METHOD FOR ANALYZING COMPOUND-SPECIFIC CARBON AND NITROGEN ISOTOPIC COMPOSITION OF PURINE AND PYRIMIDINE NUCLEOBASES IN CARBONACEOUS METEORITES: PERSPECTIVES FOR BENNU SAMPLES.

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Introduction: Nucleobases are nitrogen-containing heterocyclic compounds and are key components of nucleosides, nucleotides, and nucleic acids in terrestrial life. The detection of nucleobases in carbonaceous chondrites [1–3] emphasizes their importance in prebiotic chemistry; extraterrestrial nucleobases delivered to the early Earth could have contributed to the primordial molecular evolution leading to the origin of life.

The extraterrestrial origin of meteoritic nucleobases has been evaluated by their molecular distributions (i.e., different relative abundances with those of terrestrial life) and by the identification of unusual nucleobases (e.g., 2,6-diaminopurine, 2-aminopurine, and purine) and their structural isomers (e.g., a family of imidazole carboxylic acids), which are rare in the terrestrial biosphere [1,2]. Although the isotopic compositions of organic compounds in carbonaceous meteorites have been established as a strong proof of their extraterrestrial origin, Martins et al. [3] have measured the carbon isotope ratios of two nucleobases in the Murchison meteorite. Therefore, high-precision isotopic measurement for nucleobases in carbonaceous meteorites is required not only to provide compelling evidence for their extraterrestrial origin, but also to understand their detailed formation mechanisms in interstellar and/or asteroidal environments.

In this study, we have developed a new method of analyzing carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in underivatized pyrimidine and purine nucleobases based on two-dimensional high-performance liquid chromatography (HPLC) and ultra-small scale elemental analyzer/isotope ratio mass spectrometry (nano-EA/IRMS). In addition, we have investigated the molecular abundances and distributions of nucleobases in hot water and HCl extracts of the Murchison meteorite to optimize the extraction of nucleobases for our isotopic measurements. We plan to apply these methods to the samples of carbonaceous asteroid Bennu that will be returned to Earth this year by the OSIRIS-REx mission [4].

Analytical Methods: A standard mixture of seven nucleobases containing uracil (U), cytosine (C), thymine (T), guanine (G), adenine (A), xanthine (X), and hypoxanthine (Hx) was used for developing the analytical method for the measurement of nucleobase specific C and N isotope ratios (Fig. 1). First, individual nucleobases were separated using an HPLC system equipped with a reversed-phase pentafluorophenyl (PFP) column and collected using a fraction collector (Fig. 1a). The collected nucleobases were further isolated using a reversed-phase C18 column for the second chromatographic separation (Fig. 1b). After the two-dimensional chromatographic separations and further post-HPLC purification, the $\delta^{13}C$ and $\delta^{15}N$ values of nucleobases were measured by nano-EA/IRMS (Fig. 1c).

To evaluate the nucleobase extraction procedure, we first performed hot water extraction on a powdered sample of the Murchison meteorite (~1.0 g) using ultrapure water at 110ºC for 12 h in a flame-sealed glass ampoule. Half of the supernatant was subjected to acid-hydrolysis using ultrapure 6M HCl at 110ºC for 12 h. Then we conducted further extraction of the solid residue that remained after completing the hot water extraction step using ultrapure 6M HCl at 110ºC for 12 h (hereafter referred to as "post-HCl extract"). The unhydrolyzed hot water, acid-hydrolyzed hot water, and post-HCl extracts were individually desalted by cation-exchange chromatography. The same procedure was repeated using baked (450°C in air for 5 h) sea sand powder (~1.0 g) as a procedural blank. The hot water and post-HCl extracts of the Murchison meteorite were analyzed by HPLC coupled with heated electrospray ionization/ high-resolution mass spectrometry (HPLC/HESI/HRMS), with a mass resolution of 140,000 at a mass-to-charge ratio (*m/z*) of 200, as described elsewhere [2,7].

Results and Discussion: As shown in Fig. 1a, seven nucleobases were separated into the five fractions (Fraction #1–#5) because C and X were not separated from T and Hx, respectively, by the first PFP column. Fig. 1b shows a baseline separation of X and Hx by the

second C18 column. All seven nucleobases in Fractions #1–#5 were individually isolated into Fractions #6–#12 by the two-dimensional HPLC separations. For all nucleobases, the recovery after the two-dimensional HPLC separations was more than 90%. Fig. 1c shows the consistency for $\delta^{13}C$ and $\delta^{15}N$ values of the nucleobases before and after HPLC separation, demonstrating that there is no carbon or nitrogen isotopic fractionation during the experimental procedure.

