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Investigation of proton affinities and gas phase vibrational spectra of protonated nucleosides, deoxynucleosides, and their analogs

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Dedicated to Veronica Bierbaum, pathfinder in ion chemistry.

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ABSTRACT

DNA nucleobases make use of hydrogen bonding, whether in associating to form the Watson–Crick double-helix or in producing alternative structures such as the G-quadruplex or the *i*-motif. Nucleoside proton-bound dimers provide an avenue for investigating characteristics that they possess within the *i*-motif and related non-Watson–Crick conformations. In addition, several nucleosides are approved antiviral and anticancer agents. The nucleosides under investigation (2′-deoxycytidine, gemcitabine, and decitabine) are capable of forming proton-bound dimers (PBDs) with their conjugate acids. Protonated monomers of 2′-deoxycytidine, gemcitabine, and decitabine and proton-bound dimers of gemcitabine and decitabine have been produced in the gas phase using electrospray ionization (ESI). This paper reports proton affinities of the neutral nucleosides as well as their infrared multiple photon dissociation (IRMPD) spectra from 2800 to 3800 cm^{-1} collected using an Optical Parametric Oscillator (OPO) laser. In the case of the conjugate acid of 2′-deoxycytidine, a partial deuteration experiment suppresses overtones and combination bands, leading to the inference that a single tautomer predominates in the protonated monomer. IRMPD spectra of proton-bound dimers of gemcitabine and decitabine suggest furanose sugar ring puckering to be in the South orientation, as they are in the protonated monomers.

1. Introduction

Pairings of DNA nucleobases make use of hydrogen bonding, regardless of whether neutral nucleobases associate to create the Watson–Crick double-helix or alternative structures form, such as the G-quadruplex or the *i*-motif. DNA conformations like the latter utilize non-Watson–Crick binding when guanine-rich [1–4] and the complementary cytosine-rich [5–7] strands, respectively, separate from one another to undergo single-strand self-association.

More attention has been devoted to stabilization of the G-quadruplex than of the *i*-motif, even though both secondary structures can be expected to form complementarily to one other when the two strands separate. The *i*-motif contains proton-bound dimers (PBDs) of cytosine (also called "hemiprotonated cytosine") intercalated with one another, oriented 90° with respect to the PBDs above and below. Double-strand association *via* cytosine PBDs requires a parallel orientation [8], but if a single strand bends into a hairpin three times, intercalation among the four regions within a single strand can form within the antiparallel orientation of duplex DNA [9,10]. Hydrogen bonding between a pair of cytosines (protonation of the nitrogen of one cytosine at the 3-position leading to three hydrogen bonds with the other, neutral cytosine) provides the driving force holding the PBDs together. Ionic hydrogen bonding between cytosine and its conjugate acid has an experimental dissociation enthalpy whose experimental value ranges from 160 kJ/mol [11] to 173 kJ/mol [5,6] in the gas phase. The latter value agrees with DFT calculations [5,6].

Promoter regions of several oncogenes are believed to adopt the aforementioned secondary structures while underwound. In other words, non-Watson–Crick binding may make a contribution in the time interval between dissociation from the nucleosome and restoration of the correct linkage number by topoisomerases. If this hypothesis is correct, it offers an approach for limiting the binding of promoters and suppressing transcription of potentially harmful mRNA.

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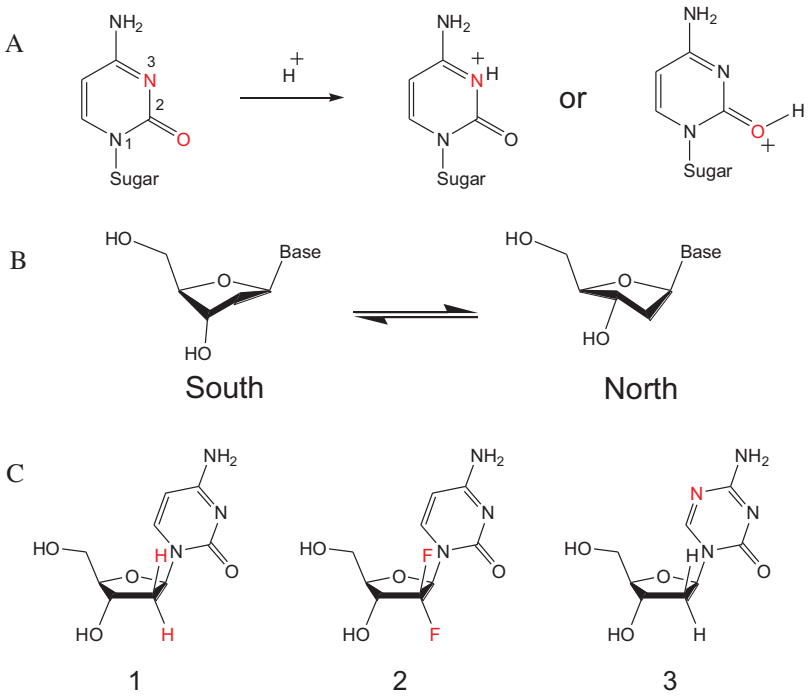

