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Discovery and sequencing of histidine and ornithine-rich polypeptide in the Helmutite phase of meteoritic carbonaceous matter☆

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Dedicated to Prof. Helmut Schwarz on the occasion of his 60th birthday

Abstract

Extraction of carbonaceous matter from a chondrite meteorite that fell in Zurich, Switzerland in November 2000 yielded a sample of Helmutite, i.e., carbon nanotubes that contained trapped polypeptides. The isolation, purification, and sequencing of these endohedral polypeptides is reported. A His-tag affinity chromatography yielded an almost pure fraction of a polypeptide, named α-tuberlin, whose sequence was unusually rich in ornithine. Sequencing was accomplished by tryptic digestion followed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry using post-source decay with ultrashort laser pulses.

Keywords: Extraterrestrial matter; Tuberlin

1. Introduction

The fall of a meteorite in the late afternoon of 13 November 2000, in the courtyard of the chemistry building of the Swiss Federal Institute of Technology (ETH) Zurich, Switzerland, caused considerable structural damage and eventually led to the entire chemistry department's move in mid-2001 to an alternate institute building at ETH's out of town campus. Luckily, nobody was hurt during the fall, because it happened at a time when almost all faculty and students were attending a seminar. Shortly after the fall, authorities sealed the surroundings of the chemistry building and prevented access to the meteorite crater because Zurich police erroneously suspected a missile attack. A team of analytical chemists managed to recover most of the meteorite mass for research purposes, which explains why the projectile that

☆ Editor Note: This paper is included as part of the Foreword Section of the Special Issue for reasons that become abundantly clear to even a mildly discerning reader. We all hope the damage done to Zurich by the arrival of Helmutite will be partially compensated for by the audacity of the work presented here. Think what would have happened if Helmut himself had actually arrived!

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Fig. 1. Photograph of the ETH meteorite (foreground) and the recovery team (background) taken on 13 November 2000.

caused the crater was never found and officially is still unaccounted for [1]. Fig. 1 shows a photograph of the meteorite after successful recovery by the team from ETH Zurich. Here we report initial results from chemical and mass spectrometric analysis of this meteorite, which was identified to belong to the class of C2 carbonaceous chondrites.

As will be shown below, this meteorite contained some highly unusual materials, in particular extraterrestrial peptides trapped in carbon nanotubes. It is not the first time that endohedral compounds are isolated in meteoritic material or fullerene-like matter. Schwarz and coworkers were the first to show that helium could be trapped in fullerene following collision experiments [2], and Becker et al. [3] used the isotopic signature of such trapped molecules in fullerene samples recovered from a meteorite crater to prove their extraterrestrial origin. The material that contains polypeptides trapped in carbon nanotubes is called "Helmutite phase," in recognition of the original experiments by Schwarz and coworkers. This present work extends the previous research to much higher molecular weights of endohedral compounds. Additional evidence for the extraterrestrial origin is provided by the highly unusual amino acid composition.

2. Experimental

A 20-g sample of the ETH meteorite was demineralized using standard procedures [4], followed by extraction in toluene (Sigma, p.a. grade, Buchs, Switzerland) at 65 °C, yielding an almost black solution/suspension of carbonaceous matter. This material (referred to as "sample 1") was directly used for X-ray fluorescence studies and atomic force microscopy investigation (mod. Explorer, Topometrix, Santa Clara, USA). Further purification by water/toluene

liquid–liquid extraction, centrifugation, and ultrafiltration resulted in a deep purple toluene solution ("sample 2") which was then evaporated to dryness for density determination and further analysis. About 8 mg of this sample was treated in an oxygen plasma in an attempt to cleave some of the carbonaceous matter and to release further organic matter. This product was then redissolved in industrial grade ethanol (Lagavulin, 16y, Lagavulin Distillery, Port Ellen, Isle of Islay) and passed over an affinity chromatography column containing Ni beads for isolation of any histidine-rich peptidic fraction. Excess solvent was consumed by members of the research team for environmentally friendly disposal. The purified peptide fraction ("sample 3") was washed from the column by adjusting the pH and then analyzed by MALDI mass spectrometric analysis. This MALDI mass spectrometer was equipped with a home-built laser source emitting ultrashort laser pulses [5] in the near-UV (nominal pulse width = 0 fs). The ability to perform MALDI with such short pulses proved to be key to laser desorb and ionize the compounds present.

