Stereochemical Course of the Adenosine Triphosphate Phosphoribosyltransferase Reaction in Histidine Biosynthesis*

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The product of the first reaction in histidine biosynthesis is shown by optical rotation measurements on three derivatives to have inverted, β stereochemistry at the newly formed bond. This is in contrast to α linkage expected on the basis of previously observed exchange, specificity, and covalent intermediate phenomena. The postulated double displacement mechanism for adenosine triphosphate phosphoribosyltransferase must be modified to account for the product stereochemistry.

Histidine is biosynthesized by microorganisms from ATP and PRibPP through a series of 10 enzymatic steps (1). The first several steps of the pathway produce PRibATP, PRibAMP, and BBM II as shown in Fig. 1. The enzymes catalyzing these steps are adenosine triphosphate phosphoribosyltransferase, PRibATP pyrophosphohydrolase, and PRibAMP cyclohydrolase, and have been given the genetic designations of G, E, and I, respectively. Since it is feedback-inhibited by histidine (2), and appears to play a role in regulation of the histidine operon (3), the first enzyme has been the most extensively studied one of the pathway (4–10). The reaction catalyzed by ATP phosphoribosyltransferase involves substitution of the C-1 ribose carbon of PRibPP, which is in α linkage to pyrophosphate (11, 12), by N1 of ATP with expulsion of pyrophosphate. The resulting PRibATP was of uncharacterized stereochemistry at the newly formed bond (13).

ATP phosphoribosyltransferase catalyzes the exchange reactions shown in Equations 1 as demonstrated by Martin (2). The exchanges are consistent with the formation of a 5-phosphoribosyl enzyme covalent intermediate (14). Such an intermediate was sought and isolated as a stable species in the presence of histidine by Bell and Koshland (15). The simplest scheme consistent with this evidence is a double displacement mechanism involving a β-linked 5-phosphoribosyl enzyme intermediate which would give PRibATP of α stereochemistry. We have tested the latter prediction by determining the stereochemistry of enzymatically synthesized PRibATP. The proton magnetic resonance spectrum of PRibATP has also been obtained in order to confirm the previously proposed structure.

\[
\text{PRibPP} + 3\text{PPi} \rightleftharpoons \text{[3\text{P}]PRibPP} + \text{PPi} \quad (\text{la})
\]

\[
\text{PRibATP} + [\text{14C}]\text{ATP} \rightleftharpoons \text{[14C]PRibAMP} + \text{ATP} \quad (\text{lb})
\]

Materials and Methods

PRibATP was synthesized by reacting 3.0 g of ATP and 1.0 g of PRibPP in 200 ml of 0.15 M KC1, 0.10 M Tris-HCl, and 0.01 M MgCl2 at pH 8.5 containing 5 units of inorganic pyrophosphatase, and 9.5 mg of histidine-activated ATP phosphoribosyltransferase (6). Reaction proceeded 1 hour at 25°C until A650 reached 1.9 after which the pH was lowered to 6.0 with HCl. The solution was freeze-dried, taken up in 27 ml of H2O, and chromatographed on a column (2.6 x 75 cm) of P-2 in water. PRibATP, which elutes ahead of PRibPP and ATP, was located by absorbance at 290 nm, pooled and chromatographed on a column (1.5 x 20 cm) of DEAE-cellulose with 200 ml total volume of a linear gradient of LiCl from 0 to 0.25 M. The pooled PRibATP was freeze-dried, rechromatographed on the P-2 column in water, located, and freeze-dried, taken up in 2.0 ml of water, and stored in liquid nitrogen. Yield was about 50 mg of pure PRibATP which exhibited the expected ultraviolet spectra at neutral and basic pH (13).

PRibATP was equilibrated in D2O for NMR spectroscopy by three freeze-dryings. Proton spectra were obtained on a Varian XL-100-15D spectrometer operating at 100.1 MHz, with deuterium lock, and Fourier transform acquisition of data. The NMR spectrum of 1-ureidoadenosine was obtained for a similarly treated sample on a Varian T-60 spectrometer.

