utilized by some organisms. The same is true for L-xylose and the lyxoses, which are rarely, if ever, encountered in nature.

The Oxidative Pathway. Nearly thirty years ago, Warburg and his coworkers[3, 4, 5] discovered the oxidation of glu-

cose 6-phosphate to 6-phosphogluconate in both red cell hemolysates and yeast autolysates. Later it was observed that 6-phosphogluconate was further metabolized in yeast extracts[6, 7] and that among the products were CO_2 and a substance with properties of pentose phosphate. It was first suggested by Dickens[8] that this might be ribose 5-phosphate.

A new coenzyme was found to be necessary for these reactions, distinct from the coenzyme of alcoholic fermentation, which had been discovered earlier by Harden and Young[9] and named cozymase by von Euler and Myrbäck.[10] Cozymase had been shown to contain adenylic acid;[11] Warburg and his coworkers found that both coenzymes also contained nicotinamide[5, 12] and named them diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), since they were distinguished only by the fact that the new coenzyme contained 3, rather than 2, phosphate groups. Thus from its discovery TPN was associated with oxidative pathways as a counterpart of DPN in the fermentative systems.

The oxidation of glucose 6-phosphate leads ultimately to ribose 5-phosphate, with ribulose 5-phosphate as an intermediate (Fig. 2).[8, 13, 14, 15] The dehydrogenases, glucose 6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase, are found in all aerobic organisms, including *Escherichia coli, Azotobacter vinlandii, Pseudomonas* sp., and others. Ribose 5-phosphate isomerase, which catalyzes the reversible conversion of ribulose 5-phosphate to ribose 5-phosphate, is even more widely distributed and has been detected in a wide variety of aerobic and anaerobic microorganisms.[16]

Warburg attempted to account for the separate functions of two similar pyridine nucleotide coenzymes in the same cell by referring to TPN as the "wasserstoffübertragendes coferment" in contrast to DPN, the coenzyme of fermentation. This difference appeared to be particularly obvious in the erythrocyte, where lactic acid production was clearly depend-

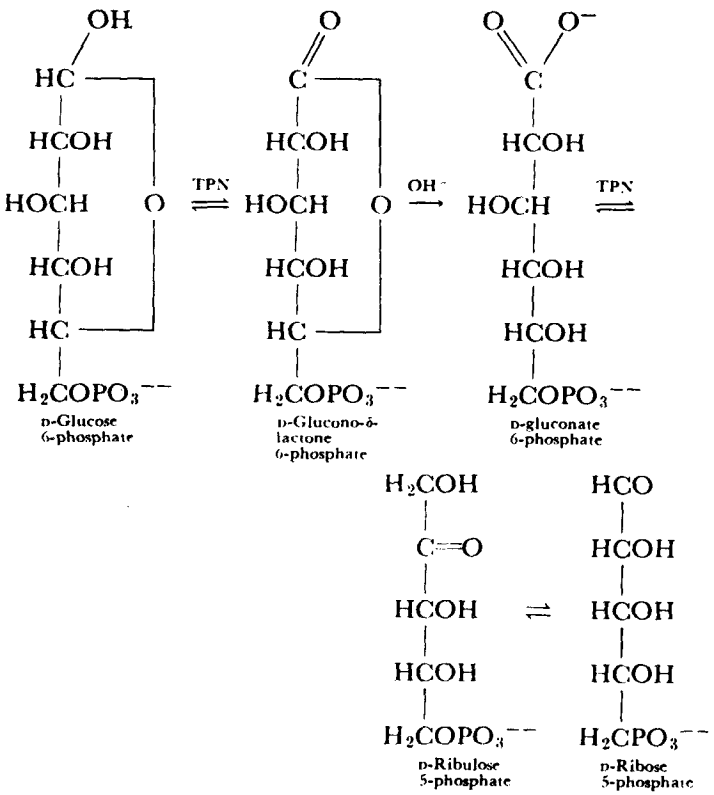

Fig. 2. The oxidation of glucose 6-phosphate and the formation of ribose 5-phosphate.

ent upon DPN, in contrast to respiration induced by the addition of methylene blue or phenyl hydrazine, which was TPN-linked and involved the oxidation of glucose 6-phosphate to 6-phosphogluconate. This notion led to an intensive search for a link between TPNH and cytochrome c and resulted in

the isolation of TPN-cytochrome reductase, first from yeast[17] and later from liver.[18]

It soon became clear, however, that the physiologically important coenzyme link to oxygen through the cytochromes is DPN, rather than TPN (Fig. 3). The oxidation of DPNH in mitochondria was found to be a rapid reaction, coupled to the esterification of ATP.[19] The oxidation of TPNH, on the other hand, was relatively sluggish and proceeded without coupled phosphorylation.[20]

These discoveries led to a reevaluation of the role of the two pyridine nucleotides.[21] There remained little doubt about the role of DPN as the specific coenzyme of glycolysis (in animal tissues) and fermentation (in microorganisms) while in the same cells it was the coenzyme of the citric acid cycle and therefore of respiration. What, then, was the function of the TPN-linked dehydrogenases? Of these the most important were the two dehydrogenases of the pentose phosphate pathway, which have already been mentioned, and isocitric dehydrogenase. The last enzyme catalyzes the only step in the citric acid cycle which appears to utilize TPN.