3. Results and discussion

Elemental analysis of sample 1 showed a large proportion of carbon (84%), accompanied by nitrogen (8%), oxygen (7%), and traces of sulfur, phosphorous, and selenium. AFM analysis of this material is shown in Fig. 2. Clearly visible are ropes or nanoworms of material that could be interpreted in various ways. One possibility is to assign these features to extraterrestrial petrified nanobacteria, based on earlier speculation in the literature [6]. However, considering the elemental composition we propose that these represent carbon nanotubes with unusually large diameters, perhaps containing trapped matter.

The second purification step (water/toluene liquid–liquid extraction, centrifugation, and ultrafiltration) gave sample 2, which was subjected to an SDS PhastGel sampling device. From the intensity of the colored spots, an estimation of the density could be obtained [7], which yielded values considerably above the known density of carbon nanotube or fullerenes. This prompted us to perform the oxygen plasma treatment of sample 2, which was believed to release any trapped matter inside the carbon nanotubes. Real-time AFM observations showed the nanoworms twisting and turning, but eventually releasing an obviously organic schlunz that proved to be best soluble in single malt scotch. Ni-bead affinity chromatography, as described in Section 2, gave sample 3 which was analyzed mass spectrometrically.

The MALDI mass spectra of sample 3, using 2,5-dihydroxybenzoic acid (DHB) as a matrix, is shown in Fig. 3a. The mass spectrum exhibits an intense signal at 5050 Da, accompanied by a number of smaller

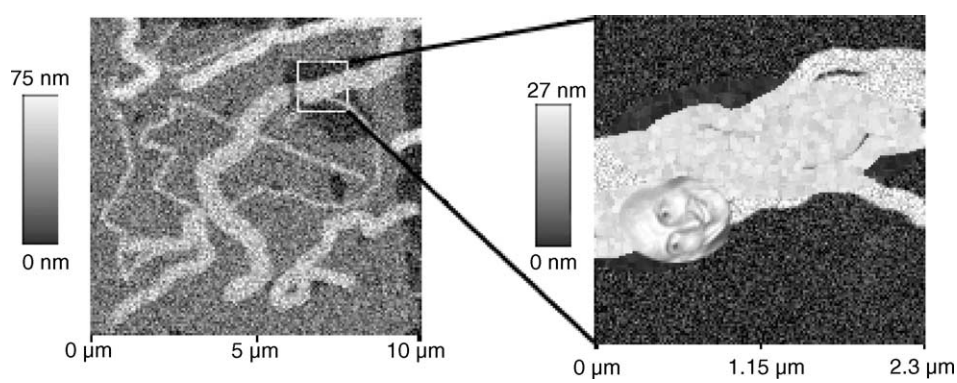

Fig. 2. Atomic force microscopy image of the carbon nanotube sample isolated from the ETH meteorite. The large features represent ropes of carbon nanotubes, the small features (right hand image) are individual nanotubes. Note the unusually large diameter of these particular carbon nanotubes.

