25th March 2015

Dear Editor,

we enclose the revised version of our paper entitled " *Free amino acids in Antarctic aerosol: potential markers for the evolution and fate of marine aerosol"* to be considered for publication in *Atmospheric Chemistry and Physics.*

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Response to Anonymous Referee #1

REF: This study reports on the size resolved measurement of free amino acids in Antarctic aerosol at two different sites, a coastal and an inland station, as well as during a cruise. Higher concentrations of amino acids were found at the coastal station originating from the sea with an enrichment of amino acids in the fine fraction compared to the inland station. Further inland, amino acids were predominantly present in the coarse fraction. The authors attribute these differences to physical and chemical processing of amino acids during atmospheric transport from the sea further inland. During the cruise the highest concentrations were found which the authors attribute to the presence of intact biological material. The manuscript presents a valuable data set and provides important insights into the chemical and microphysical characteristics of amino acids in aerosol in a sparsely studied environment. I recommend publication after a careful revision of the interpretation of results as outlined below in the "general comments" section.

General Comments:

In addition to the collected data the authors use back trajectory analysis to interpret their results. Beyond this, they rely heavily on literature for interpretation especially regarding the implications for and of ice nucleation related to the presence of amino acids in the aerosol. The authors present no measurement based evidence nor direct links to previous studies for their speculative interpretation that amino acid containing aerosol transported towards inland Antarctica has undergone ice nucleation and exhibits therefore amino acid enrichment in the coarse fraction. The single reference that is given to support this does not contain information that would directly discuss this process. Since neither evidence by the data nor from literature is provided that the observed amino acids can actually serve as ice nuclei, and since it is not at all clear from the description in the manuscript whether ice-nucleated particles were present in the coarse mode aerosol collected on the filter, I suggest removing the related passages. These are: p. 1284, l. 22-24: ": : :this is unlikely: : :", and p. 1285 l. 5-8: "The most likely process: : :". Instead it can be said that the specific reason for this enrichment is not clear based on the available data.

AC: As suggested by the referee, we removed the sentences "this is unlikely in Antarctica where the intense cold probably promotes ice-nucleation phenomena, a process that is helped by the presence of amino acids (Szyrmer and Zawadzi, 1997).". The paragraph is now reads as follows: "These fine aerosol particles can grow during long-range transport, due to condensation of molecules from the gas phase or by collision of small and large particles (coagulation) (Petzold and Karcher, 2012; Roiger et al., 2012). However, this is unlikely in Antarctica due to the very clean conditions. The specific reason for this enrichment is not clear based on the available data."

REF:Specific Comments:

Make sure that all references named in the text are present in the bibliography, there are some inconsistencies.

AC: As suggested by the referee, we checked the bibliography, removing some references missed in the manuscript,

REF:p. 1271, l. 21: Not all amino acids enhance the ice nucleating ability of aerosol, I suggest relativizing as follows: ": : : because some of them have been shown to: : :".

AC: We agree with referee and we modified the sentences as suggested by referee.

REF:p. 1274, last paragraph of the introduction: Include the years when the measurements were conducted.

AC: We introduced the years of sampling.

*REF:*p. 1274, l. 16-18: Include quantitative evidence that air masses were really not influenced by emissions from the research station.

AC: We have modified the phrase to read “It was chosen because it is located in a valley that is physically separated from the main station area by a hill, to reduce as much as possible eventual pollution from the research station.”

As we do not have recent monitoring data from that site.

REF:p. 1277, l. 5-8: The message of this sentence is very difficult to understand. Please make several sentences out of this. In addition, in line 7 the single “s” probably means “used”.

AC: I modified the sentences as follows: “In this work the amino acids were quantified using the isotope dilution method where an isotopically labeled standard was available. For other amino acids, where a labeled standard was unavailable, an internal standard was used to quantify the analytes. A detailed description of which analytes are quantified with which method can be found in Barbaro et al. (2014).”

REF: p. 1278, l. 17: What do you mean by repeatability? Do you mean standard deviation?

AC: In this case we are using the IUPAC definition of repeatability which from the IUPAC Gold Book is defined as:

“The closeness of agreement between independent results obtained with the same method on identical test material, under the same conditions (same operator, same apparatus, same laboratory and after short intervals of time). The measure of repeatability is the standard deviation qualified with the term: `repeatability' as repeatability standard deviation. In some contexts repeatability may be defined as the value below which the absolute difference between two single test results obtained under the above conditions, may be expected to lie with a specified probability.”

