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Amino acids on witness coupons collected from the ISAS/JAXA curation facility for the assessment and quality control of the Hayabusa2 sampling procedure

Haruna Sugahara[1,2*], Yoshinori Takano[1], Yuzuru Karouji[3], Kazuya Kumagai[4], Toru Yada[3], Naohiko Ohkouchi[1], Masanao Abe[3] and Hayabusa2 project team

Abstract

The Hayabusa2 mission aims to obtain pristine samples from a near-Earth carbonaceous-type (C-type) asteroid, 162173 Ryugu, and return them to Earth. One of the scientific goals of the mission is to understand the origin and evolution of organic materials through the interactions between water and minerals in the early solar system. Thus, organic materials are the main focus of the analysis on the returned samples. The analysis of extraterrestrial organic materials, however, requires great care to avoid the introduction of terrestrial contaminants and artefacts to the samples. To investigate the potential for contamination, we performed an assessment through the amino acid analysis of witness coupons that were exposed in a clean chamber in an Institute of Space and Astronautical Science/Japan Aerospace Exploration Agency (ISAS/JAXA) curation room. In the study, the witness coupons were collected at different time periods, between 1 day and 1 month, to examine the accumulation rates of the contaminants. Seven common terrestrial amino acids (glycine, alanine, valine, leucine, isoleucine, proline, aspartic acid and glutamic acid) were detected on the witness coupons. Among them, glycine was found to be most abundant, with the highest concentration of 10 pmol/cm^2 detected on the day 7 witness coupon. Alanine was found in the next highest concentration, approximately one-third that of glycine. A time-dependent profile in terms of the increasing trend observed in the concentration from days 1 to 7 was found. The contaminants were considered to have multiple origins. Our results are similar to those reported by the National Aeronautics and Space Administration/Johnson Space Center (NASA/JSC) OSIRIS-REx team, which indicates that the quality control against terrestrial contaminants in our facility is at the same quantitative level as in their facility. The knowledge obtained on the contaminants in this study will provide important information for the curation procedure of the Hayabusa2-returned samples.

Keywords: Amino acid, Hayabusa2, Curation, Witness coupons, Quality control

Introduction

The Hayabusa2 mission is the second sample return mission from an asteroid undertaken by the Japan Aerospace Exploration Agency (JAXA), following the successful Hayabusa mission between 2003 and 2010 (e.g. Fujiwara et al. 2006; Kawaguchi et al. 2008; Tsuda et al. 2013). The Hayabusa2 spacecraft is visiting a near-Earth carbonaceous-type (C-type) asteroid, 162173 Ryugu, which was previously designated as 1999 JU_3, on a sample return mission (e.g. Tachibana et al. 2014; Okazaki et al. 2017; Sawada et al. 2017; Saiki et al. 2017; Watanabe et al. 2017). C-type asteroids are considered to be related to carbonaceous chondrites that are rich in organic and volatile materials. The scientific goals of the Hayabusa2 mission include an understanding of the "diversification of organic materials through interactions with minerals and water in a planetesimal (i.e. origin and evolution of

*Correspondence: h.sugahara@jamstec.go.jp
[1] Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Natsushima, Yokosuka 237-0061, Japan
Full list of author information is available at the end of the article

volatile components in the early solar system and final state of organic matter and water prior to their delivery to the rocky planets)" (Tachibana et al. 2014). Thus, the investigation of the organic materials in the returned samples is the principal aim of the Hayabusa2 mission.

The analysis of pristine organic materials (e.g. the chemical composition, isotopic signature, abundance and morphology of the complex organic molecules) in the returned samples requires great care with regard to terrestrial contamination. Comprehensive and specific management is essential to minimise the terrestrial contamination throughout the mission. In the first Hayabusa mission, a series of assessments on terrestrial contaminants were conducted at the Extraterrestrial Sample Curation Center (ESCuC) sample curation facility, formerly called the Planetary Material Sample Curation Facility (Yada et al. 2014). The returned samples were recovered from the sample container inside clean chambers at the ESCuC. More than 800 particles on the ~ μm scale were analysed by field-emission scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (FE-SEM/EDS) and then classified into four categories based on their chemical composition. The category 1 and 2 particles (silicate particles) were confirmed to be indigenous to the asteroid, but the category 3 particles, made up of carbonaceous materials, were determined by a sequential analysis to be terrestrial or artefact materials of multiple origins, (e.g. Ito et al. 2014; Uesugi et al. 2014; Yabuta et al. 2014; Kitajima et al. 2015; Naraoka et al. 2015).

The first sample return mission from a small body was the Stardust mission, led by NASA. The mission succeeded in obtaining cometary grains from the 81P/Wild 2 comet by capturing particles using an aerogel. In the mission, the team introduced the concept "witness coupons" for the assessment of the organic contamination throughout the mission. The witness coupons were aluminium or sapphire plates and aerogel tiles used to track the origins and routes of incorporation of the contaminants found at each stage of the mission (e.g. the construction of the spacecraft, the flight and the recovery of the sample) (Sandford et al. 2010). The analysis of the witness coupons revealed that the aerogel used to capture the cometary grains contained terrestrial contaminants. These contaminants were simple forms of carbon (mostly $-CH_3$ groups) and *ε*-amino-*n*-caproic acid (EACA, $NH_2(CH_2)_5COOH$). EACA is the hydrolysis product of Nylon 6, and the source of the EACA in the aerogel was proposed to be from Nylon 6 that originated from a shipping bag (Elsila et al. 2009; Sandford et al. 2010). These past sample return missions showed that trace levels of terrestrial contamination are inevitable, even when the best efforts are made to minimise them. Thus, it is important to be able to distinguish terrestrial contaminants from other materials and investigate the level of contamination, alongside improvements to lower the level.

