Chemical analysis of organic molecules in carbonaceous meteorites

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CHAPTER 4

Amino acids in Antarctic CM1 meteorites and their relationship to other carbonaceous chondrites

CM2 carbonaceous chondrites are the most primitive material present in the solar system, and some of their subtypes, the CM and CI chondrites, contain up to 3 wt% of organic carbon. The CM2 carbonaceous chondrites contain a wide variety of complex amino acids, while the CI1 meteorites Orgueil and Ivuna display much simpler composition, with only glycine and β-alanine present in significant abundances. CM1 carbonaceous chondrites show a higher degree of aqueous alteration than CM2-types and therefore provide an important link between the CM2 and CI1 carbonaceous chondrites. Relative amino acid concentrations have been shown to be indicative for parent body processes with respect to the formation of this class of compounds. In order to understand the relationship of the amino acid composition between these three types of meteorites, we have analysed for the first time three Antarctic CM1 chondrites, MET01070, ALH88045, and LAP02277 using gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography-fluorescence detection (HPLC-FD). The concentrations of the eight most abundant amino acids in these meteorites were compared to those of the CM2s Murchison, Murray, Mighei, LEW90500, ALH83100, as well as the CI1s Orgueil and Ivuna. The total amino acid concentration in CM1 carbonaceous chondrites was found to be much lower than the average of the CM2s. Relative amino acid abundances were compared in order to identify synthetic relationships between the amino acid compositions in these meteorite classes. Our data support the hypothesis that amino acids in CM and CI-type meteorites were synthesized under different physical and chemical conditions and may best be explained with differences in the abundances of precursor compounds in the source regions of their parent bodies in combination with the decomposition of amino acids during extended aqueous alteration.

4.1 Introduction

Most current theories about the origin of life on Earth require the presence of a suite of prebiotic organic molecules on the planet's surface. About 50 years ago, Stanley Miller (1953) published the results of his famous experiment where he showed experimentally for the first time that it is possible to synthesize biologically relevant compounds such as amino acids under prebiotic conditions. Although this paper marks the beginning of experimental research in the origin of life on Earth, it later became evident that the atmospheric conditions under which the experiment was performed were probably inconsistent with the composition of the early Earth's atmosphere (Kasting 1993; Kasting and Catling 2003). As an alternative, the exogenous delivery of organic molecules to the early Earth – and to other terrestrial planets and moons – from other solar system bodies such as asteroids and comets have been proposed (Chyba *et al.* 1990; Chyba and Sagan 1992). Based on theoretical calculations it was estimated (Chyba and Sagan 1992) that the largest flux of organic carbon to the Earth is provided by interplanetary dust particles (IDPs), which deliver more than two orders of magnitude of organic carbon more than other sources, such as comet impacts and meteorites. However, more detailed work has shown that amino acids could survive cometary impacts on the Earth (Pierazzo and Chyba 1999), and that micrometeorites are heated to full-depth upon entry into Earth's atmosphere, thereby destroying present amino acids almost completely (Glavin and Bada 2001).

The organic composition of the three sources (comets, asteroids - as represented by meteorites -, and IDPs) is known to different degrees. Until recently, the organic composition of cometary nuclei could be estimated either from astronomical observations of active comets (e.g. Bockelée-Morvan *et al.* 2004) or spacecraft flybys (Kissel and Krueger 1987). The *Stardust* spacecraft captured grains from the tail of comet Wild 2 using an Aerogel collector and returned them back to Earth on January 15, 2006 (Brownlee *et al.* 2006). One of the problems with these grains is the small sample size they provide for investigations of the organic contents, pushing the sensitivity limits of current analytical technology such as high-performance liquid chromatography with fluorescence detection (HPLC-FD) or liquid chromatography-time of flight-mass spectrometry (LC-ToF-MS). The same complication is true for IDPs, although a larger amount of samples is available (Brinton *et al.* 1998; Glavin *et al.* 2004). To date, the most profound understanding about the organic composition of other solar system bodies has come from the analysis of meteorites.

Carbonaceous chondrites are among the most pristine material known in the solar system and are classified into CI, CM, CO, CV and a few other classes based on their elemental abundance ratios (Sears and Dodd 1988). From the organic composition point of view, CI and CM are the most interesting classes, in particular because they contain up to 3 wt% of organic carbon (for reviews see Botta and Bada 2002a; Sephton 2002). More than 80% of this material is locked into an insoluble macromolecule that is structurally and isotopically very complex (Gardinier *et al.* 2000; Cody *et al.* 2002; Sephton *et al.* 2004; Cody and Alexander 2005). The remaining < 20 % is comprised of a mixture of soluble organic compounds. The composition of the organic material and

its distribution as observed in carbonaceous chondrites is the result of a number of processes and depends on several factors: (1) qualitative and quantitative differences in the organics due to multiple sources, including the interstellar medium (ISM), the accretionary disk and the asteroid parent body, (2) the degree of aqueous and thermal processing in the parent body and their effects on organic content, (3) spatial inhomogeneity due to, among other things, geochromatographic separation and transport, and (4) terrestrial age and storage conditions leading to differential levels of contamination, decomposition, leaching (for example during the residence in the Antarctic ice), and perhaps microbial alteration. It is difficult to assess the relative contributions of each of these factors, but analysis of the soluble fraction of the organic content of carbonaceous chondrites is an important tool to shed light on these aspects.

Carboxylic acids are the most abundant class of compounds in the soluble fraction of meteorites with an abundance of approximately 300 parts per million (ppm), followed by the amino acids with about 60 ppm total abundance, as measured in the CM2 carbonaceous chondrite Murchison (Sephton 2002). More than 80 different amino acids have been identified in this meteorite including α-aminoisobutyric acid (α-AIB) and isovaline, which occur in only very rare cases in the terrestrial biosphere and are unambiguously extraterrestrial in origin. In fact, α-AIB was found to be the second most abundant amino acid in the Murchison meteorite, clearly in contrast to its abundance on the Earth, where it is not among the 20 genetically encoded amino acids, but is a constituent in a few fungal peptiabols that are biosynthesized through non-ribosomal enzymatic pathways (e.g. Kleinkauf and von Dohren 1996). Amino acid distributions have also been determined for a number of other CM2 carbonaceous chondrites, including Murray, Mighei and the Antarctic meteorites Lewis Cliff (LEW) 90500 (Botta and Bada 2002b) and Allan Hills (ALH) 83100 (Glavin *et al.* 2006). The total abundances of amino acids in these meteorites were found to be in the range of ~ 7760 parts per billion (ppb) in LEW90500 and ~ 15,300 ppb in Murchison. CI1 carbonaceous chondrites, such as Orgueil and Ivuna, have a distinctively different and simpler amino acid composition compared to the rich variety of amino acids observed in CM2's (Ehrenfreund *et al.* 2001).