(from http://goldbook.iupac.org/R05293.html)

In the manuscript we used the phrase “The repeatability is determined as the relative standard deviation of the analytical results for the 5 spiked filters.”

This phrase on repeatability follows IUPAC guidelines to avoid confusion with reproducibility which is defined as:

“The closeness of agreement between independent results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different laboratories and/or after different intervals of time). The measure of reproducibility is the standard deviation qualified with the term 'reproducibility' as reproducibility standard deviation. In some contexts reproducibility may be defined as the value below which the absolute difference between two single test results on identical material obtained under the above conditions, may be

expected to lie with a specified probability. Note that a complete statement of reproducibility requires specification of the experimental conditions which differ."

REF: p. 1282, l. 4: Specify which temperatures you refer to: air, sea surface etc.?

I added "air" before "temperature"

REF:p. 1284, l. 21: insert "or" in "due to condensation of molecules from the gas phase or by collision of small and large particles: : :". And continue as follows: "However, this is unlikely in Antarctica due to the very clean conditions." Remove the following sentence "This is unlikely: : :".

AC:I modified the paragraph as suggested by referee and now it is: "These fine aerosol particles can grow during long-range transport, due to condensation of molecules from the gas phase or by collision of small and large particles (coagulation) (Petzold and Karcher, 2012; Roiger et al., 2012). However, this is unlikely in Antarctica due to the very clean conditions. The specific reason for this enrichment is not clear based on the available data."

REF: p. 1286, l. 3-5: Again, not all amino acids enhance ice nucleating abilities. In addition, hydrophilicity is not a necessity for a particle to ice-nucleate. A wettable particle can do so as well (e.g. mineral dust). I suggest deleting the sentence "This is a very important indication: : :" since it does not support your conclusion regarding the water content of the aerosol.

AC:As suggested by referee I removed the sentence.

REF: Technical Comments:

p. 1270, l. 4: introduce an "and" between ": : : organic nitrogen in aerosols, and particles containing amino acids: : :"

p. 1274, l. 14: delete "the" before "the 29 November"

p. 1275, l. 5: no capitals in "Slotted Quartz Fiber filter"

p. 1277, l. 16: continue the sentence "To ensure that: : : this evaluation was carried out: : :".

p. 1277, l. 24: insert a "," between "filters, respectively."

p. 1278, l. 5: delete "%"

p. 1280, l. 20: include "," before and after "respectively"

p. 1280, l. 21: replace "an" by "a"

p. 1280, l. 25: move "respectively" to the end of the sentence.

p. 1281, l. 2: replace "find" by "found"

p. 1281, l. 8: replace "while" by "and"

p. 1281, l. 9: replace "is" by "it"

p. 1281, l. 10: remove “concentrations a high”

p. 1281, l. 12: remove “proportional”

p. 1281, l. 16: remove “the” in “that the 1 %”

p. 1283, l. 7: replace “shows” by “presents” to avoid repetition

p. 1286, l. 27: replace “internal” by “inland”

p. 1287, l. 2: replace “composition” by “contribution”

p. 1287, l. 8: remove “a” in “promoting a numerous series”

p. 1287, l. 15: remove “the” in “the 13 January”

p. 1288, l. 3: replace “where” by “that”

p. 1289, l. 25: replace “come” by “came”

p. 1290, l. 2: remove the parenthesis

p. 1290, l. 10, remove “were”

AC: I modified each point of technical comments as suggested by anonymous referee 1.