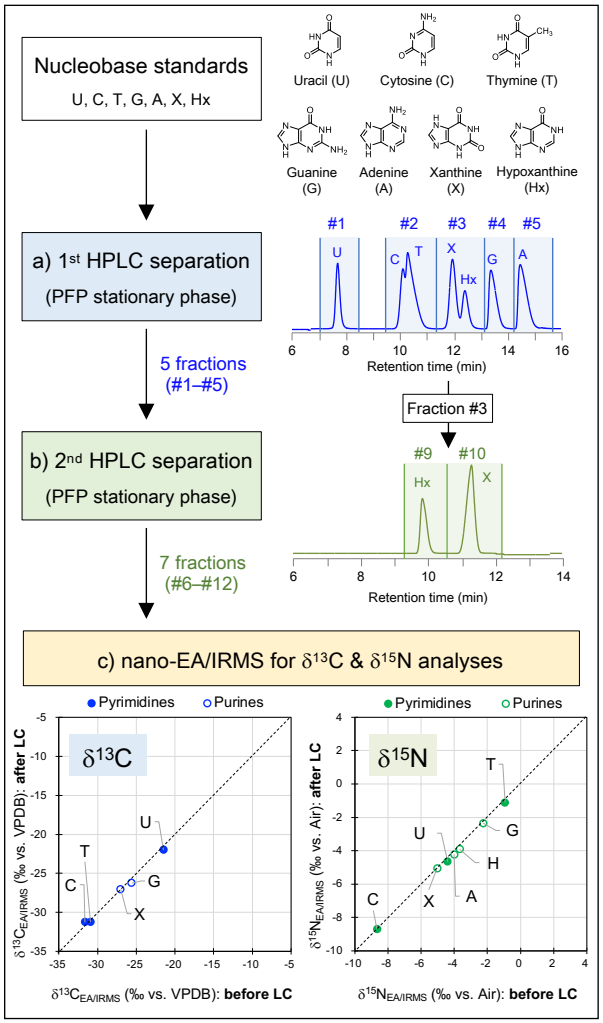

Figure 1: Flow diagram of an experimatal procedure conducted in this study for isolating nucleobases and measuring their carbon and nitrogen isotopic compositons. a) The HPLC/DAD chromatogram of seven nucleobases in the standard mixed solution at 271 nm on the PFP column for the first separation. b) The HPLC/DAD chromatograms of the isolated fraction containing xanthine and hypoxanthine at 271 nm on the C18 column for the second separation. c) $\delta^{13}C$ and $\delta^{15}N$ values of nucleobases in the seven-nucleobase standard mixture before and after the HPLC separation and purification procedures for U, C, T, G, A, X, Hx using an improved method [8].

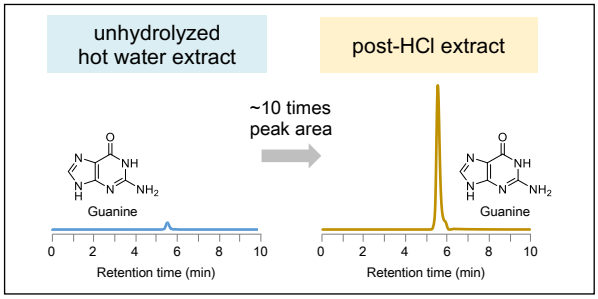

Figure 2: Mass chromatograms at *m/z* = 152.0567 (corresponding to the protonated ion of guanine) for the unhydrolyzed hot water and post-HCl extracts from the Murchison meteroite.

Figure 2 shows mass chromatograms for the hot water and post-HCl extracts from the Murchison meteorite at *m/z* = 152.0567, which corresponds to the protonated ion of guanine ($C_5H_5N_5O + H^+$). The peak area of guanine in the post-HCl extract was ~10 times that of the unhydrolyzed hot water extract. The concentration of guanine in the post-HCl extract was ~1 ppm (μg/g meteorite), which is much higher than those reported in previous studies using different extraction procedures (e.g., water extraction at room temperature: 72 ppb [2]; formic acid extraction at 100 ºC for 24 h: 56 ppb [1])—indicating that guanine in the Murchison meteorite strongly combined with mineral phases and can be efficiently released by the 6M HCl extraction. Therefore, if the samples of asteroid Bennu contain similar abundances of nucleobases as the CM-type Murchison meteorite, the optimized extraction procedure and the developed methodology can be applied to determine the carbon and nitrogen isotopic compositions of nucleobases in a ~1 g Bennu sample. With the planned comprehensive soluble and insoluble organic analyses of Bennu [9], the nucleobase isotopic analysis in the asteroid sample will have potential for providing new insights into the origin of extraterrestrial nucleobases across the Solar System.

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Acknowledgments: Supported by NASA under Contract NNM10AA11C.