The composition of the insoluble macromolecular material is also a powerful indicator to provide insight into parent body processes. Hydrous pyrolysis data obtained for Murchison, Orgueil and Cold Bokkeveld (CM2) reveal that it is qualitatively very similar in CM2 and CI1 chondrites (Sephton *et al.* 2000). However, quantitative differences regarding the ratios of one-ring and two-ring aromatics and phenols in the pyrolysates indicate that the structural differences in the macromolecular materials can be explained with different degrees of aqueous alteration on the parent body. In this model it is suggested that there is a chemical evolutionary sequence from Murchison via Cold Bokkeveld to Orgueil, and that the composition of the macromolecular material in these meteorites are related to each other by the degree of aqueous alteration. The only meteorite samples analysed so far with an aqueous alteration degree of 1 are the CI carbonaceous chondrites Orgueil and Ivuna, which show a significantly simpler amino acid distribution that had no obvious relationship to that of the CM2's. This led to the interpretation that the composition of the CI1 is *not* produced by aqueous alteration of

the CM2 composition, but is the result of different chemical compositions of the parent bodies (Ehrenfreund *et al.* 2001). In order to investigate the relationship in the amino acid composition between CM2, CM1 and CI carbonaceous chondrites, we have analysed the three Antarctic CM1 chondrites Meteorite Hills (MET) 01070, ALH88045, and LaPaz (LAP) 02277 using HPLC-FD and gas chromatography-mass spectrometry (GC-MS), and compared their amino acid composition with that of the CM2s Murchison, Murray, Mighei, the Antarctic CM2s LEW90500, ALH83100, as well as the CI1s Orgueil and Ivuna (Ehrenfreund *et al.* 2001; Botta and Bada 2002a, 2002b; Botta *et al.* 2002; Glavin *et al.* 2006). We have concentrated our investigation on the most abundant amino acids to determine their absolute and relative abundances. A complete inventory of amino acids in these meteorites is beyond the aim of this investigation.

4.2. Materials and Methods

4.2.1 Chemicals and Reagents

All tools, glassware and ceramics used were sterilised by annealing in aluminium foil at 500°C for 3 h. All tips and Eppendorf tubes used were sterilised. Amino acid standards were purchased from Sigma-Aldrich. Ammonium hydroxide (28-30wt%) was also purchased from Acros Organics. *o*-Phthaldialdehyde (OPA), N-acetyl-L-cysteine (NAC), sodium acetate trihydrate, sodium borate decahydrate as well as HPLC grade water were bought from Sigma-Aldrich. Methanol (absolute HPLC) was purchased from Biosolve Ltd. Pentafluoropropionic acid, acetylchloride and isopropanol were purchased in a derivatisation kit containing 1.5 ml ampoules of the reagents from Alltech. Dichloromethane (DCM) was purchased from Aldrich. Sodium hydroxide and hydrochloric acid (37%) were obtained from Boom. AG 50W-X8 resin was acquired from Bio-Rad. All compounds were used without further purification.

4.2.2 Meteorite Sample Preparation, Extraction and Purification

An interior chip of Murchison (1.298 g, USNM 6650) was provided to our laboratory by the Smithsonian National Museum of Natural History, Washington DC. The Antarctic CM1s MET01070 and LAP02277 were collected during the 2001/02 and 2002/03 ANSMET expeditions, respectively. A 0.622 g chip of MET01070 and a 0.255 g chip of LAP02277 (chip #6) were each provided by the Antarctic meteorite curator at the NASA Johnson Space Center, Houston. The Antarctic CM1 ALH88045 was collected during the EUROMET meteorite collection program in 1988/89, and a 0.333 g chip was provided by the EUROMET meteorite curator at the Open University, Milton Keynes, UK. A large interior piece (7.76 g) of the Orgueil meteorite was provided by the Musée National, Paris. Each sample was crushed into a fine powder using a ceramic mortar and pestle in a glove box containing argon, and was stored in a sterilized glass vial before being used in the experiments. A serpentine sample provided by the Naturhistorisches Museum, Bern, was heated to 500°C for 3 h before analysis and processed through the same steps serving as a blank.

Approximately 100 mg of powdered meteorite sample was each analysed using the established procedure (Zhao and Bada 1995; Botta *et al.* 2002) for separating and analysing amino acids in meteorites, which includes each sample being flame sealed in a test tube with 1 ml of HPLC grade water and boiled for 24 h in a heating block at a temperature of 100°C. One of two equal parts of the supernatants was then subjected to a 6 N HCl acid vapour hydrolysis for 3 h at 150°C. For LAP02277, the whole extract was acid-hydrolysed, so no non-hydrolysed extract was available for analysis. The hydrolysed and the non-hydrolysed extracts of the meteorites were desalted on a cation exchange resin (Bio-Rad AG 50W-X8). The amino acids were eluted from the resin with 5 ml of ammonium hydroxide, the resulting elutions were evaporated to dryness under vacuum and the residues dissolved in 100 μl HPLC-grade water. 10 μl aliquots of these solutions were derivatised with *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) for HPLC-FD analysis (Zhao and Bada 1995) or with pentafluoropropionic acid (PFP)/isopropanol for GC/MS (Pizzarello and Cronin 2000).

