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Composition and Chirality of Amino Acids in Aerosol/Dust from Laboratory and Residential Enclosures

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Dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday

ABSTRACT Initial results from the analyses of geological and anthropological samples for amino acids were difficult to accept because of the high enantiomeric purities of the analytes (i.e., predominantly L-amino acids). Consequently, sources of contamination had to be considered. All sources were eliminated except for direct atmospheric contamination. Essentially invisible, microscopic, aerosol/dust was found to rapidly contaminate the surface of samples and sample containers even after brief exposure times in clean laboratories. Contamination increased with exposure time. The aerosol/dust amino acids were contained predominantly in a proteinaceous material. Aerosol/dust from different locations can contain different percentages of proteinoid/amino acid material. However, the relative concentrations of the amino acids were similar for both laboratory and residential samples. The enantiomeric purity of the L-amino acids studied in aerosol/dust appears to be 99% or greater for the samples examined. Thus, even slight contamination of any sample with microscopic dust or aerosol particles can skew the results of trace amino acid analyses and amino acid e.e. determinations. *Chirality 13:153–158, 2001.*

KEY WORDS: contamination; aerosol; dust; amino acids; enantiomeric purity; geochemistry; HPLC

One of the most common and important types of analysis for biological samples involves amino acids.[1–3] The amino acids can be either free entities or, more commonly, part of larger compounds (e.g., peptides, proteins, etc.). Recently, the enantiomeric composition of many amino acids was found to provide additional information as to disease states, nutrition, age, biological role, environmental conditions, and purity.[3–10] As with all types of analyses, background contamination must be avoided in order to obtain accurate and reproducible results. Contamination is less of a problem when dealing with samples that have high concentrations of the analytes of interest, compared to the surrounding environment. This is generally the case when measuring amino acids in biological samples which contain significant quantities of amino acids, peptides, proteins, and related conjugates. However, when analyzing very small samples or samples that contain very little of the component of interest, contamination can be a very real problem. Examples of this, in the realm of amino acid analysis, include the analysis of geological samples,[11] extraterrestrial samples (meteorites, moon rock, etc.),[12,13] or even biological samples in which only a miniscule amount of material is available.[8,9,14] The analysis of such samples usually requires specialized sample pretreatments and handling, as well as ultraclean experimental environments.

Recently, we became involved in a project that involved the analysis of prehistoric cave paintings. One of the questions to be answered concerned the composition of the binder in the paint used by prehistoric artists. It was hoped that amino acid analysis (after hydrolysis) would provide information as to the nature of the binder (i.e., whether or not it was proteinaceous and perhaps the type of protein material used). Only small amounts of the samples were available and they consisted mainly of inorganic material. Analyses of the paint, as well as the underlying limestone and other samples of virgin limestone, indicated the presence of amino acids with high e.e.'s (of L-amino acids). We found these results difficult to believe, even when repeated analyses with extensive cleaning, washing, and careful handling of the samples gave similar results. Contamination was suspected but its source was difficult to confirm. After checking all reagents and equipment, empty sample vials were taken and subjected to the entire cleaning, hydrolysis, and handling process. Analysis of the "empty" vials (blanks) revealed the presence of amino acids with high e.e.'s. These amino acids could only be eliminated when the entire process was carried out in a sealed, clean, glove-

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box under positive pressure of filtered nitrogen. This indicated that the source of the contamination was atmospheric in origin. This appeared to be true even though the sample vials contained no visible material (even under 20× magnification) and may have been exposed to the atmosphere for only a few seconds. Hence, we were forced to examine the role of microscopic (and macroscopic) aerosols/dust as the main source of amino acid contamination.

Aerosols are known to be ubiquitous in most environments and a common "transport vehicle" for a variety of substances and pollutants.[15] However, we were unable to find published information on the content and chirality of amino acids in aerosols or dust from indoor environments. In this work we examine dust from several laboratory and residential enclosures and compare the results to those found from previous blank and limestone experiments.

