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Solution structure of ligands involved in purine salvage pathway

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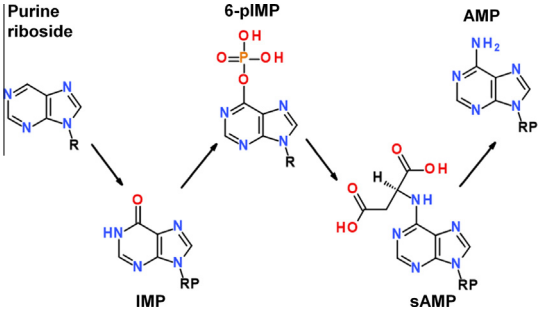
HIGHLIGHTS

- UVRR spectra of purines involved in the purine salvage pathway are reported.
- Structural parameters and vibrational spectra are determined using DFT calculations.
- Vibrations sensitive to interactions between purines-host proteins are identified.

GRAPHICAL ABSTRACT



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ABSTRACT

Analogues of intermediates involved in the purine salvage pathway can be exploited as potential drug molecules against enzymes of protozoan parasites. To develop such analogues we need knowledge of the solution structures, predominant tautomer at physiological pH and protonation-state of the corresponding natural ligand. In this regard, we have employed ultraviolet resonance Raman spectroscopy (UVRR) in combination with density functional theory (DFT) to study the solution structures of two relatively unexplored intermediates, 6-phosphoryl IMP (6-pIMP) and succinyl adenosine-5′-monophosphate (sAMP), of purine salvage pathway. These molecules are intermediates in a two step enzymatic process that converts inosine-5′-monpophosphate (IMP) to adenosine-5′-monophosphate (AMP). Experimental data on the molecular structure of these ligands is lacking. We report UVRR spectra of these two ligands, obtained at an excitation wavelength of 260 nm. Using isotope induced shifts and DFT calculations we assigned observed spectra to computed normal modes. We find that sAMP exists as neutral species at physiological pH and the predominant tautomer in solution bears proton at N10 position of purine ring. Though transient in solution, 6-pIMP is captured in the enzyme-bound form. This work provides the structural information of these ligands in solution state at physiological pH. We further compare these structures with the structures of AMP and IMP. Despite the presence of similar purine rings in AMP and sAMP, their UVRR spectra are found to be very different. Similarly, though the purine ring in 6-pIMP resembles that of IMP, UVRR spectra of the two molecules are distinct. These differences in the vibrational spectra provide direct information on the effects of exocyclic groups on the skeletal structures of these molecules. Our results identify key bands in the vibrational spectra of these ligands which may serve as markers of hydrogen bonding interactions upon binding to the active-sites of enzymes.

1. Introduction

Purine nucleotide synthesis pathways, both de novo and salvage, involve numerous intermediates. Of these, intermediates formed

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during conversion of inosine-5′-monophosphate (IMP) into adenosine-5′-monophosphate (AMP) and guanosine-5′-monophosphate (GMP) are common to both pathways. While humans exploit both these pathways, protozoan parasites like *Plasmodium*, *Leshmania*, *Tritrichomonas* and *Trypanosoma* lack the de novo synthesis of nucleotides and rely solely on their ability to salvage free nucleobases into nucleotides. Thus, enzymes of the salvage pathway are identified as potential therapeutic targets against these parasites [1–4]. Therapeutic intervention that targets these enzymes requires development of effective analogues of the natural substrates and on-enzyme intermediates [5,6]. As a first step, a thorough understanding of the structure of the natural substrates and intermediates is essential. We report here computed structures, experimental and computed vibrational spectra of the parent purine riboside and two important but least understood intermediates of the nucleotide synthesis pathway: 6-phosphoryl inosine-5′-monophosphate (6-pIMP) and succinyl-adenosine-5′-monophosphate (sAMP). Both molecules are formed during the conversion of IMP to AMP [2]. 6-pIMP is a transient intermediate in the reaction catalyzed by adenylosuccinate synthetase (ADSS) and is only stable on the enzyme [7–9]. sAMP is a stable product of this reaction that is released from ADSS in the last step of the reaction. sAMP is further transformed to AMP with the help of adenylosuccinate lyase [10,11].

