DETERMINING D/L RATIOS OF AMINO ACIDS FOUND IN ICE ABOVE LAKE VOSTOK USING ESI/CIT MASS SPECTROSCOPY. A. Tsapin[1], I. Kanik[1], L.W. Beegle[1], L. Wu[2], and R. G. Cooks[2] [1]Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr. Pasadena, Ca 91109 tsapin@email.usc.edu, ikanik@pop.jpl.nasa.gov, Luther.Beegle@jpl.nasa.gov, [2] Department of Chemistry Purdue University West Lafayette In, 47907-1393, cooks@purdue.edu

Introduction: The extent to which organisms can survive extended periods of metabolic inactivity in cold environments such as ice and permafrost is one of the key questions in the study of life in extreme environments. Cells that are not metabolically active continue to accumulate damage to DNA and other biomolecules from radiation and thermal energy [1]. If repair processes are not active during dormancy, then there must be a maximum dormancy period beyond which so much damage has been accumulated that the organism cannot resume metabolism and reproduction when conditions become more clement.

Astrobiology is an area where longevity of (micro)organisms is of great interest. Cryospheres are common phenomena in the solar system, particularly on satellites, comets and asteroids, as well as at least some of the planets. Recent data from the Mars Global Surveyor mission suggest the possibility of permafrost or perhaps even liquid water under the Martian surface [2]. These environments may be the areas in which the probability of finding life is the highest. This issue is of concern due to the probable evolution of planetary environments such as that of Mars from more hospitable to less hospitable conditions over the history of the solar system. In addition, evaluation of the possible transfer of living organisms between planets via impact ejecta [3] is dependent on knowledge of the maximum time periods over which microorganisms can remain dormant and subsequently revive and reproduce.

Amino acid racemization dating, or aminostratigraphy, has been used for many years to date biological systems, and has been examined as a possible biosignature detection technique for Mars. We have recently shown [4] that measurements racemization of amino acids in Siberian permafrost samples can provide insights into the temperature history of this environment. If we know independently the temperature history, racemization data can also give us information on the water activity in this environment over time.

Racemization analysis can indicate whether organisms in an environment have been metabolically active over a specific time period. If both the age of the sample and the environmental temperature can be independently determined, the actual rate of racemization can be compared with that expected. If the rates are similar, it can be concluded that no D-amino acid recycling has taken place over the age of the samples. If the rates are different, it indicates that metabolic repair processes have been active and have partially reset the racemization "clock" by breakdown of D-amino acids. Amino acid racemization analysis can be used as an *in situ* indicator of metabolic activity over long time periods.

We have suggested using amino acid racemization as one of the most indicative biosignatures [4]. Only life systems produce preferential synthesis of L-amino acids versus D-amino acids. Almost all amino acids in terrestrial organisms can be found only in the L-enantiomeric form.

Usually amino acid racemization is measured using HPLC with preliminary modification of amino acid presented in environmental samples with stereospecific fluorescent dyes. The whole process takes several hours. We have been developing a portable eventually flyable system, namely ElectroSpray Ionization/Cylindrical Ion Trap mass spectrometer (ESI/IMS/CIT) which is capable of measuring the D/L ratio of amino acids in aqueous samples. Utilizing the kinetic method for chiral determination [5], the CIT can be used to measure D/L ratios based upon analysis of adducts of amino acids with Cu(II)- and chiral reference compounds.

We studied the level of amino acid racemization, specifically of aspartic acid, in permafrost samples from eastern Siberia. Also we analyzed samples of ice from borehole drilled to lake Vostok, Antarctica.

