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Homochirality as the signature of life: the SETH Cigar

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Abstract. A characteristic hallmark of life is its homochirality: all biomolecules are usually of one hand, e.g. on Earth life uses only L-amino acids for protein synthesis and not their D mirror images. It is therefore suggested that a search for extra-terrestrial life can be approached as a Search for Extra-Terrestrial Homochirality (SETH). A novel miniaturized space polarimeter, called the SETH Cigar, is described which could be used to detect optical rotation as the homochiral signature of life on other planets. Moving parts are avoided by replacing the normal rotating polarizer by multiple fixed polarizers at different angles as in the eye of the bee. It is believed that homochirality will be found in the subsurface layers on Mars as a relic of extinct life. Copyright © 1996 Elsevier Science Ltd

Most biomolecules are “chiral” or handed, that is to say they exist in two mirror image forms called “enantiomers”. Non-living systems normally contain equal numbers of the left- and right-handed forms. But a characteristic hallmark of life is its “homochirality”: biochemistry uses only one enantiomer and not the other. Thus animals are made of proteins based exclusively on

left-handed (L) amino acids, coded for by DNA based exclusively on right-handed (D) deoxyribose. A few “unnatural” D-amino acids and L-sugars do occur with specific roles, as in bacterial cell walls (but not bacterial protein or nucleic acids) and antibiotics (Ulbricht, 1981; Meister, 1965), although the genetic code does not code for D-amino acids, and they are synthesized by enzymatic racemization (L/D interconversion) of the L form (Spach and Brack, 1983). Even in these cases homochirality is maintained, with the usually dominating enantiomers being excluded.

Homochirality is essential for an efficient metabolism, like the universal adoption of right-handed screws in engineering. It does not matter *which* hand is adopted so long as only *one* hand is used: the world would run just as efficiently with left-handed screws, provided they were universally adopted; and D-amino acid/L-sugar “mirror life” would be just as viable as terrestrial L-amino acid/D-sugar life. Fischer (1894) recognized this in his stereochemical “lock and key” hypothesis, which notes that a chiral molecule can “feel” the difference between the two mirror image forms of another chiral molecule, just as a left foot can feel the difference between left and right shoes, and will feel more comfortable in a left shoe. But molecules do not always interact preferentially with other molecules of the same hand, as in the example of feet and

shoes; the preference depends on the situation, e.g. a right hand prefers to shake another right hand (standing facing each other), but prefers to hold a left hand (standing side by side). Chirally discriminating interaction of this kind produces the connection between the L-amino acids and the D-sugars in life (Wolfrom *et al.*, 1949; Melcher, 1974). Similarly, whichever hand is selected in ancestral biomolecules will dictate the handedness of the rest of biology through such interactions.

The importance of homochirality in biological systems is underlined by the fact that the “wrong” hand often has destructive effects, the classic example being the 1960s sedative drug thalidomide, which caused limb deformities in babies born to mothers taking the drug during pregnancy. It was sold as a racemic mixture (equal amounts of L and D), and it seems from a study on mice (Blaschke *et al.*, 1979) that while both hands had sedative properties, only one hand was teratogenic. There is also speculation that a build-up of molecules of the “wrong” hand may play a role in the processes of ageing and carcinogenesis (Ulbricht, 1981); the body contains special enzymes called D-amino acid oxidases to eliminate amino acids of the wrong hand.

Homochirality is thus such a characteristic signature of life that a search for extra-terrestrial life could be approached as a Search for Extra-Terrestrial Homochirality (SETH). The natural choice for a SETH instrument is optical rotation, the rotation of the plane of polarized light by chiral molecules. Left- and right-handed mirror image molecules rotate the plane of polarized light by equal amounts in opposite directions, so for a racemic mixture there is zero optical rotation, whereas a non-zero rotation indicates homochirality or at least an enantiomeric excess. We describe below a novel miniaturized polarimeter, about the size of a Churchillian cigar, which we are developing to detect optical rotation as the homochiral signature of life on Mars and other solar system bodies. We call our instrument the SETH Cigar.

The advantage of looking for homochirality with optical rotation is that it is completely independent of any preconceptions about extra-terrestrial biochemistry. More conventional techniques tend to assume that the molecular basis of extra-terrestrial life is the same as on Earth, and concentrate on looking for amino acids, etc. But extra-terrestrial life might in fact be totally different. We can, however, be fairly confident that molecules sufficiently complex to support life will be chiral—only the very simplest molecules are achiral—and if we have chirality, we must have homochirality for an efficient biochemistry.