Rearrangement of PRibATP to the N7 position on the adenine moiety was studied as follows. Optical rotation at pH 6 and 25°C of 3.1 mM PRibATP in 1.35 ml of water was obtained in a 10-cm microcell in a Perkin Elmer 141 spectropolarimeter. Then 0.15 ml of 1.0 M glycine-NaOH was added to give 0.1 M glycine solution of PRibATP at pH 10.07. The polarimeter cell containing PRibATP was thermo-
stated at 45° as well as excess PRibATP at pH 10.07, and the change in optical rotation at 589, 578, 546, 436, and 365 nm followed for 3 hours, after which rotation was stable. Rotation of rearranged PRibATP also was determined at 25°. Rearranged PRibATP was periodate-oxidized by dissolving 4.0 mg of NaIO₄ in the total solution, adjusting to pH 6.0 with glacial acetic acid, and incubating at 25° for 30 min after which rotation was constant. Final pH was 7.0.

Enzymes for synthesis of BBM II were obtained from 3 liters of the hisA30 mutant of Salmonella typhimurium (16) grown as previously described (4). Harvested cells were suspended in a 2 × volume of 0.01 M MgCl₂, 0.15 M KCl, 0.005 M β-mercaptoethanol, and 0.1 M Tris-HCl at pH 8.5, sonicated, and centrifuged at 10,400 × g to remove cell debris. Two milliliters of supernatant were gel-filtered into the same buffer on a column (0.9 × 20 cm) of Sephadex G-50. One milliliter of 1.19 mM PRibATP in the same buffer was mixed with 0.2 unit of inorganic pyrophosphatase and 20 μl of the crude extract containing the E and I enzymes. The change in optical rotation was followed for 3 hours at about 35° after which it was stable.

All rotation values were corrected for buffer blanks and dilution. Molecular rotation, [M]₀, was calculated in units of degrees per dm per mol per 100 ml. PRibATP concentration was determined by absorbance at 290 nm at pH 8.50 with the extinction coefficient 3.6 × 10⁴ (17).

RESULTS

FIG. 1. The first steps of the histidine biosynthetic pathway of Salmonella typhimurium. The enzyme name and gene designation are given above each reaction step. A total of 10 steps lead to histidine. Inorganic pyrophosphate products occurring with PRibATP and PRibAMP have not been included. The stereochemical course of the first step is the subject of this investigation. PRibPP with α linkage to PP, gives PRibATP of previously undetermined stereochemistry at C-1 shining intermediate to N of the adenine moiety. The asymmetry at C-1 normally is lost in the fourth step of the pathway at the isomerase, or A gene, step.

The ultraviolet spectrum of PRibATP exhibits a pH-dependent titration centered at pH 8.8 (13). At higher pH the spectrum exhibits λmax at 260 nm, a shoulder at 265 nm, and a plateau from 285 to 300 nm. At lower pH it more closely resembles protonated ATP, with a λmax at 260 nm. No adequate theoretical approach is available yet for predicting the optical rotary behavior of this chromophore depending on the configuration of two intimately bonded asymmetric centers (19). The new absorption bands, whose transition moments are not characterized, would give unpredictable Cotton effects arising from the asymmetric perturbation of the chromophore by the two ribosyl moieties. Since these Cotton effects occur at low wavelengths they would dominate rotational behavior in the near-ultraviolet and visible. In fact, the molecular rotation of PRibATP measured at the sodium D-line doublet changes from positive to negative depending upon protonation of the chromophore, clearly demonstrating compensation from near-ultraviolet Cotton effects (Table II).

