Stereochemical Course of the *Escherichia coli* Imidazole Glycerol Phosphate Dehydratase Reaction

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The vast majority of enzyme-catalyzed dehydrations involve substrates with the departing hydrogen and hydroxyl group situated α and β, respectively, to a carbonyl or an imine group.[1] One exception to this motif is the reaction catalyzed by imidazole glycerol phosphate (IGP, **1**) dehydratase,[2] an enzyme from the histidine biosynthesis pathway[3,4] (Scheme I). Violation of a mechanistic trend poses fundamental questions regarding enzyme structure and function. For this reason and because IGP dehydratase plays a key role in the biosynthesis of an essential amino acid (and therefore is a logical target for rational inhibitor design), a thorough study of this enzyme has been initiated.

A variety of mechanisms for the IGP dehydratase reaction, some involving enol **3**, can be envisioned. If **3** were an intermediate, ketonization might be enzyme-catalyzed[10] or spontaneous; however, only in the former case is a discreet stereochemical outcome expected. As an initial test of the mechanism of the *Escherichia coli* IGP dehydratase reaction, we have determined the overall stereochemical course of the conversion of (2*R*,3*S*)-IGP (D-*erythro*-IGP]) to imidazole acetol phosphate (IAP, **2**).

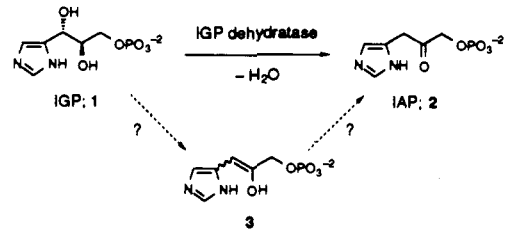
D-*erythro*-[3-^{2}H]IGP ([3-^{2}H]**1**) was prepared enzymatically (Scheme II) from D-(–)-[3-^{2}H]ribose-5-phosphate (**4**). The latter was obtained from diacetone-D-glucose via oxidation to the ketone, which was reduced with NaB^2H_4.[11] Following selective deprotection,[12] C-6 of the resulting [3-^{2}H]glucose derivative was removed by a sequence of oxidation with $NaIO_4$ and reduction with $NaBH_4$.[13] Selective phosphorylation[14] and hydrolysis of the remaining acetonide gave **4**, which was converted to 5-[3-^{2}H]phosphoribosyl 1-pyrophosphate ([3-^{2}H]PRPP) with PRPP synthetase. [3-^{2}H]**1** was produced from [3-^{2}H]PRPP by incubation with cell-free extracts of *E. coli* highly enriched in the enzymes that catalyze the first five steps of histidine biosynthesis,[3,15,16] and it was purified by ion-exchange chromatography.

For analysis of [3-$^2H_1$]**2** by NMR spectroscopy, the IGP dehydratase reaction was coupled to the next reaction in the histidine pathway, catalyzed by *hisC*-encoded IAP transaminase (Scheme III). Since the *E. coli hisB* gene encodes a bifunctional protein exhibiting both histidinol phosphate phosphatase and IGP dehydratase activities,[18] the ultimate product was (2*S*)-histidinol (**5**).[19]

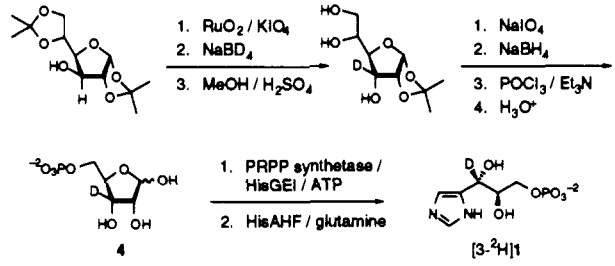
[3-^{2}H]**1** was incubated with the coupled enzyme system, and labeled **5** was purified by ion-exchange chromatography. The ^{2}H NMR spectrum of **5** derived from [3-^{2}H]**1** is shown in Figure 1b. A single C-3 deuteron resonance is observed. In a complementary experiment, unlabeled IGP was incubated with the HisB and HisC proteins in a ^2H_2O-based medium. The ^{2}H NMR spectrum of the resulting **5** is shown in Figure 1c. This spectrum exhibits two strong signals, one for the C-2 deuteron, and the other for a deuteron at C-3. In order to assign the C-3 deuteron signals (which clearly appear at unique chemical shifts) in Figure 1, a reference sample of (2*S**,3*S**)-[3-$^2H_1$]**5** was synthesized.[21]