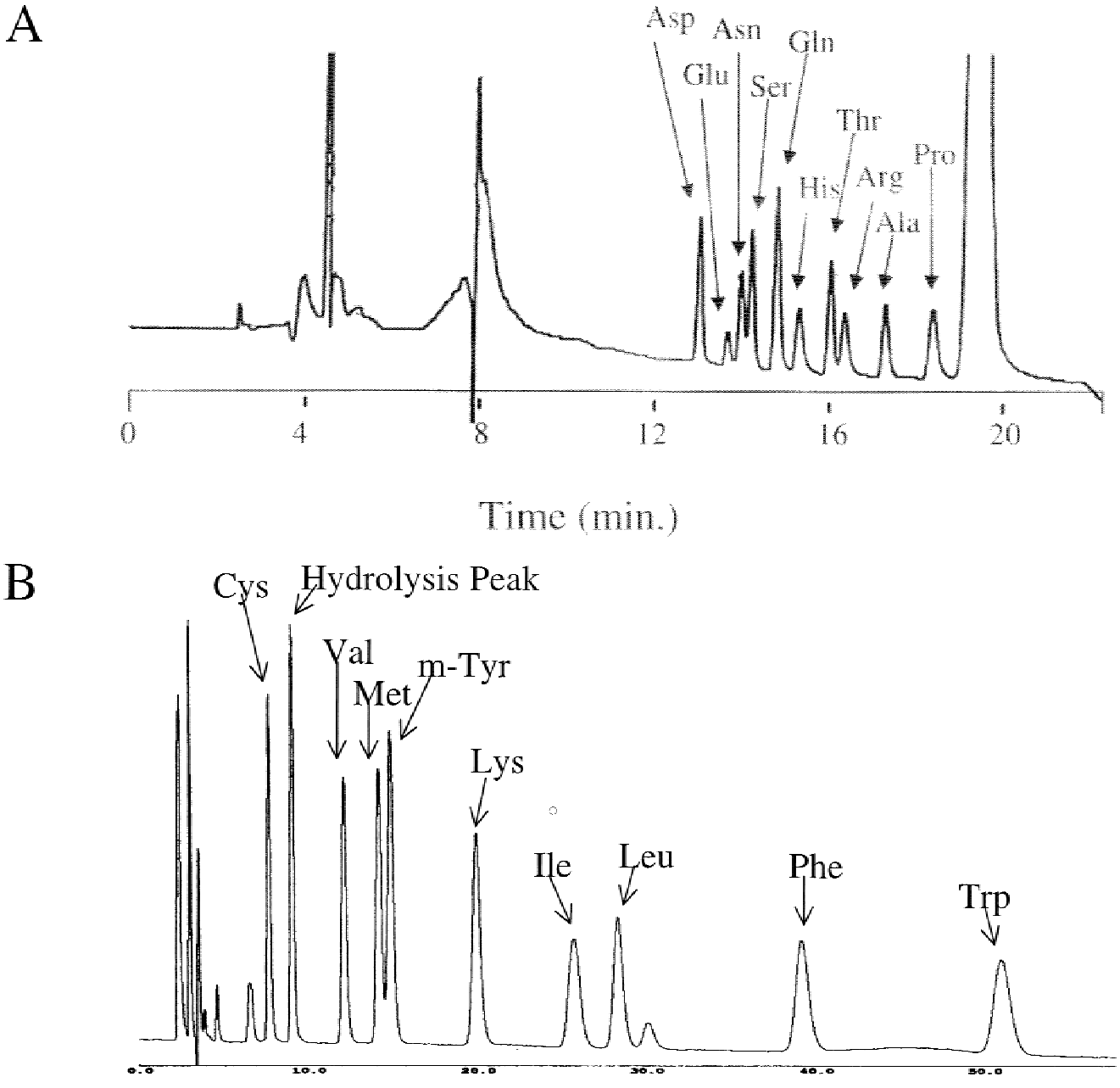

Fig. 1. A: Separation of the first 10 eluting amino acids on a C-18 column (Astec; 25 × 0.40 cm i.d.). Binary gradient elution was used. Mobile phase A consisted of 140 mM sodium acetate, 17 mM triethylamine, 1 mg/ml EDTA, pH 5.05, with H_3PO_4. Mobile phase B consisted of 60% acetonitrile, 40% water. The run started with 100% A, going to 87% A in 2 min, then 67% A at 17 min, then 100% B from 18 min until 20 min to flush the column. The column was then equilibrated with 100% A for 9 min. Flow rate 1 ml/min, UV detection at 254 nm. **B**: Separation of the last nine eluting amino acids on a C-18 column (Astec; 25 cm × 0.40 cm i.d.). Mobile phase 70% A / 30% B. Mobile phase A consisted of 140 mM sodium acetate, 17 mM triethylamine, pH 5.05, with H_3PO_4. Mobile phase B consisted of 60% acetonitrile, 40% water. Flow rate = 1 ml/min, UV detection at 254 nm.