The strategy adopted here to determine the unknown stereochemistry was to move the asymmetric center in question as far away as possible from the chromophore in a manner which did not affect its stereochemistry. The van't Hoff principle of optical superposition (20) could then be applied. This principle assumes that molecular rotation of a molecule measured at the sodium D-line will be equal to the sum of rotations separately contributed by each asymmetric unit. The principle can be valid if the asymmetric units do not strongly interact with each other (19). This was accomplished in two quite different ways, and with a third related measurement as follows.
\(^{N'}\)alkylated adenosine derivatives are known to rearrange in base essentially quantitatively to the \(^{N}\) derivative (21). This restores the adenine moiety to an electronic state resembling that of unsubstituted adenosine. Numerous mechanistic studies, including \(^{15}\)N isotopic labeling, have shown that this rearrangement proceeds through adenine ring opening without cleavage of the \(^{N'}\)alkyl bond (22). A similar rearrangement by PRibATP was demonstrated by Ames et al. (13), and is illustrated in Fig. 4. Since the intermediate phosphoryibosylaminoglycoside transiently formed during this rearrangement is conjugated to the aglycone it is not expected to be subjected to facile mutarotation at the C-1" center as is the case with an unconjugated amine (23). Thus the stereochemistry at C-1" will be unchanged in rearranged PRibATP.

In addition to moving the C-1" asymmetric center further away from the adenine moiety the rearrangement allows increased torsional motion of C-1" with respect to the adenine ring. Both changes in the relationship of C-1" to the adenine ring will minimize net interactions between them (19).

The asymmetric units of rearranged \(^{N'}\)-PRibATP can be thought of as being similar to ATP and N-glycylribosylamine (Table I). Both anomers of N-glycylribosylamine have been synthesized by others and characterized, as well as the carbonbenzoxy derivatives. Phosphorylation of the 5′ position of ribosides has little effect on rotation (19). Summing the molecular rotation for either the \(\alpha\)- or \(\beta\)-ribosylamine with that for ATP (26) yields the two predictions for \(\alpha\) or \(\beta\) C-1" linkage in rearranged \(^{N'}\)-PRibATP given in Table I.

The progress of the PRibATP rearrangement is shown in Fig. 5, where it is again apparent that a near-ultraviolet Cotton effect dominated the visible rotation of PRibATP. When rearrangement was partially complete rotation in the visible changed from positive to negative depending upon wavelength. Final molecular rotation at the sodium D-line was \(-260^\circ\), in good agreement with the prediction for the \(\beta\) configuration of C-1" (Table II).

To eliminate the possibility of any special conformational

**Table I**

| Compounds                  | Molecular rotations*  |
|---------------------------|-----------------------|
|                           | \(\alpha\) | \(\beta\) |
| \(^{N'}\)-PRibATP          | +263° (23) | -147° (23) |
| \(^{N}\)-PRibATP           | +127° (23) | -258° (23) |

*References are given in parentheses.
interactions which would invalidate the optical superposition principle, rearranged N*-PRibATP was oxidized with periodate. Periodate oxidized α or β-N-glycyrribosylamine and adenosine serve as models for this product (Table I). The molecular rotation of oxidized rearranged N*-PRibATP of −330° also is in good agreement with the prediction for β stereochemistry at C-1′ (Table II).

An independent method for confirming the stereochemistry is available by utilizing the metabolic pathway itself. PRibATP can be converted to BBM II by incubation with a bacterial extract which contains E and I enzymes but which is blocked mutationally at the A step (Fig. 1). Both E and I steps are hydrolyses requiring no cofactors other than magnesium. BBM II can be thought of as being composed of either α or β-N-glycyrribosylamine and aminooimidazolocarboxamide ribonucleotide, a well-characterized intermediate of purine biosynthesis (Table I). Incubation of PRibATP with E and I enzymes gave a product with molecular rotation of −400° in fair agreement with the prediction for β stereochemistry at C-1′ (Table II). Thus all three measurements indicate β stereochemistry in the newly formed bond of PRibATP.