Furthermore, it is now increasingly believed that homochirality is not just a consequence of life but also a pre-condition for life. This is because polymerization to give the necessary long-chain stereoregular polymers (e.g. all-L polypeptides) will not go in racemic solution: addition of the wrong hand tends to terminate the polymerization (Joyce *et al.*, 1984). An almost homochiral pre-biotic chemistry is therefore probably a pre-condition for life—and we will discuss later what chiral influences might have produced homochirality. Likely places to look for pre-biotic homochirality would be Titan and comets. On Mars, however, one would expect to find traces of homo-

chirality as a signature of extinct life. When life was starting on Earth around 3.8 billion years ago, there is evidence that conditions on Earth and Mars were very similar, so life may have evolved on both planets but then gone extinct on Mars (probably around 3.5 billion years ago) as it cooled more quickly. Bada and McDonald (1995) have made a study of amino acid racemization (D/L interconversion) and shown that this is greatly retarded in dry or frozen conditions as compared with wet conditions. They considered the racemization half-life of aspartic acid, the fastest racemizing amino acid, in order to provide a “worst case” scenario for the preservation of homochirality from extinct Martian life. Whereas its half-life is only 8×10^2 years at 300 K in wet conditions, this rises to 5×10^4 years in dry conditions. At 252 K, the freezing point of a eutectic solution of NaCl (the lowest temperature at which “wet” conditions could exist), the corresponding figures are 4×10^6 years in wet conditions and 9×10^8 years in dry conditions. The “dry” figure can be extrapolated to lower temperatures, giving racemization half-lives of 3×10^{13} years at 215 K (Martian equatorial temperatures) and 1×10^{27} years at 150 K (Martian polar temperatures). These racemization half-lives for the fastest racemizing amino acid are at least 1000 times larger than is necessary to preserve the homochiral signature of extinct life, and other amino acids such as valine could require ten times longer still to be completely racemized. Of course with optical rotation as our SETH probe we are not necessarily looking only for amino acids, and much more readily racemizable molecules could still present a residual enantiomeric excess provided their racemization half-life is 10^{10} years or more.

However, the homochiral signature of past life would be erased if it was exposed to liquid water for a significant period after extinction, which is quite possible during a slow cooling scenario. Brief periods of re-melting after freezing would do no harm if they only lasted a few thousand years, but over several million years complete racemization would occur. This could be a problem at the equatorial regions, but much less so nearer the polar regions, where the cold dry conditions would preserve homochirality beneath the surface (assuming that frozen conditions are equivalent to dry conditions with respect to racemization).

The best sites for SETH on Mars would therefore be in the more ancient southern hemisphere, as far south as possible to take advantage of colder conditions. Penetrators or drills would be needed to get below the surface oxidizing layers. However, one is not necessarily confined to the actual areas with fluvial features where life could have existed in the past. The homochiral signature of life could have been transferred to the polar regions by global dust storms, as at the terrestrial poles (Bada and McDonald, 1995), where the dry frozen conditions would preserve it beneath the surface.

The problem with using normal polarimeters for SETH is that the second polarizer is rotatable to determine the sign of the optical rotation, and needs a motor to rotate it—which is clearly very undesirable in space. We therefore propose a much more minimal polarimeter, the size of a cigar, with no moving parts. To illustrate the principle, we describe first the “A” Cigar (MacDermott and

Tranter, 1994), a simple set-up with crossed polarizers at 90° , of which we already have a prototype. We then describe a more sophisticated version called the "Bee Cigar", which is not yet built, but which we plan to develop in collaboration with space engineers at the Ruth-erford Appleton Laboratory.

The "A" Cigar has the sample solution between crossed polarizers at 90° to each other. With miniaturized diode emitters and detectors, and a 10 cm pathlength through the sample solution, this set-up is about the size of a cigar. The idea is that with racemic compounds there is zero optical rotation, which with crossed polarizers gives no light at the detector. If any light at all reaches the detector, there must be optical rotation, i.e. an enantiomeric excess—the signature of life.