EXPERIMENTAL

Materials

Amino acid standards and hydrochloric acid (constant boiling) were purchased from Sigma (St. Louis, MO). Microhydrolysis tubes (1 ml) were purchased from Kontes (Vineland, NJ). Two HPLC column-switching systems were used. System 1 was a Shimadzu (Kyoto, Japan) system consisting of two LC-6A pumps, an SCL-6A system controller, a Rheodyne 7125 injection valve, a C-18 column, an SPD-6A UV spectrophotometric detector, and a C-R6A Chromatopac. This was connected with a Rheodyne 7000 switching valve to a second HPLC system consisting of an LC-6A pump, a Rheodyne 7125 injection valve, a β-cyclodextrin column (Cyclobond I-2000; Astec, Whippany, NJ), an RF-535 fluorescence detector, and a C-R3A Chromatopac. System 2 consisted of a BAS (West Lafayette, IN) PM-80 pump, a Rheodyne 7125 injection valve, a C-18 column, and a BAS UV-116A UV-VIS detector. This was connected with a Rheodyne 3092 switching valve to a second HPLC system consisting of a Shimadzu LC-6A pump, a Rheodyne 7125 injection valve, a Cyclobond I-SN column (Astec), and a Shimadzu RF-535 fluorescence detector. Both detectors on System 2 were interfaced with a BAS DA-5 ChromGraph Interface to a Gateway 2000 computer (486, 66 MHz). All HPLC columns were purchased from Advanced Separation Technologies (Astec). The AccQ-Fluor Reagent Kit (Waters, Millford, MA) was used to derivatize the amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC).

Methods

Dust samples were collected from several laboratory and two residential properties. They were placed in glass vials previously cleaned and capped in a glove-box and only opened at the site of collection. Subsequently, all samples were handled prior to hydrolysis in a glove-box (containing a filtered N_2 atmosphere at a positive pressure) to avoid contamination from airborne sources. Approximately 3 mg of dust was weighed out and put in a hydrolysis tube with approximately 500 µl of constant boiling HCl. The tube was then closed and subjected to vacuum followed by nitrogen purge repeated three times with a final vacuum step. The hydrolysis tubes were then placed in a 100°C oven for 96 h to hydrolyze any proteins present. The samples were then transferred to a 1.5 ml Eppendorf tube and put in a vacuum centrifuge to remove the HCl. Samples were then dissolved in 100 µl 20 mM HCl and derivatized with AQC (AccQ-Fluor Reagent Kit; Waters). Originally we hydrolyzed blanks in the laboratory and from samples of limestone rock and found trace levels of amino acids, necessitating the use of the glove-box to avoid contamination from airborne sources. Additionally, extracts of samples washed with constant boiling HCl solution were run (without incu-

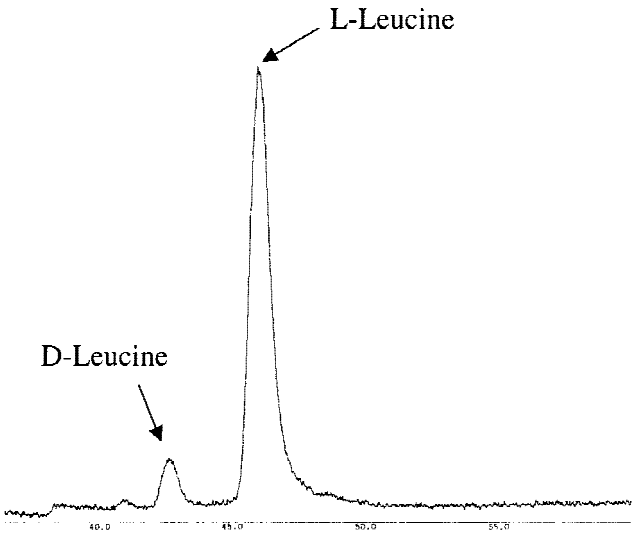

Fig. 2. Separation of AQC-D,L-leucine on a Cyclobond I-SN column after the peak was switched from the C18 separation (see Fig. 1). Mobile phase = 450:50:2:6 (MeCN:MeOH:HOAc:TEA) 1 mL/min. Fluorescence detection: Ex 250 nm, Em 395 nm.