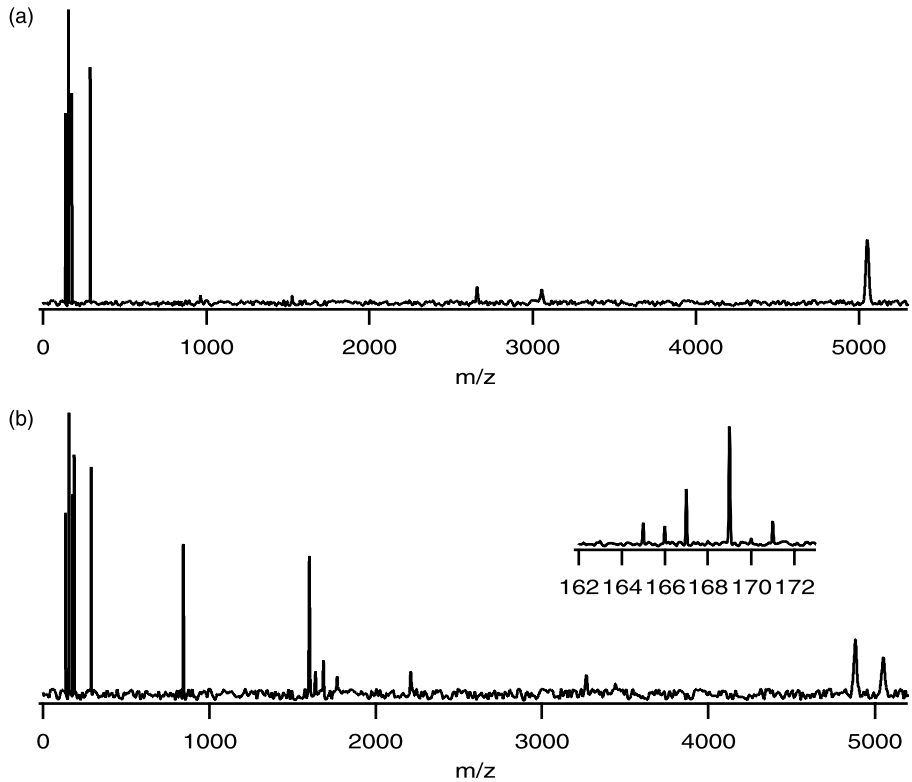

Fig. 3. MALDI mass spectra of (a) Ni^{2+} purified peptide and of (b) tryptic digest obtained from the peptidic material recovered from the carbon nanotubes. The inset in (b) shows the isotopic pattern of the signal at $m/z = 186$, identifying this peak as selenocystein.

Table 1
Peptide masses and their sequence obtained from tryptic digestion of α-tuberlin

Average *m/z*	Rel. intensity[a]	Matrix peak	Trypsin peak (auto-digestion)	Interpretation within α-tuberlin sequence
137.2	vs	*		
154.1	vs	*		
177.0	vs	*		
168.1	vs			$(P4 + H)^+$
288.4	vs	*		
842	s		*	
1599.9	s			$(P3 + H)^+$
1636.8	w			$(P1 + H)^+$
1684.8	m			$(P2 + H)^+$
1767.0	w			$(P3 + P4 + H)^+$
2211.4	w		*	
3265.9	w			$(P2 + P3 + H)^+$
3302.1	vw			$(P1 + P2 + H)^+$
3439.0	vw			$(P2 + P3 + P4 + H)^+$
4883.2	m			$(P1 + P2 + P3 + H)^+$
5050.6	m			$(P1 + P2 + P3 + P4 + H)^+$

[a] vs: very strong; s: strong; m: medium; w: weak; vw: very weak.

signals. The high degree of purity of this sample is truly astonishing. We suspected, again based on the elemental composition of sample 1 and the good MALDI response with DHB, a peptidic composition. Based on the recovery of this compound from nanotubes, we call it α-tuberlin. Tryptic digestion of α-tuberlin was attempted, and the corresponding MALDI data is reported in Fig. 3b. Obviously, this material is degraded by trypsin and yields characteristic fragments, much in the way common peptides and