But this simple set-up cannot give the all-important *sign* of the optical rotation, and is vulnerable to artifacts, especially false positives due to scattering, necessitating careful filtration to remove dust. We have therefore produced a new design, which gets around the problem of not being able to have a rotating second polarizer to get the sign by instead having many polarizers at different angles, used with a diode-array detector. Miniaturization with existing technology would permit the use of a 6–8 element diode array within a device that is still only the size of a (Churchillian) Cigar. Each diode in the array would have in front of it a polarizer at a different angle, e.g. -60° , -30° , 0° , $+30^\circ$, $+60^\circ$ and 90° deg to the first polarizer. Whereas with the second polarizer at 90° one gets no light coming through for an optically inactive or racemic sample, with the second polarizer at some other angle one gets *some* light coming through, the amount being given by a cosine squared dependence on the angle between the polarizers. For an optically active sample one can obtain the magnitude of the angle of rotation by seeing how much the actual intensity of light coming through differs from that expected for a racemic mixture. To see how to get the *sign*, consider two second polarizers at $+30^\circ$ and -30° to the first. If the sample is optically inactive or racemic, one should get the same intensity through both $+30^\circ$ and -30° polarizers. But if the sample has a small positive rotation, there will be an increased intensity through the $+30^\circ$ polarizer and a decreased intensity through the -30° polarizer (whereas it would be the other way round for a small negative rotation).

The principle of using two or more fixed polarizers (rather than a rotating one) to determine the polarization characteristics of light is used by the honey-bee (Wehner, 1976), hence we call this new design the Bee Cigar. Bees do a "dance" to indicate to other bees the direction of flowers in relation to the position of the sun. They therefore need to be able to detect the position of the sun (even on a cloudy day), and they do this by examining the polarization of sunlight scattered from the sky. Whereas sunlight itself is unpolarized, the scattered light shows a different polarization all over the sky according to the position of the sun. Bees have polarized detectors inside their head made of oriented light-absorbing rhodopsin molecules. The minimum number of independent polarized detectors needed is two, and bees have just two, at 40° to each other. We could use just this minimum number in the Bee Cigar, with two second polarizers at say $+30^\circ$

and -30° to the first, but artifacts will be reduced if we average over readings from further polarizers at say $+60^\circ$ and -60° , etc.

A Bee Cigar at a single wavelength gives the magnitude and sign of the optical rotation, but does not identify the substance. But if two or more alternative sources at different wavelengths were used (with light directed into the polarimeter with fibre optics), finding say a positive optical rotation at one wavelength and a negative optical rotation at another would give strong clues as to the identity of the molecule; indeed use of the Drude equation for the wavelength dependence of the optical rotation would allow the absorption wavelength of the sample to be deduced. Further clues from other techniques aboard the spacecraft, such as gas chromatography, would clinch the identification of the molecule.

Artifacts are eliminated by two important features of the Bee Cigar. Firstly, use of the polarized diode array eliminates false positives. With a single final polarizer, as in the "A" Cigar, false positives could arise due to scattering, etc. But sophisticated averaging over elements in the Bee Cigar's diode array will eliminate this. Secondly, use of two or more wavelengths eliminates false negatives, which could arise due to fortuitous cancellation of rotations from different compounds. But it is unlikely that exact cancellation would occur at two or three different wavelengths. Our simple, minimal polarimeter thus effectively mimics a much more sophisticated optical rotatory dispersion apparatus, by providing essentially the same information—the magnitude and sign of the optical rotation, and the absorption wavelength to enable identification of the molecule—in a tiny fraction of the mass.

An obvious problem, however, is sampling. Whereas the Cigar itself would weigh about 200 g, a further 300 g would be needed to prepare a filtered solution from a powdered sample, making 500 g overall. This includes about 20 ml of solvent (probably a methanol–water mixture, to dissolve the widest possible range of compounds) which would have to be carried and contained, plus mixing chamber, filter, and electronically actuated valves, etc., to achieve flow of solvent through the system. Clearly a large amount of development work is needed.

The sensitivity is about 0.1° with our current crude mock-up of the "A" Cigar, which can readily be increased to 0.001° in the Bee Cigar with improved sources and detectors using existing technology. This corresponds to about 10–100 μg of typical chiral molecules being present in the polarimeter. Optical rotation is thus not enormously sensitive—other techniques such as biosensors and chromatography can detect nanograms rather than micrograms—but its great virtue is that it is entirely general, does not depend on the target molecules being of any specific kind or having any specific properties such as fluorescence, and does not require different systems to detect different molecules (unlike chromatography, see below). One could, however, greatly enhance sensitivity for a range of selected target molecules by adding small amounts of complexing agents to the solvent (which would form complexes with much greater optical rotation than the target molecules themselves). Examples of effective

complexing agents include salts of copper and molybdenum.