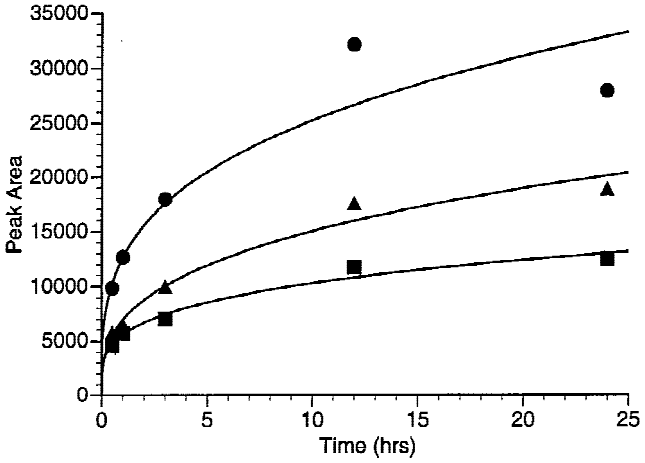

Fig. 3. Plots showing the accumulation of amino acid-containing matter in a clean 1 ml microhydrolysis tube (4 mm opening) vs. exposure time to the ambient laboratory atmosphere. Each curve represents the concentration profile of a different amino acid component of dust (● = leucine, ▲ = isoleucine, and ■ = phenylalanine) after collection, hydrolysis, and chromatographic analysis. Although different amounts of individual amino acids are found in the proteinoid part of dust/aerosols, their concentration profiles should be similar, as shown here.

TABLE 1. Amino acid levels in dust from three different locations (mg amino acid/g dust)

Sample no.	Amino acid	Laboratory	Residence 1	Residence 2
1	Asp	1.2	2.5	1.8
2	Glu	3.5	7.9	5.6
3	Ser	2.3	7.2	4.4
4	His	1.6	4.2	2.3
5	Arg	2.1	5.6	4.8
6	Thr	0.8	2.2	2.4
7	Ala	2.0	1.2	1.6
8	Pro	2.1	1.8	2.8
9	Cys	7.3	7.2	13.0
10	Val	5.4	5.7	10.8
11	Lys	8.0	8.1	14.4
12	Ile	5.6	5.7	10.7
13	Leu	6.6	6.9	11.1
14	Phe	7.3	7.5	13.2

bation at 100°C for 96 h) to analyze for free amino acids; none were found (i.e., below our limit of detection), suggesting that the main source of amino acids was proteinaceous. The time dependency for dust/aerosol accumulation in an open hydrolysis tube (4 mm opening) was examined by leaving the tubes open for specific periods of time (0.1–24 h) to the ambient air in the laboratory. Constant boiling HCl was then added as before, as well as all other aforementioned steps in the analysis of dust samples. For derivatization, 300 µl buffer was added to 100-µl samples and then 100 µl AQC (3 mM in acetonitrile) was added. The samples then sat for 1 min at room temperature and then 15–20 min at 55°C. Samples were then ready for direct injection onto the HPLC system. UV detection was performed at 254 nm and fluorescence detection at excitation of 250 nm with emission at 395 nm. Figure 1 shows the HPLC separation of the individual amino acids. Figure 2 shows the enantiomeric separation of D,L-leucine that was switched from the achiral reversed phase column.

The relative standard deviation of this method was determined by taking a macroscopic dust sample and dividing it into four approximately equal portions (of about 4 mg each). Each of the four samples was independently subjected to the entire wash, hydrolysis, derivatization, and separation procedure described in this section. Leucine was taken as a representative amino acid and its concentration was measured in all four samples. The relative standard deviation of this approach was found to be 10%.

RESULTS AND DISCUSSION

Aerosol/dust is ubiquitous in most enclosed work and living environments. While most individuals are familiar with macroscopic "dust aggregates," microscopic particles are even more prevalent, although usually unnoticed. Unless precautions are taken, this can be a problem when doing trace analyses on samples for components that are also present in aerosols/colloidal dusts.