The purpose of this study was to assess the level of terrestrial organic contamination in the clean chamber of the curation room for the Hayabusa2 mission (cf. the design of the Hayabusa2 sample container; Okazaki et al. 2017; Sawada et al. 2017). We used witness coupons made from aluminium foil to collect the contaminants in the curation room at the Institute of Space and Astronautical Science Japan/Aerospace Exploration Agency (ISAS/JAXA). To examine the accumulation rate, we collected the coupons at different exposure times, ranging from 1 day to 1 month. We analysed the concentrations of amino acids as an indicator of the contamination level. Amino acids are suitable molecules to use as an indicator because they are one of the targets of the Hayabusa2 mission, due to their importance in the evolution of organic molecules, as well as in the origins of life. However, their terrestrial contamination is often present in the analysis of extraterrestrial materials (e.g. carbonaceous chondrites and interplanetary dust particles). Amino acids are the building blocks that make up proteins, and they exist everywhere in the terrestrial environment and can thus easily become incorporated into samples. Amino acids were also used as a target species to track organic contaminants in the OSIRIS-REx mission (Dworkin et al. 2018).

Sampling Procedure

Profiles of the cleanroom and clean chambers at ISAS/JAXA

In 2008, the ESCuC was established for the reception and curation of returned samples from extraterrestrial bodies. It is mainly composed of four cleanrooms: the planetary sample handling room (class 100–1000 in FedStd. 209E), the electron microscope room (class 1000), the sample preparation room (class 1000) and the manufacturing and machining room (class 10,000). Boron-free ultra-low penetration air (ULPA) filters are set up in all of them, and they are equipped with filter fan units (FFUs). Additionally, chemical filters for volatile organics, acids and alkalis are present in the FFUs in the planetary sample handling room.

To handle the returned samples from the Hayabusa mission, clean chambers (CCs) were installed in the planetary sample handling room (Fig. 1a). They consist of two parts: clean chambers No. 1 (CC1) and No. 2 (CC2). They are mainly made of stainless steel 304, quartz and tempered glass (only for CC2) for the viewports and windows, Viton for the O-rings and gloves, and copper for the gaskets. They are filled with nitrogen gas that is

circulated using a gas purifier and ULPA filters that are set into the introduction ports for the circulated nitrogen gas. Inside CC2, an electrostatically controlled micromanipulation system is installed to handle particles that are smaller than 300 μm. It is basically made of aluminium, stainless steel 304, Teflon, quartz glass and copper wire coated with a polyimide film. Other tools used in the cleanrooms, including jigs and containers, are made of aluminium, stainless steel 304, Teflon and quartz glass. The environment inside the CCs is checked by the dew point meters present in each of the chambers and by atmospheric-pressure ionisation mass spectrometry. The dew point is normally lower than −110 °C for CC1 and lower than −90 °C for CC2, corresponding to water contents of 1.3 ppb and 92 ppb, respectively, when the gate valves or stainless steel covers of the Viton gloves are closed. As mentioned above, the CCs are equipped with multiple Viton gloves for handling tools and jigs in order to treat the Hayabusa-returned samples. However, interference gas like O_2, H_2O outside the CC could permeate nitrogen environment in the CC through these gloves, which themselves also could be the source of the interference gas. Thus, after the handling works at the CCs are finished, the gate valve between the main volume of the CC1 and the Viton gloves are closed to prevent the interference gas to permeate the CC1 environment. For CC2, all the gloves are folded and all the arm holes of the gloves are covered with stainless steal plates from outside and then the volumes between the gloves and the plates are evacuated for the same purpose with the CC1.

Gate valves are also equipped between the CC1 and the CC2 to separate their environment each other. All of the tools, jigs and containers are introduced into or extracted from the CCs via an airlock equipped beside CC2 that can be evacuated to high vacuum and purged with purified nitrogen gas.

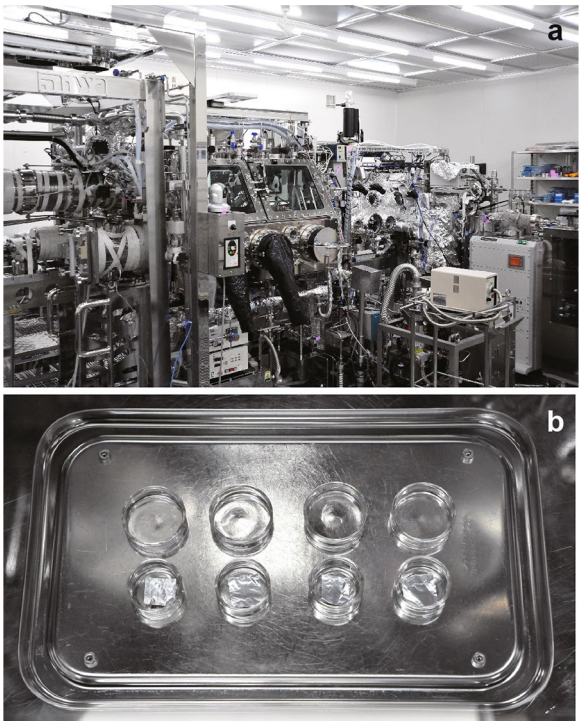


Fig. 1 **a** The clean chamber in the curation room at ISAS/JAXA and **b** the witness coupons placed in the chamber