Response to Anonymous Referee #2

REF:Review on manuscript acp-2014-1007 "Free amino acids in Antarctic aerosol: potential markers for the evolution and fate of marine aerosol" by E. Barbaro et al. This manuscript is much better than the previous version. The discussion is much clearer and the authors made some efforts to take into account the referee's comments. In particular, it is now clearly explained that the reported amino acid concentrations are corrected for blank values. The method paper Barbaro et al. Anal. Bioanal. Chem. 2014 is also available, and I was able to check that the analytical procedure is fine.
I only have one last question on the discussion, that might need to be clarified: when comparing the amino acid loadings measured in this study and at other locations in previous works (section 3.1), or between aerosol size fractions (section 3.2) is the total aerosol loading somehow taken into account ? Because larger amino acids concentration per volume of air could just be due to larger aerosol masses, not necessarily to higher amino acid concentrations in the particles.
In particular, is it clear that the "enrichment" of the coarse fraction in amino acids (and corresponding "depletion" of the fine fraction) discussed in Section 3.2 corresponds really to higher amino acid concentrations in the particles and not just to a higher aerosol mass in the coarse fraction (which is usually the case) ? An easy way to answer would be to measure the sampled aerosol mass (= weight the filters before and after sampling) and express the amino acid concentrations per mass of aerosol sample instead of m3 of air. Alternatively, the mass in each aerosol fraction could have been measured by a SMPS instrument sampling next to the filter collection : : : If this has not been taken into account, it might be worth considering in the discussion.
Other than that, the manuscript seems fit for publication.

AC: We thank the referee for this suggestion and we agree that the amino acids concentration for aerosol mass is more significant. We will consider the SMPS instrument for our next sampling campaign. In our studies, we considered the contribution of amino acids per volume and we did not measure the mass of aerosol in all sites. We have the data of aerosol collected at MZS (unpublished data), obtained by weighing the filters before and after sampling, but the data of other sites was not available. The lack of aerosol mass data for the aerosol samples collected at Dome C and during the oceanographic cruise are due to the high electrostatic charge and low humidity at Concordia making weighing to such precision virtually impossible. You can also imagine the problems in weighing to five significant figures a filter on a ship traversing the Southern Ocean. To clarify the enrichment of amino acids in the coarse fraction, we will investigate the aerosol mass in future expeditions, and we thank the referee for this suggestion. We also introduced in the manuscript the sentence: "In our future investigations, we will also evaluate the aerosols mass, which is probably a key parameter to measure that will help explain this enrichment.".
The comparison with other locations in previous works was done by considering the data for sampling volume.

Response to Anonymous Referee #3

Specific comments:

Abstract

** Some amino acids are relatively good CCN also, like l-glycine. Please, if you mention IN ability, you also have to mention CCN ability. This goes for the Introduction chapter as well. There are several papers available on the CCN activity.*

We agree with referee 3, in the past we cited the articles:

Raymond, T. M. and Pandis, S. N.: Formation of cloud droplets by multicomponent organic particles, J. Geophys. Res., 108, D15, 4469, doi:10.1029/2003JD003503, 2003.

Huff Hartz, K. E., Tischuk, J. E., Chan, M. N., Chan, C. K., Donahue, N. M., Pandis, S. N.: Cloud condesation nuclei activation of limited solubility organic aerosol, Atmos. Environ., 40, 605-617, doi: 10.1016/j.atmosenv.2005.09.076, 2006.

And

Kristensoon, A., Rosenorn, T., Bilde, M.: Could droplet activation of amino acid aerosol particles, J. Phys. Chem. A, 114, 379-386, doi:10.1021/jp9055329, 2010.

To support the ability of amino acids to act as cloud condensation nuclei. This was heavily contested by the referees when we first submitted the paper into discussion (submission ACP-2014-377) if referee 3 wishes to have a look. As such we felt obliged to remove any reference to amino acids acting as cloud condensation nuclei, as the referees stated categorically that this is not the case. We do not feel able to insert this information now, without the consensus of the other referees and editor. If referee 3 has articles that show that amino acids act as CCN that will be acceptable to the other referees and editor, we will happily insert them.

Obviously this subject in more contentious than we originally realized.

** "During the sampling cruise on the R/V Italica on the Southern Ocean, high concentrations of amino acids were found in the total suspended particles, this we attribute to the presence of intact biological material in the sample." Try to be more specific. I don't know what you mean here.*

To clarify our affirmation, we inserted in the bracket "as microorganisms or plant material" in the manuscript.

Introduction

** P 1271, L 12. "is due to", should be "depends on".*

As suggested by referee 3, we substituted "is due to" with "depends on".

** P 1273, L 28. How can antarctic aerosols give information about formation and growth. Think you have to explain this. Which formation, and which growth are you referring to?*

We agree with the referee on this point, we have changed the phrase to read “Our aim is to study aerosol particle formation and growth in Antarctica because there is minimal interference from confounding anthropogenic sources.” As this is one of the aims of our paper and we hope it becomes self explanatory to the reader.