The SETH Cigar would nicely complement gas chromatography experiments using chiral columns to separate enantiomers. A chiral column is planned for the COSAC GC-MS experiment selected for the RoLand lander of the Rosetta mission to a comet, and would also be very appropriate for Mars. However, there are two problems with chiral GC. Firstly, it is very specific: several different types of chiral column are needed to adequately separate different types of chiral molecule. With a single all-purpose column as would have to be used in space, there would be a danger of getting insufficient separation of enantiomers. Secondly, and more fundamentally, conclusive identification of chiral molecules by GC is only possible with a chiroptical detection method: the MS detectors normally used cannot distinguish between enantiomers. For example, if two GC peaks were detected in the amino acid region, it would not necessarily be clear without a chiroptical detector whether these represented the two enantiomers of one amino acid or the same enantiomer of two different amino acids. If, however, the output from the column could be directed into a chiroptical detector it would be possible to show whether their optical rotation was of the same or opposite sign. But space GC uses very small samples to minimize the amount of gas carried, with the result that typical peaks coming off the column may contain as little as 10^{12} molecules, in contrast to the 10^{16} molecules needed for detection by the Cigar. However, this gap in sensitivity might be closable by using complexing agents (specific to the molecules to which the chiral column is sensitive) in the Cigar's solvent, which could enhance detection by several orders of magnitude.

Although having the Cigar as the GC detector would thus be very useful if it could be achieved (which would be very difficult), it is in fact not necessary to separate the molecules to get a useful result from the Cigar—except in the case of cancellation of rotations from different molecules. The Cigar could equally well be used alone, or in parallel with (but separate from) a GC-MS. Note that the Cigar is better able to detect trace components when used separately from the GC than when used as the GC detector, simply because larger amounts of sample material can then be used: the concentration of the sample solution is limited purely by solubility and by the amount of powdered sub-surface sample that can be delivered by the drill (which would almost certainly be more than adequate to saturate 2 ml of solution).

The SETH Cigar is thus ideal in conjunction with chiral GC-MS, possibly as detector but more likely as independent corroboration. Without a chiroptical detector, GC-MS cannot give a totally unambiguous identification of an enantiomeric excess. A positive identification of homochirality on another planet would have such momentous implications that double confirmation is clearly desirable (especially in view of the chequered history of past attempts to detect life on Mars), and can be achieved with little extra cost and weight by adding a small Cigar to the GC. If there is no GC and the Cigar is used alone, double confirmation (of a lesser kind) is provided if positive results are obtained at two different wavelengths.

What do we expect to find with the Cigar? Are biomolecules of the same hand on different planets? Will we find L-amino acids on Mars as on Earth? And indeed *why* is terrestrial life based on L-amino acids and D-sugars and not D-amino acids and L-sugars? Several members of the SETH Cigar team have studied chiral influences which may have determined biomolecular handedness. These fall into three categories: local, solar system-wide, and universal chiral influences.

Local chiral influences have been studied by Barron (1982, 1986) and Teutsch and Thiemann (1986) and could involve combinations of magnetic and/or electric fields and/or polarized light. Such mechanisms would produce different hands on different planets. A solar system-wide chiral influence is provided by the circularly polarized radiation from neutron stars (Bonner, 1991; Greenberg *et al.*, 1994), which is of opposite hand at opposite poles of a neutron star. The idea is that the radiation from a passing neutron star would selectively eliminate one hand or the other in the pre-solar dust cloud according to which side of the neutron star the cloud passed, leading to an enantiomeric excess of the order of 10^{-1} . When the cloud later condensed to form the solar system, chiral molecules with an enantiomeric excess could then be delivered to Earth by comets. This mechanism would produce the same hand throughout any one solar system, but different hands in different solar systems.