Assessment using witness coupons

Using aluminium foil as a witness coupon material, by exposing it to CC2 for different durations, the amino acid content in CC2 was quantitatively evaluated. To compare our results with those of the National Aeronautics and Space Administration/Johnson Space Center (NASA/JSC) OSIRIS-REx team, we used the same workflow system, incorporating both quantitative and qualitative evaluation. Aluminium foil, with a thickness of 0.05 mm, was cut to 3 × 3 cm pieces and baked at 450 °C for 6 h. The baked aluminium foil was placed in cleaned Pyrex Petri dishes (ϕ28 mm) and introduced into CC2 (Fig. 1b). The cleaning procedure of the Petri dishes was based on the procedure described by Ishibashi et al. (2012). They were then baked in the same way as the aluminium foil. The baking of the aluminium foil and cleaning of the Petri dishes were done in the manufacturing and cleaning room at the ESCuC. After enclosing the pieces of aluminium foil in Petri dishes, they were brought to CC2 in the planetary sample handling room at the ESCuC. After being evacuated and purged with nitrogen in the airlock of CC2, the Petri dishes filled with aluminium foil were introduced into CC2. After their introduction into CC2, the lids of the Petri dishes were opened, and the aluminium foil was exposed to the environment in CC2. Five witness coupons were prepared and exposed for 1 day, 2 days, 8 days and 31 days, with the last used as a reference without exposure as a blank witness coupon. Each of the coupons was folded using tweezers inside CC2 and taken out of the chamber after closing the lids of the Petri dishes. The blank witness coupon was directly stored in the Petri dish after being baked together with the other witness coupons. The Petri dishes were wrapped in triplicate with baked aluminium foil and transported to the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) for analysis.

Analytical procedure

Chemicals and materials

Amino acid standards (> 98% purity) were purchased from Wako Pure Chemical Industries, Ltd. (hereafter, Wako Chemical) and Sigma-Aldrich. The derivatisation

reagents, distilled water (high-performance liquid chromatography (HPLC)-grade) and several solvents (e.g. dichloromethane (DCM), methanol and hexane) of dioxin analysis grade were purchased from Wako Chemical and used without further purification. Hydrochloric acid (HCl) (6 M, sequencing grade) was obtained from Thermo Fisher Scientific, Inc. All glass vials used in the analysis were preheated at 450 °C for 5 h to eliminate any organic contaminants.

Amino acid extraction and derivatisation

The analytical procedure for the witness coupons is summarised in Fig. 2a. The witness coupons (3-cm squares of aluminium foil) were torn into ~5-mm squares using tweezers and put into glass ampoules (10 ml). Then, 1.5 ml of distilled water was added to each of the ampoules, which were then flame-sealed after replacing the air in them with nitrogen gas. The ampoules were heated at 110 °C for 12 h to extract the amino acids. The extracts were then transferred into tapered glass ampoules (1 ml) by rinsing the witness coupons in the 10-ml ampoules with two aliquots of methanol (300 µl each). After the extracts were dried under a flow of nitrogen gas, 100 µl of 6 M HCl was added, and the ampoules were flame-sealed in the same manner as before and then subjected to acid hydrolysis at 110 °C for 12 h. Then, the solutions were transferred into new tapered glass ampoules (1 ml) by rinsing with two aliquots of methanol (300 µl each) and drying under a flow of nitrogen gas.

The hydrolysed amino acid extracts were then derivatised to *N*-pivaloyl isopropyl (Pv/iPr) ester derivatives for gas chromatographic (GC) analysis. The derivatisation method was based on that of Chikaraishi et al. (2010) and modified for the analysis of trace amounts of amino acids in this study. Analytical validations using a wide variety of sample profiles were performed prior to the present study (cf. Takano et al. 2010; Chan et al. 2016; Ohkouchi et al. 2017). The derivatisation procedure consists of two steps. The first step is the esterification of the carboxyl group. In this step, 100 µl of thionyl chloride/2-propanol (TC/iPr, 1/4, *v/v*) was added to the tapered glass ampoule (1 ml) and heated at 110 °C for 2 h. After the ampoule was cooled to room temperature, the mixture was transferred to a new tapered glass ampoule (1 ml) by washing with two aliquots of DCM (300 µl each) and then dried under a flow of nitrogen gas. The second step of the derivatisation involves the acylation of the amino group. In this step, 200 µl of pivaloyl chloride/DCM (PC/DCM, 1/4, *v/v*) was added, and the mixture was heated at 110 °C for 2 h. In both derivatisation steps, the headspace in the glass ampoules was replaced by nitrogen gas before they were flame-sealed using a hand-held burner.