Experimental section.

** P 1279, L 15. "S1-S3, it". Should be "S1-S3. It".*

We corrected this mistake.

Result section.

** "The most likely explanation for this enrichment of amino acids in the coarse fraction, is that the fine fraction has 5 been subjected to processes that increased the particle size of the aerosol. The most likely process is ice nucleation during long-range transport promoted by the intense cold over the plateau and presence of amino acids in the aerosol particles (Szyrmerand Zawadzki, 1997)." I don’t think this should be a likely explanation. i think it is more probable that these amino acids are present in primary emitted coarse mode aerosol particles, which can come from phytoplanktonic sea spray coarse mode particles (Matsumoto and Ueamatsu, 2005), or from soil dust coarse mode particles (Mace et al., 2003). Particles and their chemical constituents can travel for many weeks in the upper troposphere without being lost provided they are not subject to wet deposition, or that the compounds are reacting in the aerosol phase. You are yourselves suggesting that hydrophobic amino acids can survive long range transport. in summary: You can add that these coarse mode amino acids can have both a continental and marine origin, but that you are not sure where they come from. And with continental origin, I mean both Australia, South America, Africa, Antarctica despite that the trajectory is not showing a continental origin within the last week. The coarse mode particles can come from the continents several weeks ago).*

Thanks to the comments of referees 1 and 2 the phrase has been modified as followed.

“These fine aerosol particles can grow during long-range transport, due to condensation of molecules from the gas phase or by collision of small and large particles (coagulation) (Petzold and Karcher, 2012; Roiger et al., 2012). However, this is unlikely in Antarctica due to the very clean conditions. The specific reason for this enrichment is not clear based on the available data. In our future investigations, we will also evaluate the aerosols mass, which is probably a key parameter to measure that will help explain this enrichment.”

We do not believe that primary coarse particles can arrive at the Antarctic plateau because as stated in figure 3.8 of the book “Atmospheric physics”(A. Petzold, and B. Karcher, Atmospheric Physics - Aerosols in the Atmosphere, Springer-Verlag Berlin Heidelberg, Germany, 2012), the coarse particles have a lifetime of 1 or 2 days.

And our experimental data do not support such a mode of transport, so we agree with referee3, and as suggested by other reviewers, our experimental observations did not demonstrate this particular mechanism of enrichment. For this reason, we modified the sentence as above.

** P 1288, L 3. This is probably the main source of amino acids in our on-ship samples, this is also supported by the backtrajectory analysis (Fig. S8a–g), where demonstrate only a marine influence for that period. Should read: "This is probably the main source of amino acids in our on-ship*

samples. This is also supported by the backtrajectory analysis (Fig. S8a-g), which demonstrates only a marine influence for that period."

We agree with referee and we modified the sentences as suggested.

** P 1288, L 15. What is "Oceania"?*

Oceania is a continent that includes Polynesia (including New Zealand), Micronesia, Melanesia and Australia.

** P 1289, L 5-13. "The back-trajectory analysis (Fig. S8c–e) demonstrated that the air masses came from inland Antarctica, where no vegetation is present. The biological material present in the atmosphere with a size > 10 µm includes pollens which typically vary between 17–58 µm, fungal spores between 1–30 µm, and algal spores between 15–120 µm. Instead bacteria have a diameter between 0.25–8 µm, and viruses have diameters that 10 are typically less than 0.3 µm (Jones and Harrison, 2004). For this reason, we propose that the biological materials that influenced the concentration of the total free amino acids in the shipboard aerosols can probably be attributed to algal spores." Why only algal spores? You should not exclude pollen in this paragraph already, since it is not until the next paragraph where you use Pro to isolate algal spores as the only explanation.*

We agree with the referee and we modified the sentence as follows: “For this reason, we propose that biological materials influenced the concentration of total free amino acids in the shipboard aerosols.”

Conclusion

** "The study of aerosols with diameters > 10 µm indicated that bubble bursting processes can also emit microorganisms that are composed of a higher number of neutral amino acids." I didn’t get this from the result section?*

This is our conclusion from section 3.3, for the oceanographic cruise to explain the difference between the samples collected using the TSP sampler and the cascade impactor. This conclusion is stated on page 21, lines 491-493 at the end of the appropriate section.