Fig. 1. (A) The two most basic protonation sites of cytosine shown in red. (B) Puckering of the deoxyribose sugar ring from the (South) to (North) orientation. The South orientation has the carbon at the 3′-position downward, while the North orientation has the carbon at the 3′-position up. (C) Nucleosides in this investigation: (**1**) 2′-deoxycytidine, (**2**) gemcitabine and (**3**) decitabine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Background

NMR and gas phase vibrational studies of cytosine PBDs and their derivatives [12,13] have previously been reported, but nucleoside PBDs containing cytosine bases have not received equal attention. Yang and Rodgers have previously examined IRMPD spectra of protonated cytosine monomers and proton-bound dimers modified at the 5-position (with I, Br, F, and methyl) along with the base-paring energies of cytosine heterodimers in the gas phase using threshold collision-induced dissociation [5,6]. They found that modification at the 5-position of cytosine affects the binding energies of the cytosine proton-bound heterodimers. The proton-bound heterodimer between cytosine and 5-methylcytosine, for example, has a greater binding energy than does the proton-bound homodimer of cytosine.

NMR studies of cytosine proton-bound dimers, modified at the 1-position, have been investigated in solution by Hooley et al. [12]. They conducted variable temperature NMR studies along with acid titration experiments on 1-octylcytosine, 5-fluoro-1-methylcytosine, and 5-fluoro-1-octylcytosine homodimers. The addition of acid favors the formation of proton-bound dimers, but excess acid causes them to revert to protonated monomers. They observed proton NMR absorptions that shift downfield with decreasing pH. However, with excess acid concentration, that peak reverses direction, shifting back slightly upfield (Fig. 1).

We have previously reported the gas phase IRMPD spectra of protonated 1-methylcytosine and its PBD in the gas phase from 600 to 1800 cm^{-1}, along with solid state NMR data for the iodide salt of $(1\text{-methylcytosine})_2H^+$ [13,14]. The gas phase spectrum of the PBD in the fingerprint domain from 300 to 1800 cm^{-1} shows a distinct band at 1570 cm^{-1} that disappears upon deuteration of the exchangeable hydrogens. The same band shows up in the IRMPD spectra of PBDs of the 5-fluoro- and 1,5-dimethylcytosine homodimer analogs, as well as in the three possible heterodimers. This band is therefore assigned to be the motion associated with the bridging proton's transit from one *N3*-nitrogen to the other. A band at the same frequency also appears in powder and single crystal IR spectra, and it disappears with isotopic substitution. Solid state NMR of the crystalline iodide salt of $(1\text{-methylcytosine})_2H^+$ reveals that the bridging proton is not shared equally between the two 1-methylcytosines, but prefers to reside on one cytosine or the other, rendering all of the atoms inequivalent (consistent with gas phase vibrational spectra) [13].

Looking at the NH/OH stretching domain from 3200 to 3700 cm^{-1} in the experimental vibrational spectrum of the PBD of 1-methylcytosine, four bands are observed experimentally. Partial deuteration experiments, where four of the five hydrogens are exchanged for deuterium, indicate bands at 3240 and 3310 cm^{-1} to be overtones or combination bands [13]. By contrast, the experimental spectra in the fingerprint and NH/OH domain of the protonated monomer match a mixture of *N3*- and *O*-protonated 1-methylcytosines, suggesting both tautomers coexist in the gas phase, regardless of whether the ion arises from direct injection or from dissociation of the $(1\text{-methylcytosine})_2H^+$ dimer [14].

Speranza et al. previously reported IRMPD spectra of four protonated monomeric nucleosides: 2′-deoxycytidine, cytidine, cytarabine, and gemcitabine in the fingerprint and CH/NH/OH stretching domains [15]. They examined tautomer distributions and concluded that *N3*- and *O*-protonated conjugate acid ions are both present in the gas phase to comparable extents. Their data show the *N3*-protonated tautomer contributing prominent bands in the fingerprint domain from 1400 to 1800 cm^{-1}, while the CH/NH/OH domain from 2800 to 3800 cm^{-1}, appears to match the predicted IR spectrum of the *O*-protonated tautomer.

The partial deuteration experiment described below suggests that the extra peaks in the previously reported IRMPD spectra in the CH/NH/OH domain [15] may come from overtones and combination bands, thus indicating the presence of only one tautomer of protonated monomeric 2′-deoxycytidine in the gas phase.