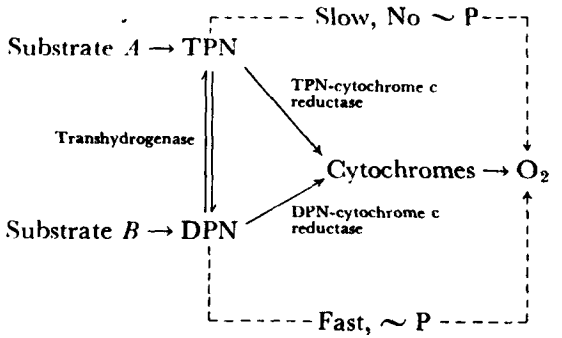

Fig. 3. Oxidation of the pyridine nucleotides.

In order to understand the function of TPNH, it is necessary to examine some of the reactions which it catalyzes. It immediately becomes evident that most of these reactions proceed in vivo in the direction of TPNH oxidation and therefore of reduction of substrate (Table 1). The only

TABLE 1

Examples of Reductive Reactions Utilizing TPNH

Reaction	Cellular Function
$\text{Pyruvate} + CO_2 \xrightarrow{\text{TPNH}} \text{malate}$	Glycogen synthesis
$\text{Crotonyl CoA} \xrightarrow{\text{TPNH}} \text{butyryl CoA}$	Fatty acid synthesis
$\alpha\text{-Ketoglutarate} + NH_3 \xrightarrow{\text{TPNH}} \text{glutamate}$	Protein synthesis
$\text{GSSG} \xrightarrow{\text{TPNH}} 2\text{GSH}$	Cell structure (?)

important exceptions are the TPN-linked dehydrogenases for D-glucose 6-phosphate, D-gluconate 6-phosphate, and isocitrate, where the equilibrium favors oxidation of substrate. The reductive carboxylation of pyruvate is an essential step in glycogen synthesis in animal cells[22] and probably participates also in the synthesis of the glucose-carbon chain in bacteria grown on alanine or lactate. The reductive amination of α-ketoglutarate in animal cells is catalyzed by either DPN or TPN;[23] on the other hand the yeast and *Escherichia coli* enzymes[24] are specific for TPNH and it is likely that this is the major reaction for amino acid synthesis in most microorganisms.* The

* A recent report by Klingenberg and Pette [*Biochem. Biophys. Res. Commun.*, **7**, 430 (1962)] provides interesting evidence for this role of TPNH in animal systems. They examined the levels of TPN, DPN, and glutamic dehydrogenase in a variety of rat and insect tissues and found a constant relation between the levels of TPN and activity of glutamic dehydrogenase. This activity was not related to the level of DPN.

last reaction shown in Table 1 is an example of what may be an important role of TPNH, namely the maintenance of essential SH groups in the reduced state. Nickerson and Falcone have implicated this type of reaction in cell division in yeast.[25]

The function of TPNH is therefore to provide reduced coenzymes for essential reduction reactions. The need for this second coenzyme is a consequence of the aerobic metabolism of the cell. Thus a cell which possesses two coenzymes is able to use one to establish a rapid and efficient transfer of electrons to oxygen without at the same time losing the ability to synthesize highly reduced cell components. We know from the studies of Glock and McLean[26] and Jacobson and Kaplan[27] that in normal respiring cells DPN is found largely in the oxidized form, while the reverse is true for TPN, which is present almost entirely as TPNH (Table 2). Jacobson and

TABLE 2

Oxidation States of Pyridine Nucleotides in Rat Liver

Ratio	Jacobson and Kaplan (1957)[27] μg/g tissue	Glock and McLean (1955)[26] μg/g tissue	% in Oxidized Form
$\frac{DPN}{DPNH}$	$\frac{421}{150}$	$\frac{370}{204}$	64–73
$\frac{TPN}{TPNH}$	$\frac{49}{352}$	$\frac{6}{205}$	3–12

Kaplan found this relation to hold even in the particulate portion of the cell, where DPN-cytochrome oxidase and transhydrogenase are located. We must conclude, therefore, that ordinarily transhydrogenase does not effectively catalyze the

oxidation of TPNH by DPN and that TPNH is not oxidized by the cytochrome pathway. In mitochondria, reduced TPN is probably formed by the action of isocitric dehydrogenase. However, most of the synthetic reduction mechanisms, such as fatty acid synthesis, are to be found in the nonparticulate portion of the cell, and here TPNH is produced at the expense of oxidation of glucose 6-phosphate and 6-phosphogluconate. A recent report from Lowry's laboratory[28] raises some questions about the state of TPN in the living cell and suggests that it may be considerably more oxidized.