Once the ampoules were cooled to room temperature, the reagent was transferred to glass vials (1.5 ml) by washing with two aliquots of DCM (300 µl each) and then dried under a flow of nitrogen gas. The derivatised amino acids were then recovered by liquid–liquid extraction using 200 µl of saturated sodium hydrogen carbonate ($NaHCO_3$) solution and DCM/*n*-hexane (98/2, *v/v*). The organic solvent containing amino acid derivatives was further dehydrated by filtering through anhydrous magnesium sulphate ($MgSO_4$) powder using a GHP Nanosep. This extraction and dehydration process was repeated three times. Then, the organic solvent was evaporated under a flow of nitrogen gas, and the residue was dissolved in 50 µl of DCM/*n*-pentane (9/1) as the final fraction.

Instrumental setup for GC/NPD and GC/MS

The derivatised amino acids were analysed by GC coupled with a nitrogen phosphorous detector (GC/NPD) for quantification and GC coupled with mass spectrometry (GC/MS) for qualification measurements. The instruments used were supplied by Agilent Technologies, 6890 N GC, with a programmable temperature vaporisation (PTV) injector. The GC/NPD apparatus was equipped with a DB-5MS fused silica capillary column (30 m × 0.53 mm i.d., 0.50 µm film thickness, Agilent Technologies), and the GC/MS apparatus was equipped with an HP-Ultra2 fused silica capillary column (25 m × 0.32 mm i.d., 0.52 µm film thickness, Agilent Technologies). The PTV temperature was programmed as follows: the initial temperature was set to 50 °C and held for 0.3 min, ramped at 600 °C/min to 350 °C and then held for 10 min. The GC oven temperature was programmed as follows: the initial temperature was set to 40 °C and held for 4 min, ramped at 30 °C/min to 90 °C, then ramped at 3 °C/min to 220 °C and held for 10 min, and then ramped at 30 °C/min to 280 °C and held for 2 min.

(See figure on next page.)

Fig. 2 **a** The procedure of the amino acid analysis of the witness coupons. Each of the steps in the extraction, acid hydrolysis and derivatisation processes was conducted in flame-sealed glass ampoules to minimise any contamination. **b** Calibration lines for the quantification of amino acids detected on the witness coupons. Nine different concentrations of authentic amino acid standard mixtures ranging from 0.5 to 1×10^{-6} nmol/injection were analysed using GC/NPD to draw these lines. Note that the error accompanying the GC/NPD analysis was within ca. 5%

a

Sample

Extraction by water (110°C, 12h)*

Acid hydrolysis by 6M-HCl (110°C, 12h)*

Derivatisation of amino acids (N-piv. iso propyl esters)*

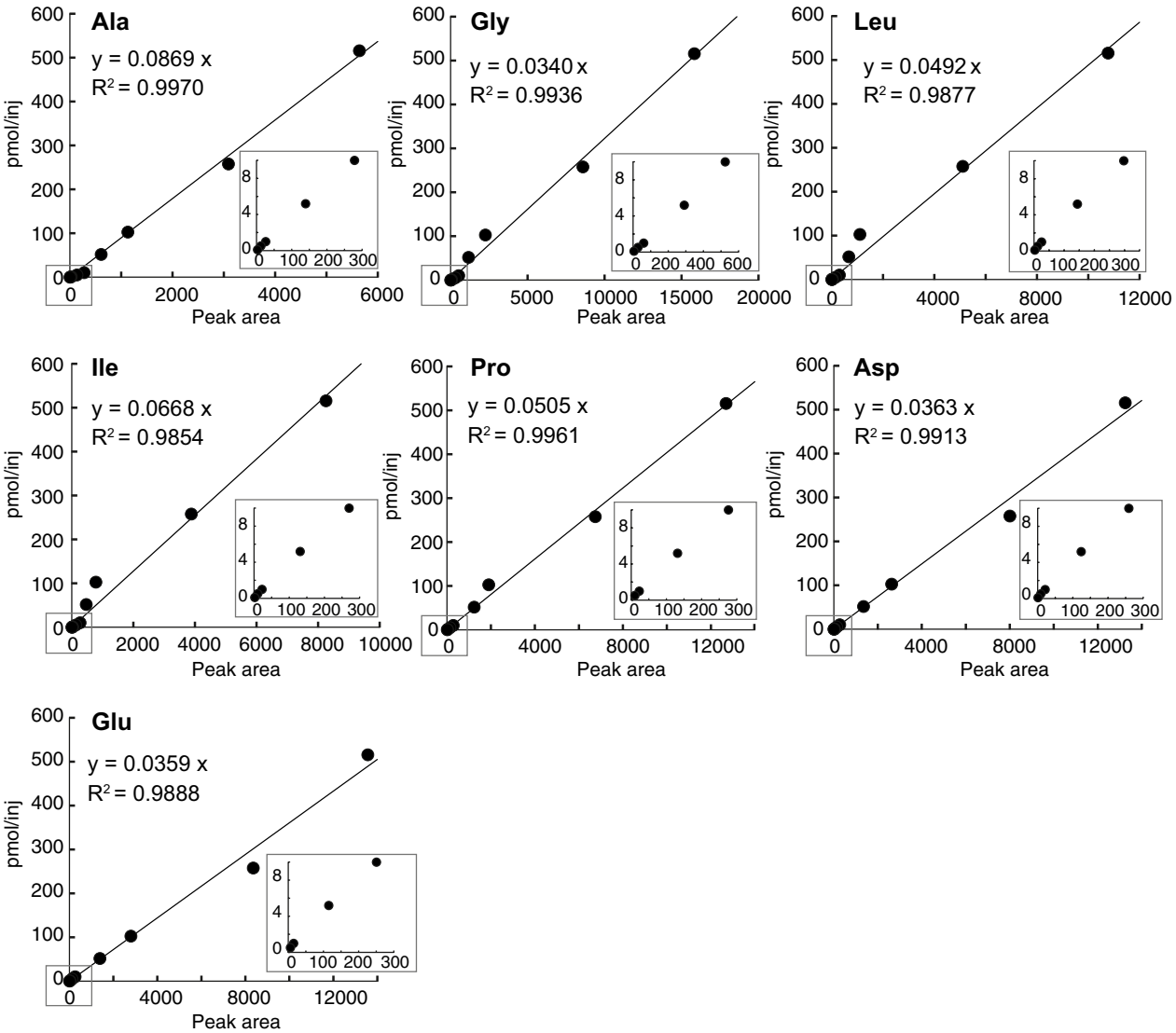
Liquid-liquid extraction of the derivatised amino acids