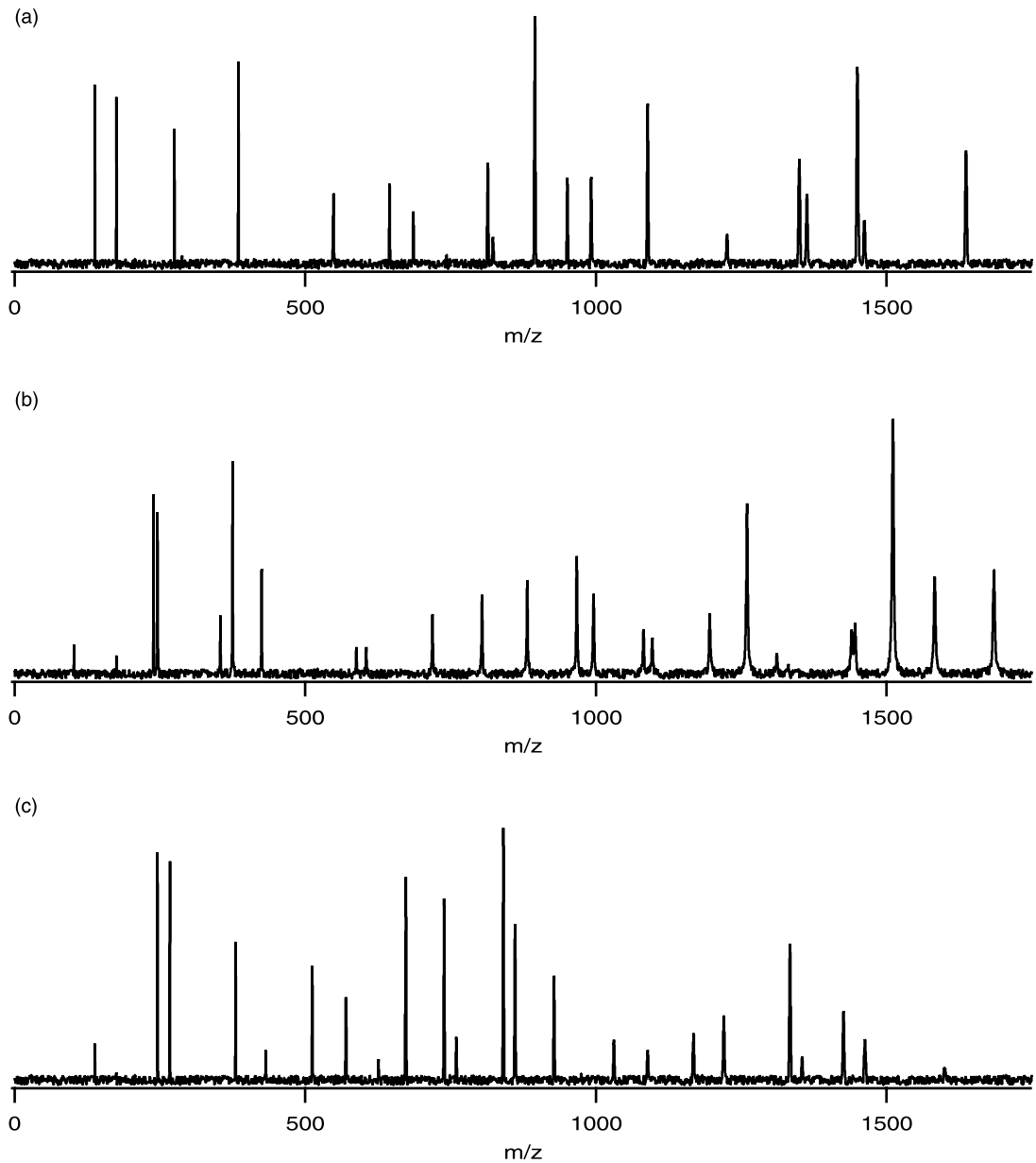

Fig. 4. (a–c) Post-source decay (MS/MS) spectra of the three main tryptic fragments identified in the tryptic digest in Fig. 3.