4.2.3 HPLC Analysis

HPLC-FD analysis of the fluorescent OPA/NAC amino acid derivatives in the meteorite and blank extracts was carried out in a C18 reverse phase (250 x 4.6 mm) Synergi 4μ Hydro-RP 80A column (from Phenomenex), with UV fluorescence detection on a Shimadzu RF-10A$_{XL}$, excitation wavelength at 340 nm and detection at 450 nm. The conditions for amino acid separations for the mobile phase at 25°C were as follows. Flow rate was 1 ml min^{-1}. Buffer A was 50 mM sodium acetate, containing 4% methanol (v/v), and buffer B was methanol. The gradient used was 0 to 5 min, 0% buffer B; 5 to 15 min, 0 to 30% buffer B; 15 to 35 min, 30 to 45% buffer B; 35 to 40 min, 45 to 60 % buffer B; 40 to 49 min, 60 % buffer B; 49 to 50 min, 60 to 0% buffer B; 50 to 60 min, 0 % buffer B.

Amino acids were identified by retention time comparison with known standards. The amino acid abundances in the meteorite samples were calculated by comparison of the integrated peak area, corrected with the abundances in the blank serpentine sample, with the integrated peak area of known amino acid standards.

4.2.4 GC-MS Analysis

The PFP/isopropanol derivatised amino acids in the meteorite and blank extracts were separated by GC-MS on a Chirasil-L-Val column (25 m, 0.25 mm ID, 0.12 μm film thickness, Varian), which was installed in a Thermo-Finnigan Trace GC Ultra gas-chromatographic system. Analyses were carried out on two identical GC systems that were coupled to a Thermo-Finnigan Trace-DSQ quadrupole mass spectrometer (QMS) and a Thermo-Finnigan time of flight-mass spectrometer (ToF-MS), respectively. Helium was used as a carrier gas, and the flow rate was kept constant at 0.6 ml min^{-1}. The injection port was kept at 220°C, and the transfer line between the GC and the MS at 280°C. The following temperature program was applied: initial temperature 60°C for 10 min; ramping to 100°C at 2°C min^{-1}; ramping to 200°C at 4°C min^{-1}; final temperature 200°C for 25 min. The mass range scanned by the MS was *m/z* 50 to 350,

and the peaks were identified in selective ion mass chromatograms using the Xcalibur software package with internal mass spectra library. No quantifications of amino acid abundances were performed in these analyses.

4.3 Results and Discussion

The aim of this study was to analyse for the first time the Antarctic CM1 carbonaceous chondrites MET01070, ALH88045, and LAP02277 to determine the concentrations of the most abundant amino acids in these meteorites. We then compared their absolute and relative amino acid abundances to those obtained for the Murchison and Orgueil meteorites as well as those calculated from literature data for other carbonaceous chondrites. It has not been the purpose of this study, and would be beyond the scope of a single analytical sequence to identify the full complement of amino acids in these meteorites. This goal, which will require larger amounts of meteorite samples, will be addressed in future analyses.

Fig. 4.1 displays typical HPLC chromatograms for all five carbonaceous chondrites as obtained from meteorite extracts after derivatization with OPA/NAC and fluorescence detection (for details see section 4.2.3). Fig. 4.1A shows the chromatograms of ALH88045 and MET01070 together with Murchison, Orgueil and a serpentine blank analysed on the same day; in Fig. 4.1B the chromatogram of LAP02277 is shown together with Murchison, Orgueil and a serpentine blank analysed together, on a different day than the chromatograms in Fig. 4.1A. In addition, we have analysed the same extracts using GC-MS in combination with pre-column derivatisation using PFP-isopropanol in order to detect additional amino acids. Due to the very small amount of meteorite samples available for this study we were not able to identify any amino acids above the detection limit of 1 pmol on both the GC-QMS and GC-ToF-MS instruments using this protocol.

4.3.1 Absolute amino acid concentrations

The absolute amino acid abundances in the HCl acid-hydrolysed hot-water extracts of the meteorites that were obtained in this study using HPLC-FD with OPA/NAC derivatisation are listed in Table 4.1. The values obtained for the Murchison meteorite are significantly lower than those in earlier studies (see Botta and Bada 2002a; Sephton 2002). Because the abundances for all amino acids considered here were reduced by approximately the same factor, this effect was probably caused by sample heterogeneity. The values for Orgueil are close to literature data and can be considered representative for this meteorite (Ehrenfreund *et al.* 2001).

Variability within the CM2 class. An initial impression about the different compositions of these meteorites can be gained by comparison of the absolute concentrations of selected amino acids. We have selected a few amino acids that represent both indigenous compounds with an extraterrestrial origin and molecules that could be potential terrestrial contamination (Botta *et al.* 2002):
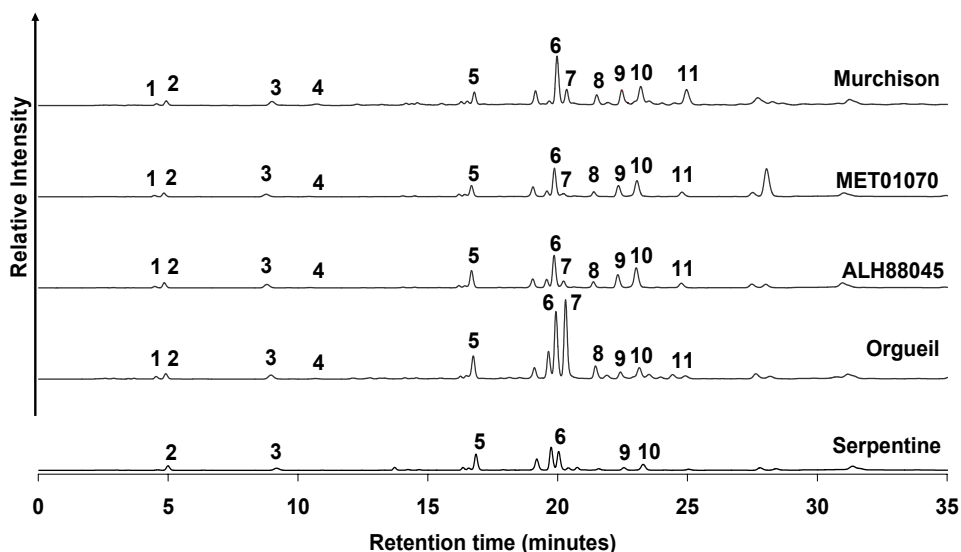
Fig. 4.1A - The 0 to 35 min region of the HPLC chromatograms (no peaks were observed outside this period). OPA/NAC derivatisation of amino acids in the 6 M HCl-hydrolysed hot-water extracts from the CM2 Murchison, CM1s MET01070 and ALH88045 and CI1 Orgueil meteorites and serpentine blank. The peaks were identified as follows: 1. D-aspartic acid; 2. L-aspartic acid; 3. L-glutamic acid; 4. D-glutamic acid; 5. D, L-serine; 6. glycine; 7. β-alanine; 8. γ-Amino-*n*-butyric acid (γ-ABA); 9. D-alanine; 10. L-alanine; 11. α-aminoisobutyric acid (α-AIB).