Advantages of Raman spectroscopy in investigating the structures of nucleic acids has been recognized several decades ago but is experiencing vigorous resurgence as ultraviolet light sources and detectors that enhance nucleobase spectra are becoming available [12–21]. We have employed ultraviolet resonance Raman (UVRR) spectroscopy and density functional theory (DFT) to determine the structure of these ligands. UVRR is a powerful vibrational technique that can unambiguously determine protonation-states and tautomers of nucleobases [22,23]. Here, we report the UVRR spectra of sAMP in aqueous solution at the excitation wavelength of 260 nm. Further, the experimental observations are complemented by DFT calculations on succinyl-adenosine using DFT at the level of B3LYP/6-31G (d, p). In addition, structures of 6-phosphoryl inosine and purine riboside were determined *in vacuo* at the same level of theory and basis set.

Fig. 1 shows the structures of three molecules investigated in this study. Purine riboside (Fig. 1a) is the parent molecule and exocyclic modification on this ring results in the formation of different purines which are the part of nucleotide synthesis pathway. 6-Phosphoryl inosine and succinyl-adenosine may be regarded as the analogues of adenosine-5′-monophosphate because of the presence of similar conjugation in all these rings.

2. Methodology

2.1. Sample preparation

Succinyl-adenosine-5′-monophosphate and $NaNO_3$ were purchased from Sigma–Aldrich and were used without further purification. Appropriate amount of sAMP was dissolved in 30 mM HEPES pH 7.0 to obtain the final concentration of 0.5 mM. Deuterium labeled sAMP was prepared by dissolving the sample in D_2O and incubating overnight to ensure complete H/D exchange. 30 mM $NaNO_3$ was used as an internal standard in all samples. Appropriate buffer controls were recorded along with the samples.

2.2. Ultraviolet resonance Raman spectroscopy

The UVRR spectra were acquired using 260 nm excitation wavelength generated by tunable Ti-Sapphire laser (Indigo, Coherent Inc.). The frequency doubled output of a nanosecond pulsed Nd:YLF laser (Evolution, Coherent Inc.) at 527 nm was used to pump Ti-Sapphire laser. The infra-red generated in turn was frequency doubled using BBO crystal to generate visible light. IR and visible outputs were mixed in another BBO crystal to generate the third harmonic UV light at 260 nm. Sample was illuminated in the spinning NMR tube with the typical power of 0.6 mW. Scattered light was collected with a 135° back-scattering geometry using a pair of fused-silica lenses and focused onto the monochromator (Jobin–Yvon) with 3600 grooves/mm grating. Slit width of 300 μm and slit height of 2 mm was used for recording all the experiments. Spectra were recorded with a back-illuminated CCD camera (Jobin–Yvon) having 1024 × 256 pixels. Calibration was done with the known band positions of solvents dimethylformamide, cyclohexane, indene, acetonitrile, trichloroethylene and isopropanol.

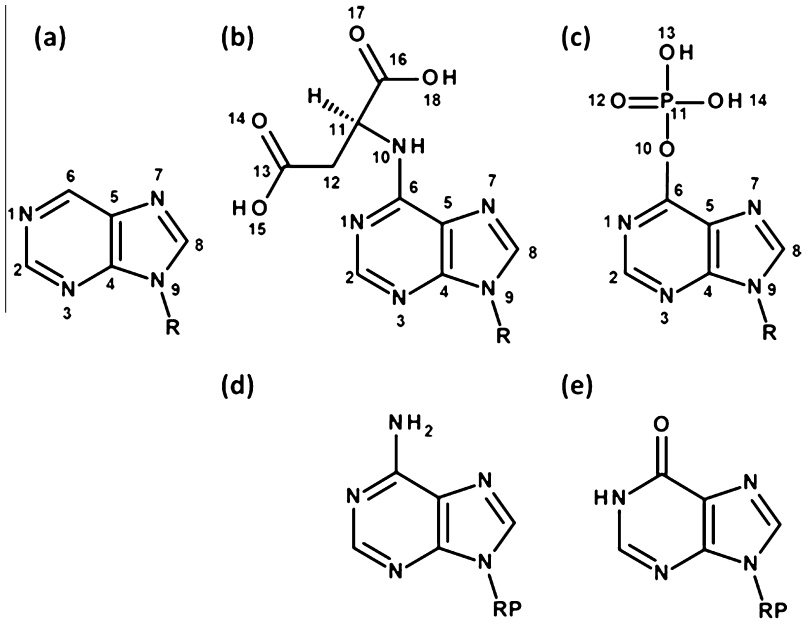

Fig. 1. Structures of (a): purine riboside, (b) succinyl adenosine, (c) 6-phosphoryl inosine, (d) adenosine-5′-monophosphate, and (e) inosine-5′-monophosphate. *Abbreviations:* R-ribose, P-phosphate.