Dust found in enclosed work and living environments is a complex mixture of many inorganic and organic components. The inorganic components can consist of a variety of minerals (e.g., quartz, clay, etc.) and salts.[16,17] The organic components can include cellulosic, proteinaceous, and microbial matter as well as synthetic compounds.[18–20] In our work, we were concerned with trace amino acid analysis. It appeared that the proteinaceous material (from skin, hair, fibers, microorganisms, etc.) of aerosols/dust severely affected our results when this material was not rigorously excluded. Figure 3 shows that amino acids from aerosol/dust proteins can enter a clean sample vial if that vial is unsealed, even briefly, in a supposedly "clean" laboratory environment. Furthermore, the amount of contamination increases rapidly for the first 3 h and more slowly after several hours (Fig. 3). The rapid onset of contamination may possibly be due to electrostatic or other surface interactions between the colloidal dust and the clean inner wall of the container (which in this case was glass).

The free amino acid content of laboratory and residential dust samples were found to be nearly negligible compared to the amino acids in bound or complexed form (e.g., proteins). The proteinaceous content of laboratory dust (7.9 ± 0.5%) was consistently lower than that of residential dusts (10.4–14.4%). The protein/amino acid content of dusts in certain agricultural environments can be even higher.[20]

Table 1 gives the concentration of 14 amino acids found in hydrolyzed dust samples from three different locations. Since alanine usually was the least prevalent amino acid (on a weight basis), all of the concentrations can be normalized to it. Figure 4 shows the normalized amino acids

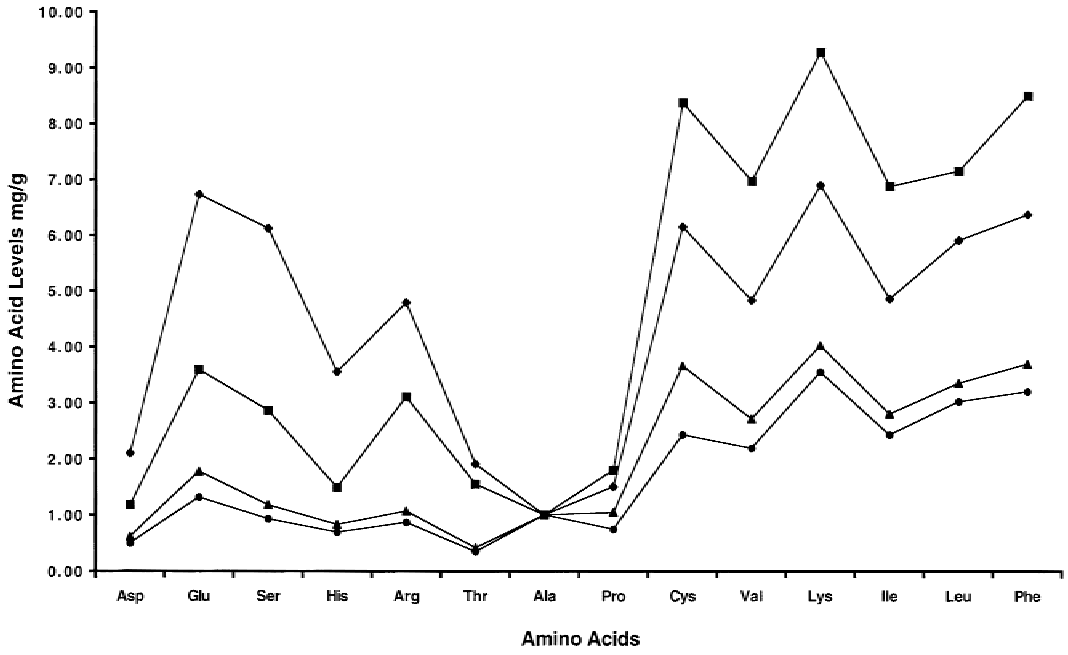

Fig. 4. Normalized amino acid levels (to alanine) of dust from three different locations and a limestone sample analyzed in ambient laboratory conditions. See footnote "c" in Table 2 for analysis conditions. Laboratory = ▲, Residence 1 = ♦, Residence 2 = ■, limestone = ●.