glycine (the smallest and simplest, but generally most abundant amino acid in meteorites), D- and L-α-alanine (the "non-natural" and biologically relevant enantiomers, respectively, of the smallest chiral amino acid), α-aminoisobutyric acid (α-AIB) (the prototypical extraterrestrial amino acid with only very limited occurrence on the Earth), β-alanine (β-Ala) (the most abundant amino acid in CI1 carbonaceous chondrites) and γ-amino-*n*-butyric acid (γ-ABA) (the smallest γ-amino acid found in meteorites).

It has been demonstrated that many of the Antarctic CM2 carbonaceous chondrites contain a similar abundance and distribution of amino acids compared with non-Antarctic CM2s (Cronin *et al.* 1979; Kotra *et al.* 1981; Shimoyama *et al.* 1979; Botta and Bada 2002a). Exceptions are the Antarctic CM2 meteorites Yamato (Y-) 791198 and ALH83100. Y-791198 contains the largest total abundance of α-AIB in any carbonaceous chondrite analysed to date (Shimoyama *et al.* 1985; Shimoyama and Ogasawara 2002). On the other hand, ALH83100 was found to be depleted in amino acids with a strikingly different amino acid distribution compared to the CM2 meteorites LEW90500 and Murchison (Glavin *et al.* 2006). These unique features may indicate that these meteorites originated from chemically distinct parent bodies from most other CM2 meteorites. The Antarctic CM2 meteorites Y-793321 and Belgica (B-) 7904 have been
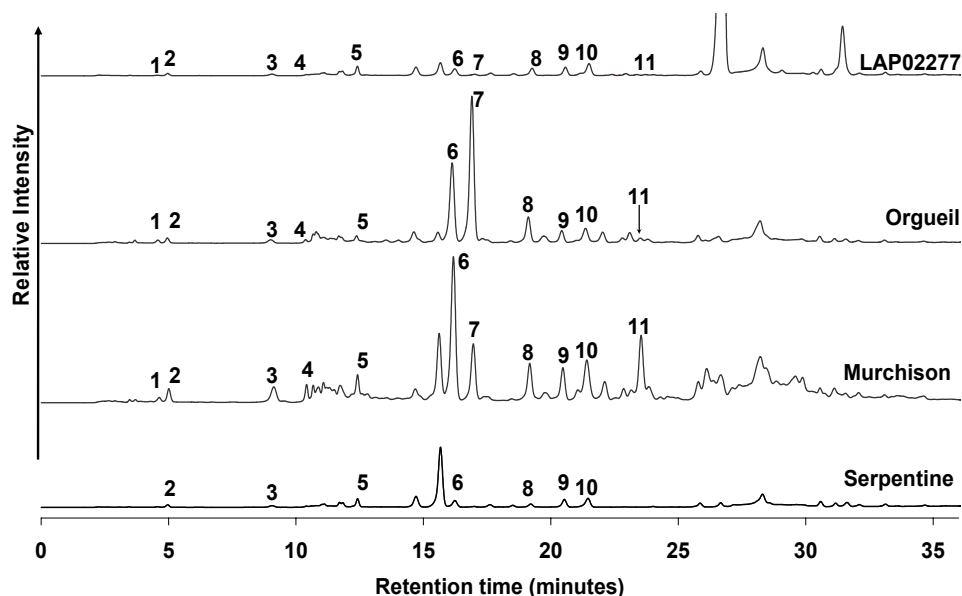
Fig. 4.1B - The 0 to 35 min region of the HPLC chromatograms (no peaks were observed outside this period). OPA/NAC derivatisation of amino acids in the 6 M HCl-hydrolysed hot-water extracts from the CM1 LAP02277 (zoomed), CM2 Murchison and CI1 Orgueil meteorites and serpentine blank. The peaks were identified by comparison of the retention time to those in the amino acid standard run on the same day: 1. D-aspartic acid; 2. L-aspartic acid; 3. L-glutamic acid; 4. D-glutamic acid; 5. D, L-serine; 6. glycine; 7. β-alanine; 8. γ-ABA; 9. D-alanine; 10. L-alanine; 11. α-AIB.

reported to be depleted in amino acids and to show a unique organo-chemical distribution (Shimoyama and Harada, 1984). These authors considered aqueous alteration or leaching during the residence time of the meteorites in the Antarctic ice as possible causes for the amino acid depletion. On the other hand, several Antarctic meteorites, including LEW90500, Y-74662 and ALH77306, show amino acid compositions that are almost identical to the typical CM2s Murchison and Murray, which make leaching an unlikely process to be responsible for these high depletions.

A summary of the abundances (in nmol g^{-1}) of the selected amino acids referred above is shown in Table 4.2. In all CM2 meteorites, glycine and α-AIB are the two most abundant amino acids. Because other organic compound classes such as the carboxylic acids in meteorites show that the abundance of a compound decreases with increasing number of carbon atoms it should be expected that the abundance of alanine is higher than that of α-AIB (Cronin and Chang 1993). However, the alanine concentration is significantly lower than that of α-AIB in all CM2s measured so far with the exception of the unusual CM2 ALH83100 (Glavin *et al.* 2006). For example, in Murchison in our analysis we found a total α-alanine concentration of 9.3 nmol g^{-1}, while the α-AIB concentration was found to be 18.6 nmol g^{-1}, a factor of two higher (Table 4.2, column 3). The β-alanine concentrations for the non-Antarctic CM2 meteorites are in a similar range, again with the exception of ALH83100.

