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BERNARD L. HORECKER and EARL R. STADTMAN

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J. ZAPF, E. R. FROESCH, AND R. E. HUMBEL

Regulation of the Histidine Operon: Translation-Controlled Transcription Termination (A Mechanism Common to Several Biosynthetic Operons)

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I. Introduction

Most bacterial species have the ability to synthesize the amino acid histidine. The most extensive studies on this pathway have been conducted in the enteric bacteria, *Salmonella typhimurium* and *Escherichia coli,* and in yeasts. The biochemical pathway leading to the biosynthesis of histidine was elucidated by B. N. Ames and his colleagues in the late 1950s and early 1960s. Interest in the genetic orga-

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nization of this system arose quite early [for a review see P. E. Hartman *et al.* (*61*)] and these early genetic studies helped elucidate some aspects of the operon theory as originally proposed by F. Jacob and J. Monod (*3, 70*). Several interesting phenomena have been investigated using the histidine operon as a model system: the existence of polycistronic messenger RNAs (*91*), polarity (*94*), and regulation of operon expression (*30, 55*).

Very extensive reviews on the histidine operon and its regulation were published between 1970 and 1972 (*30, 49, 57, 93*). Material referred to in those articles will be only briefly discussed here, the emphasis being given mostly to more recently unreviewed results. This article will not try to cover all aspects of histidine biosynthesis but rather will focus on those areas in which considerable progress has been made in the last few years.

II. Histidine Biosynthesis: The Reactions, the Enzymes

Figure 1 shows the ten enzymatic steps used by *S. typhimurium* to synthesize histidine. No differences have been found in *E. coli* or in yeasts. Some of the enzymes have been isolated from *S. typhimurium* or *E. coli* and studied in detail. Information on the histidine pathway and on the histidine enzymes up to about 1972 has been reviewed (*30, 57, 93*); this information is summarized as follows:

1. The pathway consists of ten enzymatic steps, without any branch point leading to the synthesis of other metabolites required for growth. An adenine requirement is imposed on histidine auxotrophs when grown with limiting histidine or on *hisG* feedback-resistant, *hisT* double mutants. The requirement may result from depletion in the intracellular adenine pool, caused by the uncontrolled use of ATP in histidine biosynthesis, and provides a powerful selection for *hisG* and *his* promoter mutants (*72*).
2. Histidine biosynthesis is regulated both genetically and enzymatically. Enzymatic regulation results from feedback inhibition of the first biosynthetic enzyme by the end product of the pathway, histidine, at the concentration of the internal histidine pool (15 μM). Histidine acts at an allosteric site (*92*) causing a conformation change of the enzyme (*22*).
3. The histidine biosynthetic pathway *in vivo* operates at an overall rate well below its maximal capabilities. This is completely a result of feedback inhibition as shown by the drastic increase in the rate of histidine production in feedback-resistant mutants (*30*).