Determination of chirality: Although mass spectrometry itself is not intrinsically enantiomer-specific, there are methods to determine quantitatively chiral determination methods [5-7]. The method in this study is based on gas phase trimeric metal ion-bound cluster ions, which are formed in an ESI source, mass selected, and then subjected to collision-induced dissociation (CID) to undergo competitive ligand loss. The method borrows from ligand exchange chromatography (a method for chiral resolution), the idea of creating complexes whose stability will differ, depending upon the chiral configuration of the complexing analyte. The complex is created by coordinating the chiral analyte (viz., amino acid) to a metal cation that is also complexed by additional chiral ligands. The chiral amino acid interacts with the other chiral ligands; steric interactions between the amino acid and the other ligands will differ slightly, depending upon the chirality of the

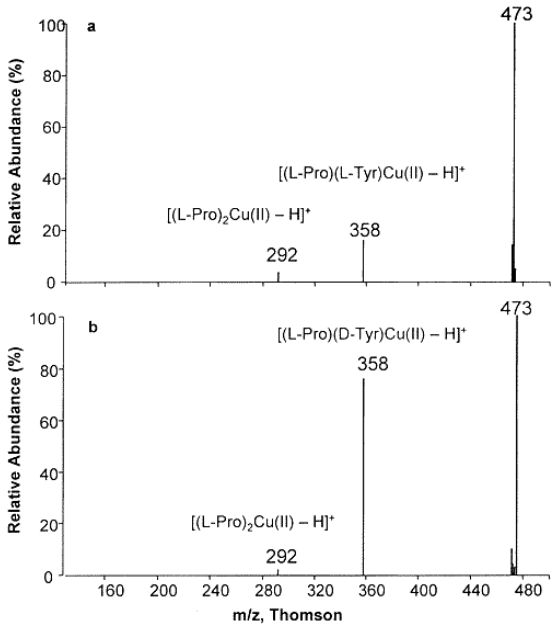

Fig. 1 MS/MS product ion spectra of $[Cu^{II}(L\text{-}Pro)_2(Tyr)\text{ -}H]^+$(mass 473 a.m.u.) the upper spectra is pure L-Tyr while the lower one is pure D-Tyr. The ratio of intensities between mass 358 and 292 is determinate on the ration of D and L-Tyr in the original sample.

amino acid, causing the two different forms of the complexes to differ slightly in stability.

The metal ion complex fragmentation products are formed in different proportions as a result of this energy difference, depending upon the chiral composition of the amino acid ligand as shown in Fig. 1. This method is an adaptation of the kinetic method [8, 9], which is capable of converting extremely small differences in energy into relatively large differences in fragment ion branching ratios.

Trimeric clusters $[Cu^{II}(A)(ref^*)_2\text{-}H]^+$, where Cu^{II} is a divalent metal ion, H is proton, A is an amino acid and ref* is the reference amino acid, via two competitive channels upon CID. Therefore, relative rates of the two competitive dissociations (k_A and k_{ref}) can be expressed as the relative abundance ratio:

$$R = k_A / k_{ref} = [Cu^{II}(A)(ref^*) - H]^+ / [Cu^{II}(ref^*)_2 - H]^+ \quad \textbf{(1)}$$

From the kinetic method [9] the natural logarithm of the ratio of rate constants (k_A and k_{ref}), R, is proportional to the differences between the Cu (II) affinities of the two dimeric products associated with D- or L-enantiomeric form of the analyte as shown in equation **(1)**.Using this method there is a linear relationship between ln(R) and the enantiomeric composition of the amino acid sample, which allows enantiomeric excess to be determined to within 3% for most amino acids [6].

More recently, our laboratory has further extended this method to the analysis of amino acids mixtures. Preliminary results show that several important amino acids, such as alanine, serine, and asparatic acid, can be determined in a mixture containing these three amino acids to levels as low as 2% enantiomeric excess with average relative errors of 3%-7%. [10].

This method has been demonstrated under laboratory conditions. However due to the great importance of detecting chiral molecules in the search for signatures of life on planetary bodies (such as Mars and Europa), the method described above is being used to determine the enantiomer excess of terrestrial environmental samples and to compare them with the ratios determined from HPLC.

Acknowledgment: The research described here was carried out at the Jet Propulsion Laboratory, California Institute of Technology, and Purdue University and was sponsored by the National Aeronautics and Space Administration's PIDDP and ASTID program offices. We thank J. Priscu for providing us with samples from Lake Vostok.

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