Table 4.1 - Summary of the average serpentine blank-corrected amino acid concentrations in the non-acid-hydrolysed (free) and the 6 M HCl acid-hydrolysed (total = free plus bound) hot-water extracts of Murchison, ALH88045, MET01070, and the 6 M HCl acid-hydrolysed (total) hot-water extracts of LAP02277 and Orgueil, as measured in this study.*

Amino Acid	Murchison		ALH88045		MET01070		LAP02277	Orgueil
	Free	Total	Free	Total	Free	Total	Total	Total
D-Aspartic Acid	62 ± 18	112 ± 29	52 ± 13	68 ± 18	50 ± 8	106 ± 27	10 ± 1	59 ± 14
L-Aspartic Acid	101 ± 35	136 ± 45	94 ± 18	99 ± 39	117 ± 17	201 ± 61	29 ± 13	58 ± 8
L-Glutamic Acid	213 ± 83	287 ± 88	160 ± 37	174 ± 50	252 ± 31	274 ± 74	64 ± 10	101 ± 27
D-Glutamic Acid	58 ± 9	245 ± 30	24 ± 9	33 ± 9	29 ± 8	54 ± 14	24 ± 3	35 ± 5
D,L-Serine	<146‡	189 ± 38	<138‡	<174‡	69 ± 16	147 ± 52	101 ± 13	145 ± 12
Glycine	857 ± 99	1249 ± 137	258 ± 52	369 ± 117	223 ± 45	575 ± 159	55 ± 17	664 ± 34
β-Alanine	153 ± 12	809 ± 39	73 ± 14	197 ± 21	43 ± 5	169 ± 39	16 ± 6	1579 ± 44
γ-ABA	137 ± 33	510 ± 66	168 ± 42	168 ± 35	207 ± 11	259 ± 61	95 ± 10	279 ± 7
D-Alanine	414 ± 113	415 ± 104	229 ± 74	389 ± 87	546 ± 151	630 ± 135	201 ± 14	102 ± 22
L-Alanine	443 ± 100	473 ± 127	271 ± 91	605 ± 149	1007 ± 41	1028 ± 239	228 ± 45	133 ± 52
α-AIB	1071 ± 43	1921 ± 269	7 ± 2	380 ± 97	19 ± 3	881 ± 280	28 ± 10	181 ± 25

* The values are reported in parts-per-billion (ppb). Quantification of the amino acids included background level correction using the serpentine blank and a comparison of the peak areas with those of a known amino acid standard. The uncertainties (δx) of the values are based on the standard deviation of the average value of between four and six separate measurements (N) with the standard error $\delta x = \sigma x \cdot N^{-1/2}$.

‡ This concentration was very similar to blank levels and therefore is considered to be a maximum value.

Abbreviations: α-AIB, α-aminoisobutyric acid; ABA, amino-*n*-butyric acid.

Table 4.2 - Summary of the average total (free plus bound[†]) absolute amino acid concentrations in blank-corrected 6 M HCl acid-hydrolysed hot-water extracts of carbonaceous chondrites.*

Amino Acid	CM2						CM1			CI1		
	Murchison [1,2]	Murchison	Murray [1,2]	Mighei [1]	LEW90500 [3]	ALH83100 [4]	ALH88045	MET01070	LAP02277	Orgueil [1,2]	Orgueil	Ivuna [1,2]
Gly	38.9	16.6	28.1	10.5	17.8	4.0	4.9	7.7	0.7	9.4	8.8	8.2
β-Ala	14.2	9.1	11.9	10.1	6.4	3.8	2.2	1.9	0.2	23.0	17.7	15.7
γ-ABA	12.9	5.0	7.0	11.0	9.5	<3.0	1.6	2.5	0.9	6.1	2.7	~5.8
D-Ala	8.1	4.7	6.9	2.7	3.7	1.2	4.4	7.1	2.3	0.8	1.1	0.9
L-Ala	10.7	4.6	7.2	3.9	5.3	1.5	6.8	11.5	2.6	0.8	1.5	1.8
α-AIB	28.1	18.6	19.1	7.2	21.4	2.4	3.7	8.6	0.3	0.4	1.8	0.5

* Values are reported in nmol g^{-1}. Uncertainties are not shown, but are known for all the literature values and are reported for this study in Table 4.1.
† Free amino acids are those that are readily detectable in the non-hydrolysed fraction; bound amino acids are those that were liberated during acid hydrolysis, making them accessible for detection with OPA/NAC.
Abbreviations: LEW, Lewis Cliff; ALH, Allan Hills; MET, Meteorite Hills; LAP, LaPaz; Gly, glycine; Ala, alanine; ABA, amino-*n*-butyric acid; α-AIB, α-aminoisobutyric acid.
References: [1] Botta *et al.* 2002; [2] Ehrenfreund *et al.* 2001; [3] Botta and Bada 2002b; [4] Glavin *et al.* 2006.

Variability within the CI class. The only two CI carbonaceous chondrites that have been analysed for their amino acid composition are Orgueil and Ivuna (Ehrenfreund *et al.* 2001), and their absolute and relative compositions have been found to be very similar (Botta *et al.* 2002). Generally, the most abundant amino acid in this meteorite class is β-alanine with values between 15.7 and 23 nmol g^{-1}, respectively. Only glycine and γ-ABA were found at significant abundances while all other amino acids are present at concentrations lower than 1.8 nmol g^{-1} (Table 4.2). This very simple amino acid distribution has been attributed to processes occurring on a parent body at very low temperature (for example an extinct comet in an asteroidal orbit) or originating in a region with depleted organic starting material (Ehrenfreund *et al.* 2001).

