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Kinetic isotope effects of nucleoside hydrolase from *Escherichia coli*

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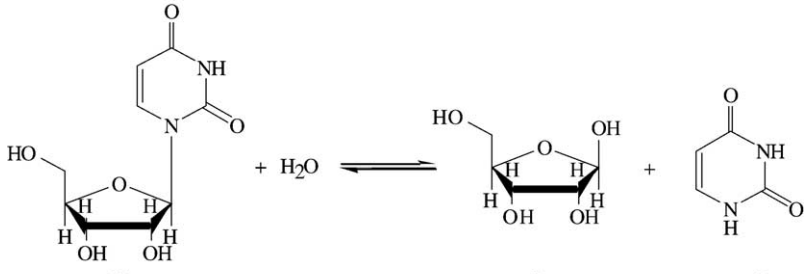
Abstract

rihC is one of a group of three ribonucleoside hydrolases found in *Escherichia coli* (*E. coli*). The enzyme catalyzes the hydrolysis of selected nucleosides to ribose and the corresponding base. A family of V_{max}/K_m kinetic isotope effects using uridine labeled with stable isotopes, such as ^{2}H, ^{13}C, and ^{15}N, were determined by liquid chromatography/mass spectrometry (LC/MS). The kinetic isotope effects were 1.012 ± 0.006, 1.027 ± 0.005, 1.134 ± 0.007, 1.122 ± 0.008, and 1.002 ± 0.004 for [1′-^{13}C], [1-^{15}N], [1′-^{2}H], [2′-^{2}H], and [5′-$^{2}H_2$] uridine, respectively. A transition state based upon a bond-energy bond-order vibrational analysis (BEBOVIB) of the observed kinetic isotope effects is proposed. The main features of this transition state are activation of the heterocyclic base by protonation of/or hydrogen bonding to O2, an extensively broken C-N glycosidic bond, formation of an oxocarbenium ion in the ribose ring, C3′-*exo* ribose ring conformation, and almost no bond formation to the attacking nucleophile. The proposed transition state for the prokaryotic *E. coli* nucleoside hydrolase is compared to that of a similar enzyme isolated from *Crithidia fasciculata* (*C. fasciculata*).

Keywords: Kinetic isotope effect; Nucleoside hydrolase; Transition state

1. Introduction

Nucleoside hydrolases are a group of enzymes that catalyze the hydrolysis of different nucleosides to ribose and the corresponding base.



Abbreviations: LC/MS, liquid chromatography/mass spectrometry; IU–NH, inosine/uridine nucleoside hydrolase; GI–NH, guanosine/inosine preferring nucleoside hydrolase; KIE, kinetic isotope effects; LB, Luria–Bertani; IPTG, isopropyl β-D-thiogalactopyranoside; Tris, tris(hydroxymethyl)aminomethane; ODS, octadecyl; API-ES, atmospheric pressure interface-electrospray mass spectrometer; BEBOVIB, bond-energy bond-order vibrational analysis; SIM, selective ion monitoring; GC/MS, gas chromatography/mass spectrometry; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

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These enzymes have been isolated or the genes of the enzymes have been identified from a number of sources including bacteria, parasitic protozoans, and yeast [1–5]. Parasitic protozoans, which lack a de novo pathway to synthesize purine nucleosides, rely on nucleoside hydrolases to supply purine nucleosides by salvaging them from the host [3]. Mammals, including humans, lack these enzymes, relying on a different set of metabolic reactions to supply their nucleoside requirements. For purine nucleosides, mammals rely on de novo synthesis or a salvage pathway containing purine nucleoside phosphorylases [6]. For pyrimidine nucleosides, mammals lacking nucleoside hydrolases rely on de novo synthesis for their supply. These differences make bacterial nucleoside hydrolases an attractive target for drug discovery [7].