The Nonoxidative Pentose Phosphate Pathway. The reactions shown in Fig. 2 serve the dual function of generating TPNH for synthetic mechanisms and at the same time provide ribose 5-phosphate essential for the production of nucleic acid and coenzymes. In most cells, however, this is an unreliable and inadequate source, since it depends on the rate at which TPNH is reoxidized. In the majority of microorganisms pentose phosphate is formed from hexose monophosphate by a nonoxidative mechanism.

The existence of this pathway for the interconversion of hexose and pentose phosphate was discovered by Dische[29] more than twenty years ago, when he observed that red cell hemolysates catalyzed the phosphorolytic cleavage of adenosine and the conversion of the ribose portion of the molecule to a mixture of triose and hexose phosphates. This work was extended by Schlenk and Waldvogel,[30] who isolated and identified glucose 6-phosphate as a product of ribose 5-phosphate metabolism in liver extracts. The first evidence for these reactions in microorganisms was provided by Racker[31] and by Sable,[32] who demonstrated with extracts of *Escherichia coli* and yeast, respectively, that triose phosphate was formed from ribose 5-phosphate. These observations suggested that pentose phosphate was undergoing a C_2-C_3 split and that the C_3-fragments were converted to hexose monophosphate by

way of fructose diphosphate (Fig. 4) and led to an intensive but unsuccessful search for the hypothetical C_2-fragment.

The hypothesis itself was brought into question by Dische's report[33] that hemolysates which produced hexose monophosphate from ribose 5-phosphate did not catalyze the hydrolysis of fructose diphosphate. Furthermore, careful balance experiments carried out by Dische[34] and by Glock[35] proved that hexose formed accounted for significantly more than three-fifths of the pentose carbon atoms. This required that at least a portion of the C_2-fragment be utilized for hexose synthesis.

Purification of the enzyme system responsible for pentose phosphate conversion to hexose monophosphate revealed that at least two enzymes were involved. Studies in a number of laboratories[36–44] (for an excellent review see Dickens[44]) revealed the following facts concerning the first of these enzymes: (1) the first step in pentose phosphate cleavage is catalyzed by a diphosphothiamine enzyme which has been named transketolase; (2) the substrate for cleavage is not ribose 5-phosphate, but xylulose 5-phosphate; (3) the reaction requires both a substrate for cleavage and an acceptor to combine with the C_2-fragment, which otherwise remains tightly

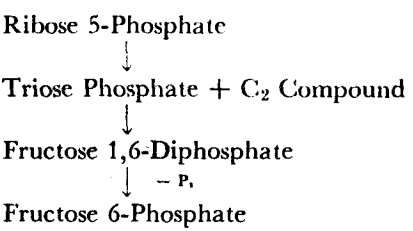

Fig. 4. Hypothetical pathway for hexose monophosphate formation from pentose phosphate.

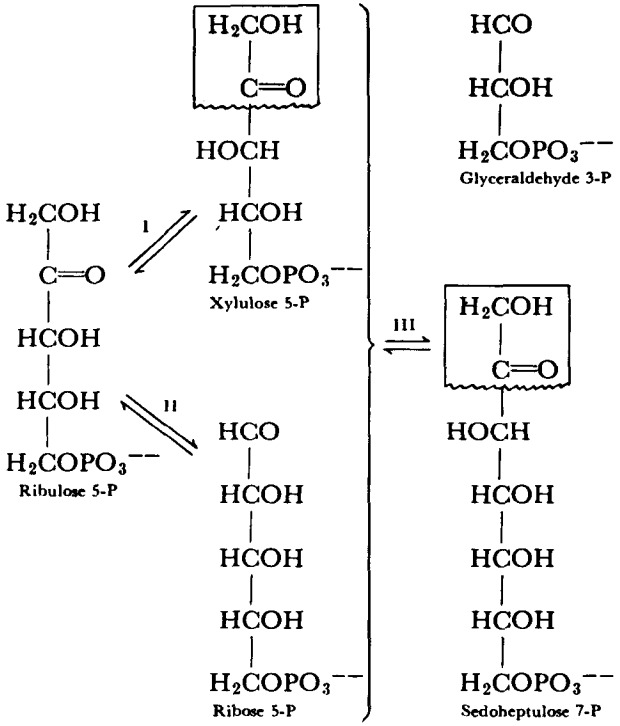

Fig. 5. The formation of sedoheptulose 7-phosphate from pentose phospate.

attached to the enzyme-diphosphothiamine complex; (4) ribose 5-phosphate can serve as such an acceptor, in which case the end product is sedoheptulose 7-phosphate. These facts are summarized in Fig. 5.

Ribulose 5-phosphate, formed in the oxidation of D-gluconate 6-phosphate, is converted to xylulose 5-phosphate by the enzyme D-xyluplose 5-phosphate 3-epimerase (1),[45-51] and to