The ^{2}H signal in the spectrum of the reference sample (Figure 1a) is clearly distinct from that of **5** made from [3-^{2}H]**1** (Figure 1b), but taking into account the isotope effect[25] owing to ^{2}H at

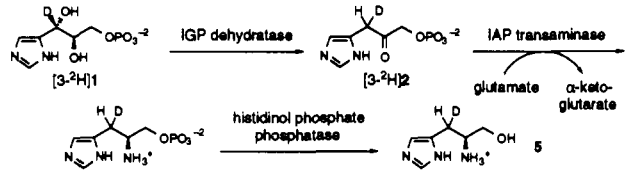
Scheme I



Scheme II



Scheme III



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(21) (2*S**,3*S**)-[3-$^2H_1$]Histidinol was made from 4(5)-[*formyl*-^{2}H]-formylimidazole[22] by conversion to (*Z*)-2-benzamido-3-[3-^{2}H][imidazol-4(5)-yl]acrylic acid.[23] The latter compound was hydrogenated to *N*-benzoyl-(2*S**,3*S**)-[3-$^2H_1$]histidine, which was converted to (2*S**,3*S**)-[3-$^2H_1$]histidinol by the method of Bauer et al.[24]
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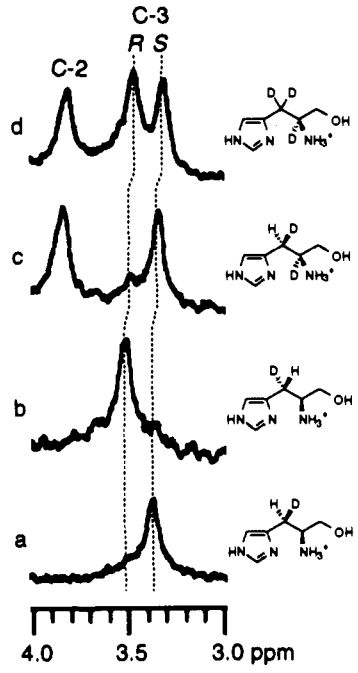

Figure 1. ^2H NMR spectra (76.728 MHz) of [^2H]histidinol (**5**) in CH_3OH. The spectra are referenced to a $CDCl_3$ external standard. (a) Synthetic (2*S**,3*S**)-[3-$^2H_1$]**5**; (b) (2*S*,3*R*)-[3-$^2H_1$]**5**, generated enzymatically from [3-^2H]IGP (**1**) in ^1H_2O; (c) (2*S*,3*S*)-[2,3-$^2H_2$]**5**, generated enzymatically from unlabeled **1** in ^2H_2O; (d) (2*S*)-[2,3,3-$^2H_3$]**5**, generated enzymatically from unlabeled **1** in ^2H_2O but using minimal IAP transaminase.

C-2, it matches well the C-3 ^2H signal in Figure 1c. As a control, the ^2H NMR spectrum of [2,3,3-$^2H_3$]**5** (from incubation of unlabeled IGP in a mixture that included minimal IAP transaminase, thus permitting complete exchange of the C-3 protons of IAP) is portrayed in Figure 1d. Allowing for the double ^2H isotope effect, the chemical shifts of the C-3 deuteron signals are consistent with the assignments in the other spectra.

By comparison of the ^2H NMR spectrum of the synthetic standard (Figure 1a) with those of the enzymatically produced histidinol samples, it is apparent that (a) the "new" hydrogen at C-3 of IAP comes from the aqueous medium. It is also clear that (b) the IGP dehydratase reaction is highly stereoselective and (c) proceeds with *inversion of configuration* at C-3. The high stereoselectivity of the IGP dehydratase reaction is inconsistent with the release of enol **3** into the solution. Whether there is an enol intermediate that is ketonized stereoselectively by IGP dehydratase remains to be determined on the basis of additional experiments under way in our laboratory.

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Supplementary Material Available: ^1H NMR spectra of unlabeled IGP and [3-^2H]IGP (2 pages). Ordering information is given on any current masthead page.

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