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CHIRALITY

Homochirality in Life: Two Equal Runners, One Tripped

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Abstract Strong arguments can be found in the literature addressed to the question of the origin of homochirality in life, supporting the hypothesis that primordial life could have evolved in both homochiral forms and that early on when life was still rarely found, random events led to the survival of only one of these living mirror images. This proposal is an alternative to the generally accepted view that small enantiomeric excesses of biologically important molecules were amplified to homochirality prior to life's origin. Acceptance of the possibility of "two equal runners" leads to the importance of research investigations on routes to formation of ensembles of racemic mixtures of isotactic biologically interesting polymers, supramolecular entities and aggregates.

Keywords Polymers · Racemic homochiral life · Chirality · Origin of life · Origin of biological homochirality

Scientists have long wondered about the connection between life and chirality, an interest that can be broken down into two questions: *why* does life always make chiral choices by choosing one mirror form and excluding the other, and *how* did it come to be that life is homochiral considering the likely origin of small biological molecules as mixtures of enantiomers?

It may be valuable to remind ourselves of possible answers to these questions, which have long existed in the literature, answers that could alter our thinking and even the focus of our experiments.

Let's start with the first question, *why*, where there is general agreement on the answer, but where there may be value in seeing an answer to this question that has not been prominently displayed, and is relevant to the *how* question.

Thirty two years ago Jeremy Knowles asked a question that is apparently different from the *why* question. How perfect a catalyst can an enzyme be? He published his work on

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triosephosphate isomerase, an enzyme which maximizes the number of adenosine triphosphate molecules produced in muscle glycolysis (Knowles and Albery 1977). Knowles pointed to a powerful evolutionary force in perfecting the action of this enzyme using the anecdote of the fox chasing the rabbit, both animals needing to maximize their speed. Knowles concluded:

> "*It is clear from the free-energy profile that the enzyme has reached the end of its evolutionary development, the maximum flux of substrate being determined by a nonerodable diffusion limitation*."

In other words the enzyme is perfect in its catalytic action.

Much was already known about the detailed mechanism of the action of triosephosphate isomerase when Knowles reported his findings, including that in the conversion of a HO-CH_2- group to an aldehyde group in one of the substrates, one of the hydrogen atoms is taken to the exclusion of the other although reaction using either hydrogen atom yields the identical product. In fact one could not know about this selection except by the use of an isotope of hydrogen. This kind of choice between structurally identical atoms or groups of atoms within a molecule is in fact the rule in enzymatic reactions. A beautiful example is the enzyme catalyzed reaction of one of the $CH_2CO_2^-$ groups in citrate to the exclusion of the other structurally identical group in forming cis-aconitate. Here the enzyme perfectly retains the carbon atoms which most recently arrived in citrate from acetyl coenzyme A, and sends the "older ones" on their way to carbon dioxide. The choices made by triosephosphate and by aconitase are well known to be chiral choices between enantiotopic atoms or groups of atoms. The critical point here is that there are a multitude of enzyme catalyzed reactions distinguishing enantiotopic groups in which the product of the reaction would be identical whichever of the enantiotopic groups were chosen (Arigoni and Eliel 1969; Benner et al. 1989; Cornforth 1974). Therefore, why should an enzyme bother to make the distinction?

In fact it is no bother at all for enzymes to distinguish enantiotopic atoms or groups of atoms. The chiral distinction is nothing more than a byproduct of the excellence, near perfection in some cases, of the catalysis (Knowles and Albery 1977). It is impossible for a chiral catalyst to maximally speed the rate of a chemical reaction without distinguishing enantiomers or enantiotopic moieties. John W. Cornforth in the year before he won the Nobel Prize came to this conclusion from his studies on the stereospecificity of biological reactions (Cornforth 1974). Here are his words:

> "*We are thus forced to conclude that stereospecificity is inherent in the catalytic action of enzymes, and that enzymes would not be equally efficient as catalysts of non-stereospecific reactions."*

One way to put the answer to the question of *why* posed above is that life strives for efficiency and chiral choice is a byproduct of that efficiency. This arises from the complex folded structures which are characteristic of polymers, which makes enzyme catalysis possible, and is one of the reasons that polymers are the essential molecular form of life. Life is full of mystery and wonder but the fact that life is stereospecific is not one of those wonders.

But *how* did life came to be homochiral? We've just seen Cornforth's view that living processes have to be stereospecific for maximum efficiency. That efficiency, as in Cornforth's focus on enzymes, directs our attention in all biological functions to the properties of polymers, necessary for both metabolic and genetic functions. When we ask *how* life came to be homochiral, it is reasonable to propose polymers as playing a central

role. This was the view that George Wald presented at the Polytechnic Institute of Brooklyn at a lecture in 1955 (Wald 1957). In Wald's own words:

> "*If one could grow such polymers in a reversible system in which syntheses was partly balanced by hydrolysis, the opportunity for selection would be greatly improved."……If the synthetic system also were reversible, any disadvantageous sequences formed initially could be corrected later by exchanges between the forming polymer and the solution, until the most stable configuration had been achieved."*
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> "*The essential idea is that from unit molecules provided by geochemical processes in racemic mixtures, single optical isomers were selected in the process of composing structures of higher order: polypeptides and proteins from amino acids, nucleic acids from nucleotides, and so on. The basis of the selection was that the structures of higher order gained stability and other advantages by assuming physical configurations demanding that only one optical configuration be employed. The final choice of one of the two optical isomers in each instance is assumed to be arbitrary."*
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> *"As such molecules aggregated with others to form structures of still higher order, organized aggregates and, eventually, living organisms, the importance of forming helices and the consequent pressure to select single optical isomers, must have grown steadily."*