GC/NPD analysis for quantification

GC/MS analysis for identification

* These processes were conducted in sealed glass ampules.

b



Results and discussion

Qualification and quantification

The amino acids in the sample solutions were identified by GC/MS analysis by comparing their retention times and mass fragmentation patterns with those of authentic standards that include 24 amino acids in total (Fig. 3b) that were derivatised in the same manner. Eight amino acids (glycine, alanine, valine, leucine, isoleucine, proline, aspartic acid and glutamic acid) were detected. All of the detected amino acids are common terrestrial amino acids that make up proteins. The non-biological but characteristic amino acids in carbonaceous chondrites, such as aminoisobutyric acid and isovaline, were not detected. We also checked for the existence of EACA that was found to originate from Nylon 6 (Elsila et al. 2009; Sandford et al. 2010), but it was not detected on our witness coupons. The absence of EACA on our witness coupons indicated that nylon materials are not contaminants in the ISAS/JAXA curation room.

The quantification of the amino acids was performed based on a GC/NPD analysis by drawing calibration lines using the authentic standard solutions of amino acids (Fig. 2b). Seven out of the eight identified amino acids were detected by GC/MS analysis and quantified by GC/NPD analysis, but valine was found to be under the quantification limit (Fig. 3a). Among the 7 amino acids found on the witness coupons, glycine was most abundant, and the highest amount of 10 pmol/cm^2 was found on the day 7 witness coupon, a magnitude higher than that of the blank witness coupon (Table 1; Fig. 4). The second most abundant amino acid was alanine, and the abundance was approximately one-third that of glycine. Aspartic acid and glutamic acid were also detected on all of the witness coupons, although their abundances were relatively close to that of the blank witness coupon. Leucine, isoleucine and proline were also detected on the day 2 witness coupon, but they were not always detected on the other witness coupons.

All of the detected amino acids are proteinogenic amino acids, and they are suspected to be derived from biologically related materials. The relatively high abundance of glycine among all of the detected amino acids is consistent with the amino acid composition of hydrolysed human skin (Jacobson et al. 1990). However, the absence of serine and threonine and relatively low abundances of aspartic acid and glutamic acids, which are also major constituents of the human skin, indicate that human skin was not the major contaminant of the witness coupons. Abiotic processes could also synthesise amino acids. For example, polymers based on polyamides and polyimides have potential risk to be degraded into amino acids by several physicochemical processes such as thermal decomposition, hydrolysis and photo-oxidation (e.g. Jikei and Kakimoto 2004; Sanda and Endo 1999; García et al. 2010; Levchik et al. 1999; Kroes 1963; Herrera et al. 2001). Thus, the contributions of other materials that contain amino acids or can be degraded into amino acids by acid hydrolysis are considerable.

Time-course profile during the exposure monitoring

The abundances of the four major amino acids (glycine, alanine, aspartic acid and glutamic acid) that were detected on all of the witness coupons were plotted against the exposure time, and the results are shown in Fig. 5. There was found to be an increase in the abundances for an exposure time of up to 7 days. In particular, glycine showed a linear increase with the exposure time, i.e. the rate of increase over 7 days was calculated to be 1.2 pmol/cm^2/day upon subtracting the concentration of the blank witness coupon. For the other amino acids, the average rate increases over 7 days were calculated to be 0.6 pmol/cm^2/day for alanine, 0.1 pmol/cm^2/day for aspartic acid and 0.2 pmol/cm^2/day for glutamic acid, although they were variable due to their low concentrations.

Here, we note that the day 31 witness coupon showed an apparent decrease in the amino acid abundance, similar to that of the day 1 witness coupon. A plausible explanation for the dropping off is that the contaminants that accumulated on the witness coupon over time were eventually blown off and lost at some point between days 7 and 31. Another possibility is the heterogeneity of the contaminants on the witness coupons. Further investigation of this phenomenon is necessary, including the determination of the morphology of the contaminants.