Table 2
PSD data for peptides obtained from tryptic digestion of α-tuberlin

Peptide 1													
b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	**1637**	
138	275	(412)	(549)	686	823	895	992	1089	(1253)	1350	1462	(1619)	
	y12 + 2	y11 + 2	y10 + 2	y9 + 2	y8 + 2	y7 + 2	y6 + 2	y5 + 2	y4 + 2	y3 + 2	y2 + 2	y1 + 2	
1637	1450	1363	1226	1089	951	814	(743)	645	548	385	(288)	175.1	
Peptide 2													
b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	b13	
102	239	354	425	588	(690)	804	967	1082	1196	1311	1440	1511	**1685**
	y13 + 2	y12 + 2	y11 + 2	y10 + 2	y9 + 2	y8 + 2	y7 + 2	y6 + 2	y5 + 2	y4 + 2	y3 + 2	y2 + 2	y1 + 2
1685	1583	1446	(1331)	1260	1097	996	882	719	605	(490)	375	246	175.2
Peptide 3													
b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12		
138	267	380	512	626	739	841	928	1031	1168	1355	1426	**1600**	
	y12 + 2	y11 + 2	y10 + 2	y9 + 2	y8 + 2	y7 + 2	y6 + 2	y5 + 2	y4 + 2	y3 + 2	y2 + 2	y1 + 2	
1600	1463	1334	1220	1089	(975)	861	760	673	570	432	246	(175)	

Values in parentheses were fragments not observed in the PSD spectra, bold values are the *m/z* values of the protonated peptide peaks selected for PSD. Amino acid sequences were deduced from the mass differences between the PSD fragment peaks.

proteins would react. A detailed peak list of the peptidic fragments is given in Table 1. Of special interest is the intense signal observed around $m/z = 168$ in the midst of the matrix peaks. It exhibits an unusual isotope pattern characteristic for selenium. A database search (SWISSPROT database) of the tryptic map did not yield any hits. Only a poor correlation with a number of known proteins was found, among them porcine hilariousine (12%), monkey tuberculase (8%), and human hybris growth factor B (7%). The weak signals in the mass range above 1700 Da are interpreted as incompletely digested. A very surprising result was the fairly strong basicity of all four tryptic fragments. Isoelectric points (pI values) were determined for all of them, and they added up to exactly 60, which seems to be some kind of "magic number".

We thus attempted de novo sequencing by post-source decay (PSD) after mass spectrometric isolation of the three most intense tryptic fragments. The corresponding PSD spectra are shown in Fig. 4a–c. This experiment resulted in an almost complete sequence coverage dominated by y and b fragment ions. This assignment is based on the carboxy-terminal mass difference, which must be due to Arg (or Lys, which was, however, not found) following tryptic digestion. The complete interpretation of the PSD signals is given in Table 2. Based on mass differences alone, we are able to determine the nature of the individual amino acids in the sequence with very few ambiguities. This is reported in Table 3. A few points deserve further comment: α-tuberlin was found to be rich in His and Orn. The former is not surprising, given the purification with the Ni-bead affinity chromatography; in fact, the amino-terminal His_6 region is a "His tag" that is expected to be selectively retained by the column used. Orn (residue composition $C_5H_{10}ON_2$) was differentiated from Asn ($C_4H_6O_2N_2$) by exact mass measurement done specifically for the residues in question. CH_4 is slightly heavier than O, by

Table 3
Most probable amino acid composition of α-tuberlin

Peptide 1
His His His His His His Ala Pro Pro Tyr Pro Ile Arg
Peptide 2
Thr His Asp Ala Tyr Thr Orn Tyr Orn Orn Asp Glu Ala Arg
Peptide 3
His Glu Leu Met Orn Orn Thr Ser Cys His Trp Ala Arg
Carboxy-terminal peptide: seleno-Cys
Consensus sequence (1 letter code)
HHHHH HAPPY BIRTH DAYTO YOODE ARHEL MOOTS CHWARZ

36 mDa, which was comfortably resolved for all the mass differences in question. Finally, the carboxy terminal residue was assigned to seleno-Cys based on its isotopic pattern (see inset in Fig. 3).

4. Conclusions

An extraterrestrial peptide named α-tuberlin (molecular weight 5051 Da) was found trapped in meteoritic carbon nanotubes, extracted, sequenced, and identified. The most probable amino acid sequence is given in Table 3. The amino acid sequence is surprisingly rich in histidine and ornithine and carries a carboxy-terminal selenocysteine. Ornithine and selenocysteine are not usually found in terrestrial peptides and proteins, further confirming the extraterrestrial nature of the sample. At present, it is neither clear what the function of this material might be, nor whether it carries any message, although close inspection of the consensus sequence given in Table 3 might provide a clue to careful readers.

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