Comparison with the CM1 class. The amino acid abundances for the CM1 carbonaceous chondrites are summarized in Table 4.1 (in ppb, including uncertainties) and Table 4.2 (in nmol g^{-1}). The results in Table 4.2 are graphically represented in Fig. 4.2, where it can be seen that the CM1 average abundances of the five selected amino acids are lower than in the CM2 and CI1 meteorites. Exceptions are the glycine abundances in Orgueil and MET01070, which are almost identical, and the D-alanine abundances, which shows a wide variability between all meteorites and which is even higher in MET01070 than in Murchison. The high α-AIB content in MET01070 indicates an indigenous extraterrestrial origin. This is confirmed by the relatively high D/L alanine ratios in all three CM1s (0.65, 0.62 and 0.88 respectively for ALH88045, MET01070 and LAP02277). It is possible that the lower abundances of amino acids in the CM1 carbonaceous chondrites are a consequence of extended aqueous alteration on the parent body, and that the high α-alanine abundance in these meteorites was caused by an exceptionally high abundance of acetaldehyde in the parent body. The amino acid abundances in LAP02277 are even lower than those in MET01070 and ALH88045, with only D- and L-alanine present at abundances of approximately 200 ppb (Table 4.1). The abundances of glycine and α-AIB in LAP02277, the most abundant amino acids in CM2s, are very low (only a few ten ppb). This meteorite seems to be unique in terms of its amino acid composition.

Comparison between non-hydrolysed and hydrolysed extracts of ALH88045 and MET01070. The non-hydrolysed fractions of the meteorite extracts were analysed for Murchison, ALH88045 and MET01070 (Table 4.1). No analysis of the non-hydrolysed fraction was possible for LAP02277 because the whole sample extract had to be hydrolysed due to the low abundances. Comparison between the non-hydrolysed and hydrolysed extracts of ALH88045 and MET01070 shows, with the exception of α-AIB, increases in the total amino acid concentrations between approximately a factor of 1 for γ-ABA in ALH88045 and a factor of 4 for β-alanine in MET01070. These increases are in the same range as observed in Murchison (Cronin and Chang 1993). One major difference, however, concerns the α-AIB concentration. While α-AIB can be found in high abundances already in the non-acid-hydrolysed extract of Murchison (Table 4.1) and LEW 90500 (Glavin *et al.* 2006), only very low concentrations of free α-AIB were detected in the two CM1 (7 ppb in ALH88045 and 19 ppb, in MET01070). In ALH83100, which has a much lower amino acid concentration than Murchison and LEW90500, the free α-AIB concentration was also found to be only about a factor of 2
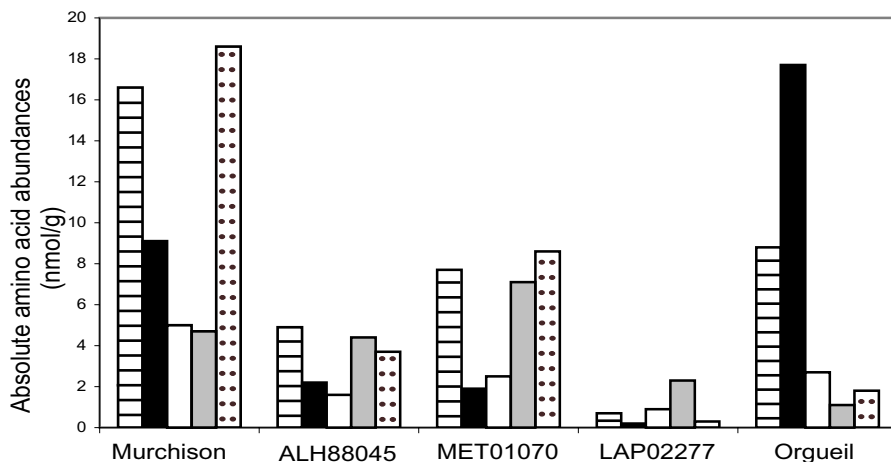
Fig. 4.2 – Average amino acid abundances (in nmol g^{-1}) for the amino acids glycine (horizontal lines), β-alanine (black), γ-amino-*n*-butyric acid (white), D-alanine (grey) and α-aminoisobutyric acid (dots) in the CM2 Murchison, the CM1s ALH88045, MET01070 and LAP02277, and the CI1 Orgueil (data taken from Table 4.2).

lower than the total α-AIB concentration (Glavin *et al.* 2006). This finding indicates that α-AIB is primarily present as an acid-labile precursor molecule in ALH88045 and MET01070, which may point to another synthetic pathway for α-AIB on their parent bodies. Another option is selective leaching of α-AIB in the Antarctic ice during the terrestrial residence time, which would primarily affect the free amino acids. However, this should have affected the CM2s LEW90500 and ALH83100 as well, but their free α-AIB concentrations are in the same range as that of Murchison, indicating that they did not suffer any leaching in the Antarctic ice. Thus, apart of the very low free α-AIB concentration in the two CM1s, the increase of the amino acid concentrations by acid hydrolysis is consistent with what has been observed in CM2 meteorites.

4.3.2. Relative amino acid abundances

Comparing relative amino acid concentrations, in particular relative to glycine, has been shown to be a powerful indicator for parent body processes (Botta *et al.* 2002). The calculated relative abundances in nmol g^{-1} are slightly different to the weight (ppb) ratios calculated previously (Botta *et al.* 2002), but the general trends remain unaffected. There is a significant difference in the α-AIB/glycine (gly) ratio for Murchison between the literature value of 0.72 (Ehrenfreund *et al.* 2001, Botta and Bada 2002b) and the one from our study of 1.12 (Table 4.3). Since all other values for Murchison are very similar, it is likely that differences in the peak integration procedures could be responsible for this effect.