Inter-laboratory evaluation of witness coupons between the NASA/JSC and ISAS/JAXA cleanroom facilities

The species and the abundance of amino acids on our witness coupons are similar to what was found on those reported by the NASA/JSC OSIRIS-REx team (Dworkin et al. 2018; McLain et al., 2015, *Contamination Knowledge Report JSC Curation Cabinets*, personal communication). The NASA/JSC OSIRIS-REx team investigated the contamination level of cabinet desiccator boxes located in an ISO 7 cleanroom at Johnson Space Center (JSC) that were reserved for the storage of OSIRIS-REx-returned samples. The desiccator boxes were purged with curation-grade nitrogen gas, and they analysed a set of Al foil witness plates (10 cm^2 each) that were placed in the cabinet desiccator for 1–365 days. Glycine and alanine were found to be the most abundant amino acids on their witness plates, consistent with the findings in our study. Serine was also a common amino acid detected on their witness plates, but it was not found on our witness coupons. Instead, aspartic acid and glutamic acid were major species found on our witness coupons, which were not common on their witness plates. The total abundance of

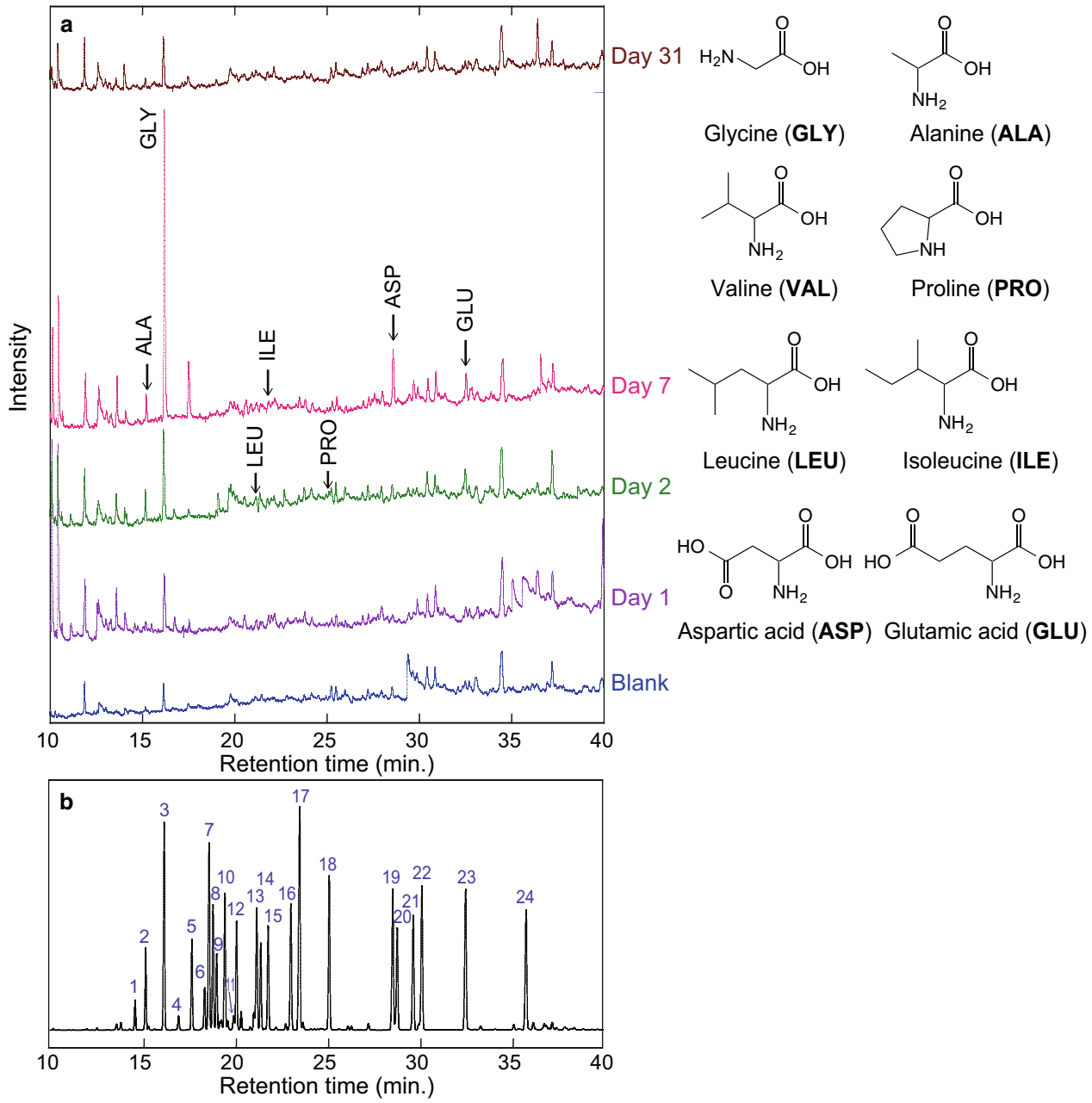


Fig. 3 **a** GC/NPD chromatograms of the witness coupons and chemical structures of the detected amino acids. **b** GC/NPD chromatograms of the authentic amino acid standards. The numbered peaks refer to the following: 1. α-aminoisobutyric acid; 2. α-alanine; 3. glycine; 4. isovaline; 5. α-aminobutyric acid; 6. sarcosine; 7. β-alanine; 8. β-aminobutyric acid; 9. valine; 10. β-aminoisobutyric acid; 11. N-ethylglycine; 12. norvaline; 13. leucine; 14. *allo*-isoleucine; 15. isoleucine; 16. norleucine; 17. γ-aminobutyric acid; 18. proline; 19. aspartic acid; 20. threonine; 21. serine; 22. methionine; 23. glutamic acid; 24. α-aminoadipic acid

amino acids in weight ranged from 0.05 to 0.8 ng/cm^2 for their witness plates, and ours were also in the same range (0.3 to 1.2 ng/cm^2, blank-corrected). They also reported that the lowest total abundance was on the 365-day witness plate and the highest abundance was detected on the 120-day witness plate, and thus, the abundance did not correlate with the exposure time. This observation may correspond to our results that the day 31 witness coupon showed the lowest total concentration. An explanation for this is that any contamination materials may have been blown away by the gases in the chamber when they accumulated beyond a certain amount.