A universal chiral influence is provided by the weak force. This is one of the four forces of nature—electromagnetic, weak, strong and gravitational—and it is the only one which is chiral, i.e. it can tell the difference between left and right. The weak force produces a slight parity-violating energy difference (PVED) between enantiomers (Hegstrom *et al.*, 1979, 1980), which has been calculated by *ab initio* methods (Mason and Tranter, 1984, 1985; MacDermott and Tranter, 1989; MacDermott *et al.*, 1992; MacDermott, 1994) for a large number of important biomolecules, giving results in the range 10^{-20} – 10^{-17} hartree, corresponding to 10^{-17} – 10^{-14} kT at room temperature. The PVED produces a very slight excess of the more stable enantiomer, which could be amplified to homochirality over long periods (see later). In almost all cases the PVED calculation predicts the correct hand, e.g. the natural L-amino acids are indeed more stable than their D mirror images, natural D-deoxyribose is indeed more stable than L-deoxyribose, and right-handed DNA is also PVED stabilized. If biomolecular handedness was determined by the PVED, one would expect to find the same hand throughout the universe—but of course we will have to wait for interstellar travel before the universal effect of the weak force can be distinguished from mere solar system-wide influences!

The weak force can also have an effect through the beta radiation emitted by radioactive isotopes (Hegstrom, 1982; Hegstrom *et al.*, 1985). This radiation is known to be practically homochiral, left handed for beta electrons and right handed for beta positrons. The radiolysis of racemic mixtures with beta radiation can therefore lead to a small enantiomeric excess which, as in the case of the PVED, would require amplification before reaching observable levels. The enantiomeric excess from beta radiolysis could be as high as 10^{-12} —much higher and more

easily amplifiable than the 10^{-17} – 10^{-14} from the PVED. Likely isotopes which could produce such an effect include ^{40}K , ^{235}U , ^{238}U .

The small enantiomeric excesses from the various chiral influences would need amplifying to reach homochirality. Kondepudi (1987) has devised a kinetic amplification scheme involving autocatalysis and enantiomeric antagonism—that is, the presence of one hand encourages production of itself but inhibits production of its mirror image. Kondepudi showed that an enantiomeric excess of 10^{-17} from the PVED can be amplified to homochirality within 10^4 years, and the amplification time decreases by four orders of magnitude for each order of magnitude increase in the initial enantiomeric excess, so the larger excesses from beta-rays or neutron stars could be amplified very easily. Computer simulations of this sort of stereospecific autocatalysis have produced promising results (Buhse *et al.*, 1993). Many polymerization reactions essential to life show the autocatalysis and enantiomeric antagonism features of the Kondepudi amplification mechanism, and some enantiomeric enrichment has been demonstrated in the laboratory (Brack and Spach, 1979, 1980; Darge and Thiemann, 1974; Thiemann and Teutsch, 1990).

Finding the same hand on different planets would thus lend support to the more global theories of the origin of homochirality, such as the weak force. But even finding the “wrong” hand on Mars would be of enormous significance because it could still be the homochiral signature of life.

It is sometimes argued that exobiology instruments should not be flown on Mars missions in the immediate future, but should await later missions so as to give earlier missions a chance to identify suitable landing sites. Such caution might well be appropriate for some of the larger and more complicated exobiology experiments, but the Cigar is small and simple enough to take on an earlier mission—indeed it could assist in the identification of suitable sites for exobiology. As mentioned earlier, we believe we already know what sites would be suitable from current information: clearly we are looking for ancient sediments, almost certainly in the southern hemisphere, and at as high a latitude as possible to take advantage of dry, frozen conditions and lower temperatures. Dust storms would in any case have been likely to distribute biological materials all over the planet. The high resolution camera and IR on the Mars Global Surveyor will further assist in identifying exact sites. It has been suggested that the three or four landers on the InterMarsNet mission would all carry an identical core payload for seismological and other measurements that take advantage of networking, but that each lander would have additional space for other instruments, so that one lander could carry exobiology experiments. The exobiology lander would need a 1 m drill to sample the subsurface layers, and could carry a GC-MS (ideally with chiral columns) to identify organics, plus the Cigar to give an unequivocal identification of homochirality.

The possibility of finding signs of life on other planets is the most effective generator of public interest in space missions necessary to ensure funding, so it is extremely important to be making at least some attempt to look for

life sooner rather than later. We believe the SETH Cigar provides a simple and cost-effective way to do this, because it is small and cheap enough to take routinely on all solar system missions to test for the homochiral signature of life.

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