TABLE 2. D-Amino acid levels found in hydrolyzed dust from three different locations

		Laboratory	Residence 1	Residence 2	Hydrolyzed protein standard[a]	Unprotected blank[b]	Limestone[c]
1	Pro	15.8%	10.8%	12.6%	9.7%	12.8%	24.5%
2	Ala	10.6%	9.3%	8.4%	11.6%	12.6%	29.1%
3	Asp	18.2%	17.4%	15.3%	15.2%	NA	NA
4	Leu	7.6%	7.5%	6.9%	5.1%	6.3%	34.4%
5	Phe	7.2%	7.2%	6.9%	6.7%	6.0%	30.7%

[a]The protein standard consisted of α-chymotrypsin (see Experimental section for details).
[b]These values came from exposing a clean, dry glass hydrolysis tube (4 mm opening) to ambient laboratory air for 30 min (see Experimental as well as Results and Discussion sections). No visible contamination was in the device.
[c]A 3 mg sample of freshly exposed limestone (i.e., from the center of a limestone rock ~10 cm diameter that was broken in half) was taken washed with 2N NaOH solution and placed in a hydrolysis tube (see Experimental). The closed sample was acidified with constant boiling HCl and digested at 100°C for 96 h. The resulting solution was analyzed for amino acid content and enantiomeric purity.

levels for laboratory dust, two residential dusts, and a previously analyzed sample of limestone where the cleaned sample and the hydrolysis vessel were exposed to ambient laboratory air for 30 min. From this figure it appears that even though the protein amino acid levels can vary considerably in different dust samples, the relative amounts of amino acids in these particular dusts are somewhat similar. Note the similarities in the patterns of the normalized amino acid level plots in Figure 4. The pattern from the contaminated limestone sample is similar as well.

Some generalizations have been made about the frequency of occurrence of different amino acids in proteins.[21] For example, amino acids do not occur in equimolar frequency in any known protein, but they do occur in fixed molar ratios to one another.[21] Different proteins can have very different amino acid compositions and some proteins lack one or more of the 20 amino acids normally found in proteins. Examples are: 1) basic proteins such as histones have high levels of amino acids with positively charged R-groups (e.g., histidine, lysine, and arginine); 2) fibrous proteins such as collagen, α-keratin, silk, etc., contain a high percentage of hydrophobic amino acids (e.g., valine, leucine, isoleucine, etc.); and 3) ribonuclease lacks tryptophan, while the fibrous proteins lack several amino acids.[21] The protein material in dust/aerosols can come from many different sources, depending on the local environment. The dust in this study seemed to be characterized by relatively high levels of hydrophobic amino acids as well as appreciable amounts of cysteine, lysine, and glutamic acid. The relative level of alanine in our samples was fairly low compared to its concentration in microorganisms such as *E. coli,* where it has a higher relative frequency.[21]

The enantiomeric purity of amino acids in some samples can sometimes be correlated to their age (in the case of some geological or anthropological samples)[5–7] or to their environmental history or storage environment (in the case of some foods, drugs, etc.).[3,4,8–11] Since aerosol/dust is a ubiquitous contaminant that contains protein/amino acids, it would be useful to know the enantiomeric composition of its constituent amino acids. Table 2 gives the enantiomeric composition of five amino acids from three different dust samples, as well as a pure protein standard. The e.e. of these amino acids in the protein standards was >99%. The D-amino acid levels in the hydrolyzed standards (which ranged from ~5–15.2%) resulted from the acid hydrolysis of the protein (see Experimental). The D-amino acid levels of the hydrolyzed dust samples were all fairly similar to those of the standard protein (within experimental error). Furthermore, the "unprotected blank" sample gave analogous results. If we assumed that the partial racemization of the amino acids in dust and the protein standard amino acids is similar during the acid hydrolysis step, then the enantiomeric purity of these dust amino acids in laboratory or residential environments also is similar (~99%). Therefore, the amino acids in the aerosol/dust in this study were predominantly of the L-configuration. Thus, contamination of any sample containing trace amounts of amino acids, with even small amounts of microscopic aerosol/dust, tends to produce artificially elevated levels of L-amino acids. The limestone samples clearly contain higher levels of D-amino acids even though they were contaminated in the same manner as the unprotected "blank" (Table 2). This indicates that they also contain indigenous amino acids that were either racemic or had high levels of D-amino acids.

CONCLUSION

Microscopic aerosol/dust can be a significant contamination problem when doing trace amino acid analysis and enantiomeric purity determination for amino acids. Microscopic and macroscopic laboratory and residential dusts have similar, relative amino acid levels and high enantiomeric purities. These trends and values may be useful in identifying contaminated samples. Contamination increases with exposure time, but leveled off after several hours. Limestone appears to contain small amounts of indigenous D-amino acids.

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