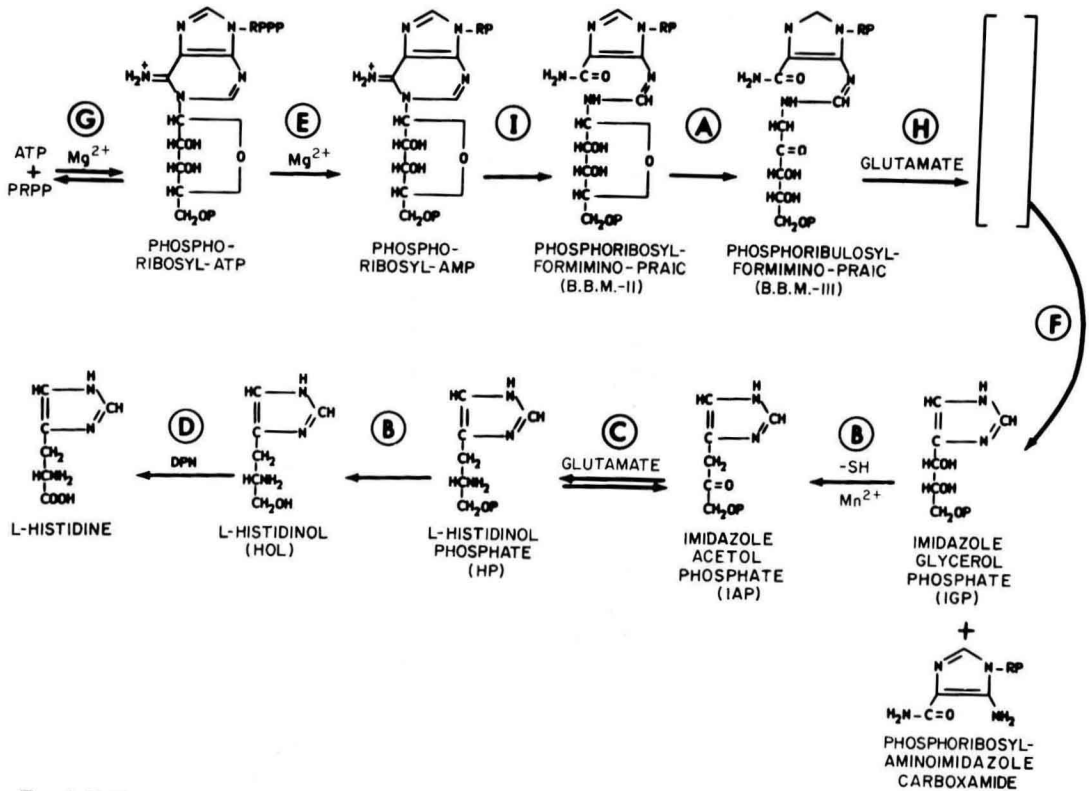

FIG. 1. Pathway of histidine biosynthesis. The enzymes are represented by circled letters according to the gene involved. Reproduced from Brenner and Ames (*30*) by permission of the authors and the publisher.

A. The First Enzyme

Table I summarizes the structural information on the histidine biosynthetic enzymes. The data are taken from R. G. Martin *et al.* (*93*) and are revised according to more recent information that will be discussed later. The available data on the *E. coli* enzymes have been included.

The first enzyme of the biosynthetic pathway, *N*-1-(5′-phosphoribosyl)adenosine phosphoribosyltransferase (abbreviated: ATP phosphoribosyltransferase) (EC 2.4.2.17), the product of the *hisG* gene (hence sometimes called the G enzyme), catalyzes the first step of the pathway and is inhibited by histidine. Preliminary work on this enzyme required partial reevaluation when it was discovered that purified preparations contained a contaminant, histidase. A method is now available (*106*) for the rapid isolation of ATP phosphoribosyltransferase free of contaminants. This method uses a strain containing a *gal chl hut bioA uvrB* deletion as the source of enzyme and employs only precipitation steps (heat, ammonium sulfate, acid pH) in the presence or absence of histidine, taking advantage of the conformational changes induced by the inhibitor (*106*). The purified enzyme is a hexamer of molecular weight (MW) 215,000, composed of identical subunits of MW 33,200 (*111*). Under assay conditions the enzyme is present in a hexameric form, but multiple aggregation states can be demonstrated under other conditions (*107*). The enzyme is inactivated by bulky alkylating reagents acting on a unique reactive group. At least five conformations of the native enzyme must be present at significant levels to account for the inactivation behavior (*109*). At low temperature, species of lower and higher aggregation states than the hexamer can be demonstrated; at low ionic strength or at alkaline pH, the enzyme is a dimer, which appears to be the basic oligomeric unit. Thus, the hexameric enzyme appears to be a trimer of dimers. Combination of alkaline pH and low ionic strength leads the dimer to further dissociate into monomers. The substrates (ATP and PRPP) or histidine, in the presence of sodium ions, stabilize the hexameric form. Aggregation of the enzyme may be adequately described by the equilibria of Fig. 2; each state of aggregation predominates under different sets of conditions. The time required for interconversion of the various states may be on the order of minutes and is influenced by the presence of histidine or of the substrates (*15*). In early work, exchange reactions in the presence of only one substrate (PRPP) suggested that an intermediate covalently bound to the enzyme was formed (*92,13*). More recent studies could find no evidence for such an