Table 1 Quantification results of the witness coupons in the clean chamber of the ISAS/JAXA curation room

pmol/cm^2	Blank	Day 1	Day 2	Day 7	Day 31
Alanine	0.26	0.68	2.3	2.1	0.66
Glycine	0.88	2.1	3.2	10	1.5
Valine	< LOQ[a]	< LOQ	< LOQ	< LOQ	< LOQ
Leucine	< LOQ	0.67	0.88	< LOQ	0.25
Isoleucine	< LOQ	1.0	0.61	0.42	0.55
Proline	< LOQ	< LOQ	0.38	< LOQ	< LOQ
Aspartic acid	0.49	0.25	0.60	2.2	0.26
Glutamic acid	0.33	0.28	1.1	1.0	0.43

[a] < LOQ: under the limit of quantification by GC/NPD analysis, although identified by GC/MS analysis

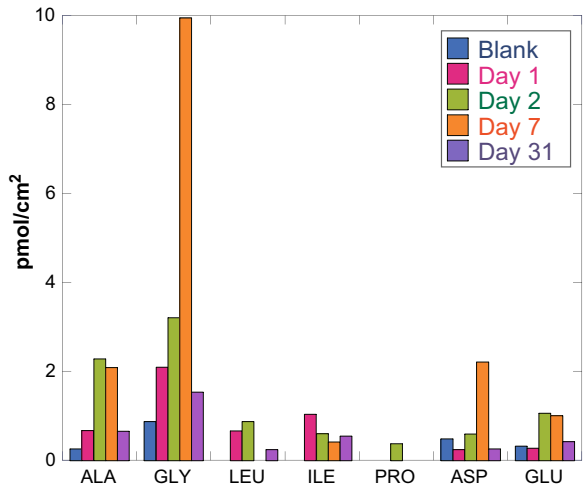


Fig. 4 A bar chart showing the quantification results of amino acids on the witness coupons

The similarity in the total abundance of amino acids between the NASA/JSC cabinet desiccator boxes and our ISAS/JAXA clean chambers indicates that the contamination levels in both facilities were on a similar level. The difference in the common species of amino acids between the two facilities may be attributed to the origins of the contaminants. Through luminosity analysis, Dworkin et al. (2018) found that the total abundance on the NASA/JSC witness plates could not be explained only by the presence of viable cells on the witness plates. Thus, several other organic materials in the cleanroom could also have been the source of the amino acids.

Assessing the clean chambers and feedback to the Hayabusa2 project

The analytical results of the witness coupons in CC2 of the ESCuC showed the existence of amino acid contamination, such as alanine, glycine, aspartic acid and glutamic acid. The total contamination level in the ESCuC of 0.3–1.2 ng/cm^2 (blank-corrected) is comparable to that in the NASA OSIRIS-REx team curation cabinet, 0.05–0.8 ng/cm^2. Dworkin et al. (2018) concluded that the contamination level of the NASA/JSC curation cabinet was satisfactory enough to receive samples. In the OSIRIS-REx mission, they set up a contamination requirement for amino acids at 180 ng/cm^2. This value was based on the contamination level of the foil investigated in the Stardust mission, assuming the collection of a minimum of 60 g of asteroidal samples inside the touch-and-go sample acquisition mechanism (TAGSAM) head that has a surface area of 1916 cm^2. In the case of the Hayabusa2 mission, the expected minimum sample (> 100 mg) is lower than that of the OSIRIS-REx mission, and the sample container is also different (Tachibana et al. 2014). Thus, the OSIRIS-REx contamination requirement cannot be applied straightforwardly to the Hayabusa2 mission; the requirement for the Hayabusa2 mission must be stricter.

The amino acid abundance of carbonaceous chondrites will give a basic overview as Ryugu sample analogues. One reasonable example would be the Murchison meteorite (CM2). The concentration of glycine is 2600 ppb, and that of alanine is 1300 ppb (Glavin et al. 2011). The abundances of these amino acids in a 100-mg sample are calculated to be 3500 pmol and 1500 pmol, respectively. The maximum contamination levels of ESCuC [10 pmol/cm^2 for glycine and 2 pmol/cm^2 for alanine (Fig. 4)] are low enough that they are unlikely to interfere with the original sample. However, it has to be noted here that there is a wide variation in the amino acid abundances by orders of magnitude among carbonaceous chondrites. As Dworkin et al. (2018) discussed, the "worst case" could be 25 pmol/100 mg for glycine and 66 pmol/100 mg for alanine if the asteroid is depleted in soluble organic molecules (Yamato 980115 CI meteorite; Burton et al. 2014). In the OSIRIS-REx mission, they set a contamination level on measurements of "± 30% precision and accuracy" (National Research Council 2007) to define that the material is pristine (Dworkin et al. 2018). This constraint will serve as a useful reference for the Hayabusa2 mission.