Variability within the CM2 class. Some of the relative amino acid abundances in the five CM2 meteorites listed in Table 4.3 show very little variability between the different

samples. For example, the D-alanine (D-ala)/gly and the L-ala/gly ratios range from 0.21 to 0.30 and 0.26 to 0.38, respectively. The higher L-ala/gly in Mighei may be a signature of terrestrial contamination of this meteorite (as is also indicated from the D/L ratio for alanine of 0.69), whereas ALH83100 has been shown to possess a unique amino acid distribution (Glavin *et al.* 2006). The same arguments can be used to explain the enhanced β-ala/gly ratios in these two meteorites compared to Murchison, Murray and LEW90500. Interestingly, the α-AIB/gly ratio in ALH83100 is consistent with the literature values for Murchison, Murray and Mighei. Because α-AIB is extremely rare in the terrestrial biosphere, an α-AIB contamination is unlikely to occur. In contrast, LEW90500 shows an unusually high α-AIB/gly abundance ratio, and although there is a relatively large uncertainty associated with the α-AIB measurements in this meteorite (Botta and Bada 2002b), this ratio is significantly larger than any other CM2, with the notable exception of Y-791198 (Shimoyama and Ogasawara 2002). In summary, the α-AIB/gly abundance ratio in CM2 carbonaceous chondrites may vary by a factor of 2 between 0.60 and 1.20, although a value of ~0.7 is probably typical.

Variability within the CI class. As expected from the absolute abundance data, the relative amino acid abundances calculated for Orgueil and Ivuna are very similar as well (Table 4.3). The most outstanding feature is the very high β-ala/gly ratio, which is unmatched by any of the CM2s (Ehrenfreund *et al.* 2001). With the exception if the γ-ABA/gly ratio (0.31-0.71), the other relative amino acid abundances are very low (< 0.22).

Comparison with the CM1 class. The most distinctive feature of the amino acid distribution in the CM1 carbonaceous chondrites is their high D-ala/gly ratio. In ALH88045 and MET01070 the D-ala abundances are almost as high as the glycine abundances, and the combined relative α-ala/gly ratio is almost 2.5 (Table 4.3). With the exception of this feature, the relative abundances of the other three amino acids measured in ALH88045 and MET01070 are very similar to those observed in the CM2 carbonaceous chondrites (Figure 4.3). In contrast, the amino acid distribution in LAP02277 is unique in this data set displaying a high D-ala/gly ratio of 3.3 and a γ-ABA/gly ratio of 1.3.

None of the CM1 displays a signature that is similar to the CI1 meteorites. The high absolute and relative abundance of β-alanine in Orgueil and Ivuna does not have any equivalence in the CM1s and therefore remains a unique feature to the CI carbonaceous chondrites (Ehrenfreund *et al.* 2001).

These observations suggest that there may be a synthetic link between the CM1 and CM2 carbonaceous chondrites in that the amino acid distribution of the CM1s may be a result of CM2 distribution that was depleted in absolute abundances due to extensive aqueous alteration (see section 4.3.3 for more detailed discussion). On the other hand, no synthetic connection can be inferred between the amino acids in the CM1 and the CI1 meteorites.

Table 4.3 - Summary of the relative amino acid abundances in blank-corrected 6 M HCl acid-hydrolysed hot-water extracts of carbonaceous chondrites.*

Amino Acid	CM2						CM1			CI1		
	Murchison [1,2]	Murchison	Murray [1,2]	Mighei [1]	LEW90500 [3]	ALH83100 [4]	ALH88045	MET01070	LAP02277	Orgueil [1,2]	Orgueil	Ivuna [1,2]
β-Ala	0.36	0.55	0.42	0.96	0.36	0.95	0.45	0.26	0.29	2.45	2.01	1.91
γ-ABA	0.33	0.30	0.25	1.05	0.55	<0.75	0.33	0.33	1.29	0.65	0.31	~0.71
D-Ala	0.21	0.28	0.25	0.26	0.21	0.30	0.90	0.92	3.29	0.09	0.13	0.11
L-Ala	0.28	0.28	0.26	0.37	0.30	0.38	1.38	1.50	3.71	0.09	0.17	0.22
α-AIB	0.72	1.12	0.68	0.69	1.20	0.60	0.76	1.11	0.43	0.04	0.20	0.06

* Values are reported as ratios of the amino acid abundances in nmol g^{-1} to the glycine abundance in nmol g^{-1}.
Abbreviations: LEW, Lewis Cliff; ALH, Allan Hills; MET, Meteorite Hills; LAP, LaPaz; Ala, alanine; ABA, amino-*n*-butyric acid; α-AIB, α-aminoisobutyric acid.
References: [1] Botta *et al.* 2002; [2] Ehrenfreund *et al.* 2001; [3] Botta and Bada 2002b; [4] Glavin *et al.* 2006.
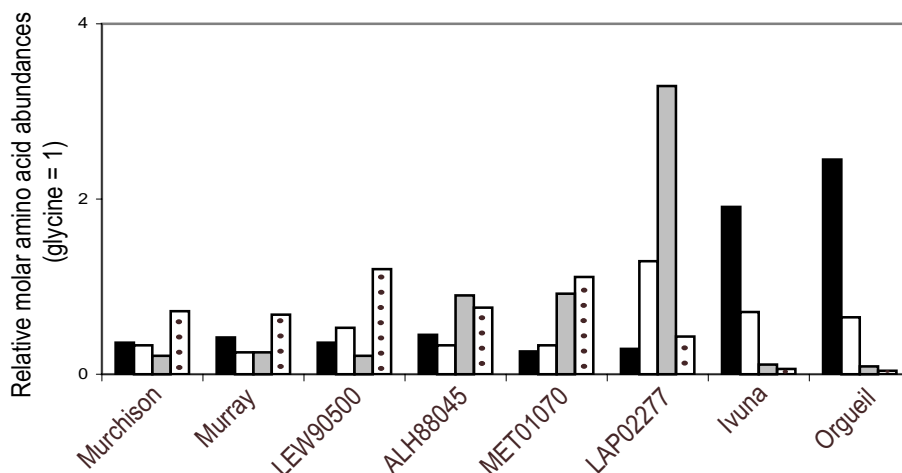
Fig. 4.3 – Average molar relative (glycine = 1) amino acid abundances for the amino acids β-alanine (black), γ-amino-*n*-butyric acid (white), D-alanine (grey) and α-aminoisobutyric acid (dots) in the CM2s Murchison, Murray and LEW90500, the CM1s ALH88045, MET01070 and LAP02277, and the CI1s Ivuna and Orgueil (data taken from Table 4.3).