In *E. coli*, there are multiple pathways for the salvage of nucleosides. One of these pathways consists of nucleoside phosphorylases and cytidine deaminase [8–10]. The second pathway consists of a group of hydrolases capable of breaking down nucleosides. The *E. coli* genome contains genes for three such ribonucleoside hydrolases, *ybeK*, *yeiK*, and *yaaF* [1]. Based upon their substrate specificities, these genes were renamed *rihA*, *rihB*, and *rihC*, respectively. *rihA* and *rihB* are pyrimidine-specific ribonucleoside hydrolases, while *rihC* hydrolyzes both purine and pyrimidine ribonucleosides [1]. The deoxyribonucleosides are not substrates for any of these enzymes. The physiological role of these enzymes is not well understood, as their salvage activity can be accomplished by the nucleoside phosphorylases. *rihA* and *rihC* are subject to catabolite repression in the presence of glucose.

Two nucleoside hydrolases have been isolated from *C. fasciculata*. One, designated inosine/uridine nucleoside hydrolase (IU–NH), catalyzes the hydrolysis of both purine and pyrimidine nucleosides [3]. The second is designated guanosine/inosine preferring nucleoside hydrolase (GI–NH) and catalyzes the hydrolysis of selected purine nucleosides [2]. IU–NH is the most extensively studied of the nucleoside hydrolases. Its transition state has been determined using kinetic isotope effects [11]. In addition, the enzyme has been cloned and its crystal structure determined in the presence and absence of the tight-binding transition state inhibitor, p-aminophenyliminoribitol [12–14].

The transition states of a number of nucleoside metabolizing enzymes, including nucleoside hydrolase from *C. fasciculata*, have been determined. The transition states of these enzymes involve the formation of an oxocarbenium ion, which may be reached by different pathways [15]. These pathways include activation of the leaving group, stabilization of the oxocarbenium ion by interactions between the ribosyl group and the enzyme, and stabilization of the oxocarbenium ion by ionization of the 2′-hydroxyl group. For example, the IU–NH enzyme draws most of its catalytic power from the conversion of the ribose moiety to an oxocarbenium ion, while the GI–NH enzyme obtains nearly equal amounts of transition state stabilization from leaving-group activation and oxocarbenium ion formation [11,15].

Knowledge of the structure of the transition state provides information on the interactions in enzyme-catalyzed reactions. In addition, the transition state provides a “blueprint” for the design of tight binding inhibitors. As the binding of a transition state inhibitor is proportional to the rate enhancement achieved by the enzyme, transition state inhibitors have the potential to attain near-irreversible binding with high specificity against the target enzyme [16]. It is the purpose of this study to determine the transition state of nucleoside hydrolase from *E. coli* using kinetic isotope effects (KIE) and compare this transition state to that of nucleoside hydrolase from *C. fasciculata*.

2. Materials and methods

2.1. Materials

Uridine, uracil, 2′-3′-*O*-isopropylidineuridine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside were purchased from Sigma. Hexamethyldisilazane, trimethylsilyl trifluoromethanesulfonate, anhydrous ammonia, dicyclohexylcarbodiimide, deuterated acetic acid, platinum oxide, and deuterated sodium borohydride were purchased from Aldrich. The Xpress protein purification system and EKMax™ enterokinase kit were purchased from Invitrogen. QIAquick PCR purification kit was obtained from Qiagen, while T4 DNA ligase was obtained from Roche. [^{15}N] Potassium cyanide was purchased from Cambridge Isotope Labs. [1′-^{13}C] and [1′-^{2}H] Uridine were obtained from Omicron Biochemicals. All others compounds were reagent grade.

2.2. Preparation of enzyme

2.2.1. Cloning

The pZErO-2.1*yaaF* plasmid containing the *yaaF* (*rihC*) gene was obtained from Dr. Gerard O’Donovan. *yaaF* was subsequently subcloned into pTrcHis2C vector. The pTrcHis2C vector encodes the *myc* epitope and six histidine residues at the C-terminal end to aid in purification by metal affinity chromatography.

2.2.2. Expression

Two primers *yaaF/BstBI* (5′-TGAAGTCGATTAAGTCATTAGCAGCCTAAGTTATTCGAAAATGCCGGTCTTGTTACC-3′) and *yaaF/SnaBI* (5′-CGCCAGAGCCAGCACCTCAGCTACGTACTGCTGGAAGCCTTTCACATCCAGATCCAA3′) were used in conjunction with pZErO-2.1-*yaaF* as a template in a PCR reaction. The PCR product was purified from a 0.8%