TABLE I

STRUCTURE OF THE HISTIDINE BIOSYNTHETIC ENZYMES

Enzyme	Gene	Organism	MW of gene product	Method employed[a]	Native size (MW)	References
PR-ATP synthetase	*hisG*	*S. typhimurium*	33,216	a.a. sequence	200,000	(*111*)
PR-ATP synthetase	*hisG*	*E. coli*	33,000	SDS–PAGE	200,000	(*35, 77a*)
Histidinol dehydrogenase	*hisD*	*S. typhimurium*	47,000	a.a. sequence	95,000	J. Roth
Histidinol dehydrogenase	*hisD*	*E. coli*	47,000	SDS–PAGE	95,000	(*21*)
Aminotransferase[b]	*hisC*	*S. typhimurium*	29,000	Ultracentrifuge	59,000	(*93*)
Dehydratase–phosphate[c]	*hisB*	*S. typhimurium*	95,000	SDS–PAGE	145,000	(*93, 128*)
Amidotransferase	*hisH*	*S. typhimurium*	Unknown	Sucrose gradient	44,000	(*93*)
Isomerase[d]	*hisA*	*S. typhimurium*	29,000	SDS–PAGE	29,000	(*90*)
Cyclase	*hisF*	*S. typhimurium*	Unknown	Sucrose gradient	41,000	(*93*)
PR-AMP-1,6-cyclohydrolase	*hisI*	*S. typhimurium*	Unknown	Sucrose gradient	48,000	(*93*)
PR-ATP pyrophospho-hydrolase	*hisE*	*S. typhimurium*	Unknown	Sucrose gradient	43,000	(*93*)

[a] Abbreviations used: a.a., amino acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

[b] Imidazolylacetolphosphate: L-glutamate aminotransferase.

[c] Imidazolylglycerolphosphate dehydratase: histidinol phosphatase.

[d] Phosphoribosylformiminophosphoribosylaminoimidazolecarboxamide ketolisomerase.

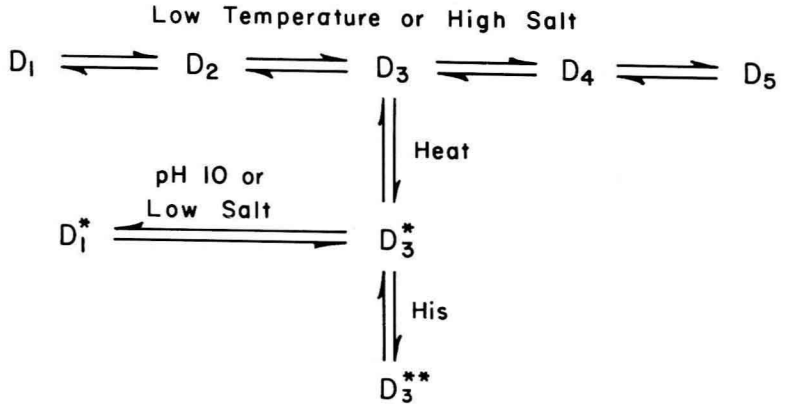

FIG. 2. Aggregation states of ATP phosphoribosyltransferase. At low temperature or high salt, the enzyme aggregates in the indefinite continuous manner indicated on the top line. The dimer D_1 is the basic aggregation unit. Above 22°C in 0.1 *M* salt at pH 7.5 or 8.5, the enzyme is predominantly the hexamer D_3. Low ionic strength or pH 10 will cause the high-temperature hexamer (D_3^*) to dissociate to dimer D_1^*. Substrates and inhibitor (histidine) each lock the enzyme into the hexameric state. However the hexamer created by histidine has a different sensitivity toward inhibition by histidine in the assay than the hexamer created by high temperature. Reproduced from Parsons and Koshland (*107*) by permission of the authors and the publisher.

intermediate (*27*). The stereochemistry of the reaction product, phosphoribosyl ATP, also speaks against the formation of a covalent intermediate generated by a double displacement mechanism. Possibly the early workers were misled by the presence of the other substrate, ATP, as an impurity in the commercial batches of phosphoribosylpyrophosphate (PRPP) (*27*).

The enzyme purified from *E. coli* (*77*) appears to have properties very similar to those of *S. typhimurium,* i.e., subunit size (*77a*), inhibition by histidine (77), and presence of several aggregation states (*76*). The basic oligomeric unit is also a dimer (*132*). The equilibrium between the aggregation states is also shifted toward the hexameric form by histidine (*133*).

A major advance in our knowledge of ATP phosphoribosyltransferase has been the determination of its primary structure (*111*), which is reported in Fig. 3. The enzyme is composed of 299 amino acids, has 5 cysteines and no disulfide bridge. Several data argue that a cysteine is essential for activity (*109, 14*). The position of this cysteine, however, has not yet been identified. From the sequence, the authors noticed that a 40-residue segment of ATP phosphoribosyltransferase, which contains a cysteine, may have partial homology (14 amino acids out of 40)