**DISCUSSION**

The generally good agreement between observed and predicted rotations for all β linkage in PRibATP is strong support for inversion of configuration at the newly formed linkage. Since α linkage should have yielded positive rotations, it is highly unlikely that any special conformational effects accidentally would cause inversion of sign to give negative rotation for all three measurements. The occurrence of the β configuration in PRibATP makes it very unlikely that it arose from α-PRibPP by a double displacement mechanism. Chemical studies on ribofuranosides give no evidence for front-side displacement reactions at C-1 (27). Thus the simple double displacement mechanism for ATP phosphoribosyltransferase must be modified.

There are at least three reaction mechanisms, shown in Fig. 6, that can account for the exchange reactions, formation of a phosphoribosylated enzyme, and the β stereochemistry of product. These are the simplest mechanisms which incorporate essential elements characteristic of other enzymatic glycosyl reactions, such as those of lysozyme (28), sucrose phosphorlylase (29), γ-galactosidase (30), other phosphoribosyltransferases (31), and purine nucleoside phosphorylase (32). Possible mechanisms which invoke anchimeric assistance from the substrate itself are unlikely since the C-2 hydroxyl of PRibPP is cis to the

### Table II

**Predicted and observed rotations for three derivatives of PRibATP**

| Compound       | pH      | Predicted rotation [M]₀ | Observed rotation [M]₀ |
|----------------|---------|-------------------------|------------------------|
| PRibATP        | 6°      | α: +100                 | β: +100                |
| N*-PRibATP     | 10.07°  | α: +110                 | β: −280                |
| ox-α-N*-PRibATP| 7°      | α: +30                  | β: −350                |
| BBM II         | 8.5°    | α: +100                 | β: −290                |

*All rotations measured at 25° except for BBM II at 40°.*
*The sodium salt in water.*
*The sodium salt in 0.1 M glycine-NaOH.*
*The sodium salt in 0.1 M glycine-NaCl.*
*The lithium salt in 0.1 M Tris, 0.15 M KCl, 0.01 M MgCl₂.*

**Fig. 6.** Possible mechanisms for ATP phosphoribosyltransferase. These are mechanisms which would account for exchange of PT, and ATP into PRibPP or PRibATP, respectively, the extreme specificity of the enzyme (Enz), formation of a covalent intermediate with PRibPP in the presence of histidine, and inverted stereochemistry in the product. Only part of the PRibATP molecule is shown on the right. N, and N₂ are nucleophiles on the enzyme surface which can become phosphoribosylated by PRibPP.

Pyrophosphate leaving group and the C-5 phosphate can attack C-1 only in a strained conformation.

A triple S₂N₂ displacement could occur involving two phosphoribosylated enzyme intermediates both on the normal pathway. Pyrophosphate might exchange with the first intermediate and ATP with the second. Histidine could bind to either one or both intermediates to stabilize them (15). Alternatively, the apparent phosphoribosylated enzyme intermediate might not be on the pathway, but could be trapped in a reversible side reaction by histidine. The normal enzymatic pathway then could involve either a single S₂N₂ displacement, as shown on the second line of Fig. 6, or a carbonium ion intermediate, as shown on the third line, which could give either α- or β-PRibATP. In the latter two cases reversible side reactions also would have to occur in the presence of PRibATP in order to account for exchange reactions.

Steady state initial velocity rate determinations of enzymatic activity as a function of substrate and histidine could be useful in limiting mechanistic possibilities. However, previous studies are very contradictory (2, 6, 7, 10) as a result of uncharacterized conformational and dissociative equilibria of ATP phosphoribosyltransferase (5) and contaminating histidase (4). Rate studies have not been performed on the exchange reactions. More information will be required to decide among the possibilities in Fig. 6, in addition to the possibility that some of the previous data were incomplete or incorrect. Observation of inversion in the adenosine triphosphate phosphoribosyltransferase reaction as shown here indicates that exchange, specificity and “covalent intermediate” criteria (15) must be used with care to predict the double displacement mechanism.

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