4.3.3 Implications for Parent Body Processes

Given that these amino acids were synthesized on the asteroidal parent bodies of these meteorites in the first few million years of solar system history and are not interstellar molecules, the most accepted pathway for abiotic amino acid synthesis is via Strecker-cyanohydrin reaction (Peltzer and Bada 1978; Lerner and Cooper 2005). Interstellar and/or solar system precursor compounds, including HCN, ammonia and carbonyl compounds such as formaldehyde, acetaldehyde and acetone would react under aqueous conditions at neutral to basic pH to form in equilibrium cyanohydrins and α-aminocyanides. These intermediates would irreversibly hydrolyse to the corresponding hydroxy- and amino acids (Peltzer *et al.* 1984; Cronin and Chang 1993; Botta and Bada 2002a). Assuming that the reaction rates for the formation of the aminocyanides and the hydrolysis steps are approximately equal, the abundance of the amino acids depends on (1) the abundance of the precursor carbonyl compound and (2) the stability of the amino acid to decomposition or leaching during the lifetime of the meteorite (which includes its residence time on the Earth). This trend is clearly observable in the CM2 amino acid distribution and has been seen for other organic compounds such as carboxylic acids as well (e. g. Cronin *et al.* 1993). From the relatively high α-AIB concentrations seen in the CM2 carbonaceous chondrites it can be inferred that either acetone was present at unusually high abundances on the CM2 parent bodies or that α-AIB is exceptionally stable against decomposition compared to other amino acids. On the other hand, the high α-alanine abundances in the CM1s suggests a higher than usual acetaldehyde abundance

on the parent bodies of these meteorites and therefore a different source region for these parent bodies. The unique amino acid distribution of LAP02277 indicates that this meteorite originated on a distinct parent body following a similar argumentation as for the unique amino acid distribution of ALH83100 (Glavin *et al.* 2006).

The mechanism for the extraterrestrial formation of non-α-amino acids has not been investigated in detail in laboratory studies. The only suggestion by Cronin and Chang (1993) was that Michael-type addition of ammonia to α,β-unsaturated nitriles such as cyanoacetylene or acrylonitrile or higher analogues followed by reduction/hydrolysis steps could be the responsible pathway. Some of these precursor molecules were identified in the interstellar medium and could be present in solar system bodies and are obvious candidates as precursors for this reaction scheme (see Ehrenfreund *et al.* 2002 for a review). Chemical pathways such as Michael additions could occur on parent bodies of meteorites. The high abundances of β-alanine in the CI meteorites indicate either that (1) their parent bodies were depleted in the Strecker-cyanohydrin precursor molecules such as carbonyl compounds at their place of origin, (2) β-alanine synthesis is decoupled from aqueous alteration, which points towards a formation mechanism for this amino acid, and perhaps all non-α-amino acids in meteorites, that does not require the presence of liquid water in the parent body, or (3) the more complex amino acids were selectively decomposed after their formation, leaving only glycine and β-alanine behind in significant abundances. For example, α-decarboxylation, the primary decomposition process for amino acids during thermal alteration of the organic material, could lead to the formation of β-alanine (from aspartic acid) and γ-ABA (from glutamic acid). However, the high concentrations of aspartic and glutamic acids necessary for the observed β-alanine and γ-ABA abundances in CIs, which are not direct products of Strecker-cyanohydrin synthesis, require further explanation. One possible precursor for glutamic acid is the hydrolysis of pyroglutamic acid, a member of the carboxylactams that were identified in the Murchison meteorite (Cooper and Cronin 1995).

4.4 Summary and Conclusion

This study aimed at finding a generic link in the amino acid composition between CM2, CM1 and CI1 carbonaceous chondrites. We have extracted for the first time samples of the CM1 meteorites ALH88045, MET01070 and LAP02277 and determined the concentrations of the eight most abundant amino acids in these meteorites (Table 4.1). Comparing the absolute and relative abundances of five crucial amino acids with those of the CM2 and CI1 carbonaceous chondrites led to the following observations:

(1) The total amino acid concentration in CM1 carbonaceous chondrites was found to be significantly lower than the average of the CM2s. The only exception is the abundance of α-alanine, which is as abundant as in Murchison. Among the analysed CM1s, MET01070 contains the highest individual amino acid abundances, followed by ALH88045.

(2) The relative abundances of D-alanine, α-AIB, β-alanine and γ-ABA to glycine allow the CM1 meteorites to be separated into two groups. ALH88045 and MET01070 are

very similar in their relative amino acids abundances. They show a high relative α-alanine abundances compared to the CM2s, but are very similar to the CM2s with respect to the other amino acids. LAP02277 show a unique amino acid distribution that cannot be synthetically linked to either of the other meteorite classes.

(3) Our data show that there is no relation between the amino acids content of CM1 and CI meteorites. The differences in the amino acid distributions in the three classes of carbonaceous chondrites may best be explained with differences in the abundances of precursor compounds in the source regions of their parent bodies in combination with the decomposition of amino acids during extended aqueous alteration. For the CI1 meteorites, a high abundance of the α,β-unsaturated alkylnitriles cyanoacetylene or acrylonitrile, undergoing reaction with ammonia followed by reduction/hydrolysis, would lead to the large amounts of β-alanine observed in the CI1s (Ehrenfreund *et al.* 2001). In contrast, a low abundance of carbonyl compounds in the same source region would result in limited amounts of α-amino acids. Based on the similar relative amino acid abundances seen between the CM2 and the CM1 class meteorites and the lower overall abundances in the CM1s, it is likely that decomposition of a CM2-like amino acid distribution during extended aqueous environment in the CM1s has led to a low concentration of amino acids in these meteorites, leaving the relative abundances approximately equal. The relatively high abundance of α-alanine, which would indicate a higher interstellar acetaldehyde concentration in the CM1 parent body, indicates that CM2 and CM1 parent bodies probably did not originate in the same source region. In summary, these results suggest that, very likely, the chemistries in these three types of meteorites reflect compositional characteristics of different types of parent bodies.

In order to investigate the network of possibilities for the formation of these amino acid distributions, laboratory studies for amino acids synthesis and decomposition under realistic parent body conditions should be carried out.

Acknowledgements

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