On the other hand, our analytical method for detecting amino acids is highly sensitive, and the concentrations of the detected amino acids on our witness coupons are similar to those on the blank witness coupon. This result means that our method can detect most amino acids that exist at levels only a few times higher than those found on the blank as primary signals from the samples handled in CC2. In CC2, most of the tools, jigs and containers were

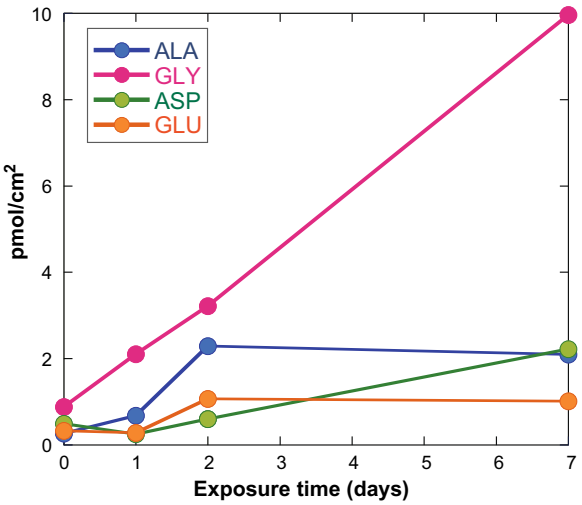


Fig. 5 A time-course profile up to 7 days of selected amino acids common to all of the witness coupons. The result of the day 31 witness coupon, which is not plotted, showed a similar value to that of the day 1 coupon

introduced just after being ultrasonically cleaned by following the methods described by Ishibashi et al. (2012). Baking the items before introduction to CC2 resulted in a decrease in the level of contamination. In addition, it is important to continue monitoring the amino acid contamination level in CC2 on a regular basis to maintain the cleanliness and to detect any accidental increases in the contamination level. It is also useful to understand the detection limits of amino acids in the Hayabusa2-returned samples processed in CC2.

The Hayabusa2 spacecraft arrived at the target asteroid, Ryugu, on 27 June 2018. After a series of remote-sensing analyses, the spacecraft will attempt touchdown and sample recoveries on the asteroid three times, and there are plans for it to return the samples at the end of 2020. At the ESCuC, newly designed clean chambers for the Hayabusa2-returned samples will be installed in the summer of 2018. These chambers are also the subjects of our assessment of amino acid contamination levels.

Summary and future issues

We investigated the contamination levels of CC2 at the ESCuC/JAXA, where the Hayabusa2-returned samples are due to be stored. We used witness coupons to collect contaminants, following the example of the Stardust mission. All of the detected amino acids on our witness coupons were found to be common amino acids of terrestrial origin. The amino acids were thought to be of multiple origins, the nature of which will be determined in future studies. Nevertheless, the results of this study indicate that most of the amino acids were found to be several times more abundant on the witness coupons than on the blank coupon, as determined by primary signals from the samples handled in CC2. Contamination control, especially related to organic contaminants of terrestrial origin, is an essential part of sample return missions from C-type asteroids. Inter-laboratory evaluation of the witness coupons between our ISAS/JAXA curation facility and other related missions such as the OSIRIS-REx mission (NASA/JSC) will contribute to our understanding of the nature of potential contaminants and how to further minimise the levels of such contaminants.

Abbreviations

JAXA: Japan Aerospace Exploration Agency; PMSCF/JAXA: The Planetary Material Sample Curation Facility of JAXA; ISAS/JAXA: Institute of Space and Astronautical Science/JAXA; ESCuC: The Extraterrestrial Sample Curation Center; FE-SEM/EDS: field-emission scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy; ULPA: ultra-low penetration air; FFUs: filter fan units; CC: clean chamber; NASA: National Aeronautics and Space Administration; JSC: Johnson Space Center; JAMSTEC: Japan Agency for Marine-Earth Science and Technology; TAGSAM: touch-and-go sample acquisition mechanism; C-type: carbonaceous type; EACA: *ε*-amino-*n*-caproic acid; DCM: dichloromethane; Pv/iPr: *N*-pivaloyl isopropyl; TC/iPr: thionyl chloride/2-propanol; PC: pivaloyl chloride; GC: gas chromatography; GC/NPD: GC coupled with nitrogen phosphorous detector; GC/MS: GC coupled with mass spectrometry; PTV: programmable temperature vaporisation; GLY: glycine; ALA: alanine; VAL: valine; LEU: leucine; ILE: isoleucine; PRO: proline; ASP: aspartic acid; GLU: glutamic acid.

Authors' contributions

HS conducted the amino acid analysis of the witness coupons, interpreted the data and drafted the manuscript. YT participated in the design of the witness coupons, interpretation of data and drafting of the manuscript. YK, KK and TY designed and prepared witness coupons in ISAS/JAXA and participated in the interpretation of the data and drafting of the manuscript. NO and MA participated in the interpretation of the data and drafting of the manuscript. All authors read and approved the final manuscript.

Author details

[1] Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Natsushima, Yokosuka 237-0061, Japan. [2] Present Address: Institut de Chimie de Nice, Université Côte d'Azur, CNRS, UMR 7272, 28 Avenue Valrose, 06108 Nice, France. [3] Japan Aerospace Exploration Agency (JAXA), Yohinodai, Sagamihara 252-5210, Japan. [4] Marine Works Japan Ltd., Oppamahigashi, Yokosuka 237-0063, Japan.

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Competing interests

The authors declare that they have no competing interests.

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