In the beginning, Wald reasoned, it is difficult to imagine primordial versions of polymers, which are poised to play a biological role, and therefore heterostructural within each chain, as anything but heterochiral. Biologically significant molecules such as amino acids and sugars are well suited to condensation polymerization and many, if not all of these *unit molecules* were likely racemic or with an array of enantiomeric excess. Wald imagined such an array of heterochiral-heterostructural polymers with random chiral configurations for each of the units within each chain. In this way of thinking we put the formation of the polymers first, although a mess of polymers; no two chains alike in terms of structural units and their positions within each chain and each chiral unit of uncertain configuration. Wald imagined that these polymers were then driven toward homochirality within individual chains first by intramacromolecular forces that favored a uniform chirality and then by the higher order states associated with primordial replication and metabolic functions for these polymers. But he saw this drive toward homochirality without favoring one mirror form over the other, allowing both to form. Wald takes us to the conclusion that the reversibility/equilibration he saw as necessary for these polymers/oligomers/aggregates must produce the racemic state, a principle seen in a recent example (Blackmond 2009).

Hans Kuhn, in the early 1970s, with prescient detailed arguments, although arriving at the racemic state for a different reason, also saw the necessity for homochiral macromolecular/aggregate biologically primordial states (Kuhn 1972). Kuhn allowed the possibility that such biologically primordial states would arise in large numbers by replication mechanisms from a few homochiral oligomeric entities, which although improbable, nevertheless when formed were exceptionally effective replicators. Here are his words:

> *"One can imagine the chance formation of an RNA molecule with, say, 21 bases and containing exclusively d-ribose as sugar component……Among 10^6 molecules…. there is on average one molecule that happens to contain only d- or only l-ribose"* (Kuhn 1972).

Kuhn saw these homochiral oligomers and their aggregates as competitive to the eventual exclusion of the more numerous heterochiral isomers. But as in Wald's hypothetical picture, Kuhn also saw no favoring of one mirror form over the other (Wald 1957; Kuhn 1972). He saw the resolution of this mixture as a competition. Here are Kuhn's words:

> *"It is conceivable that evolution of organisms with l-ribose could take place in one locality while organisms with d-ribose develop elsewhere. As soon as these two populations come into competition….."*(Kuhn 1972).

Kuhn went on to develop his ideas, on the connection between the entangled origin of life and origin of homochirality, in an increasingly convincing manner (Kuhn and Waser 1983a; Kuhn 2008). As for the prevalence of one mirror form we see in life, Kuhn imagined the necessary competition to arise from differing resources in the localities or even one chiral form that is more advanced, inflicting damage on the other (Kuhn 1972).

Vladimir Prelog in his Nobel Prize winning lecture, also allowed equal probability of both mirror forms of primordial life, but avoided the competition necessary in both Wald's and Kuhn's hypotheses by suggesting that life arose from a single event, a view that has been recently reinforced (Prelog 1976; Wald 1957; Kuhn 1972; Fuss 2009).

Prelog wrote:

> "*Many hypotheses have been conceived about this subject, which can be regarded as one of the first problems of molecular theology. One possible explanation is that the creation of living matter was an extremely improbable event, which occurred only once"* (Prelog 1976).

The fundamental basis of Wald's seminal hypothesis (Wald 1957) was based on the fact that biologically significant polymers take specific secondary structures, the helix being prominent ("*physical configurations*" in the quoted passage). He saw the helix in consort with evolving life's functions as the selecting mode to enforce the tendency from heterochirality within individual chains toward homochirality within each individual chain even if the ensemble of chains were racemic. Wald understood that a helical conformation could be maintained even in a polymer constructed of units of opposing helical sense preference, which has been experimentally demonstrated in synthetic polymers as the concept of "majority rule" a characteristic of the helical state seen earlier by the Italian School in studying the properties of stereoregular polymers (Green et al. 1995, 1999; Brack 2007). Higher stability would then lead to a homochiral helix by equilibration/epimerization events (Green et al. 1999).

Indeed, in the more than half century since Wald considered these ideas, research has shown that certain peptide conformations, β-sheets, tend to homochirality within each sheet, although a racemic ensemble (Illos et al. 2008; Weissbuch et al. 2009). While isolated α-helices favor this tendency toward homochiral chains to a lesser extent, research on self-replication in α-helical coiled coil peptides demonstrates high enantioselectivity in what is termed a chiroselective autocatalytic cycle (Brack and Spach 1981; Brack 2007; Lee et al. 1996; Saghatelian et al. 2001).

Long known replication phenomena in oligonucleotides, arising from base paired double helical structures, have been connected to hypotheses concerned with the origin of life and the origin of homochirality (Joyce and Orgel 1986; Kiedrowski et al. 1991; Sievers and Kiedrowski 1994; Naylor and Gilham 1966; Kuhn 1972, 2008; Kuhn and Waser 1983b; Orgel 1992; Inoue et al. 1984; Kiedrowski 1986; Paul and Joyce 2004). Recent advances in this area in addition to discoveries of plausible prebiotic routes to nucleotide structures reinforce the early theoretical ideas of both Wald (1957) and Kuhn (2008) in pointing to

oligomers/polymers as the critical entities in the origin of homochirality: chiral studies in the replication area have shown, as noted above for peptides and especially for oligonucleotides, that high enantioselectivity is the rule (Lincoln and Joyce 2009; Powner et al. 2009; Saghatelian et al. 2001; Joyce et al. 1984; Goldanskii et al. 1986; Avetisov and Goldanskii 1996). Analogously, intermolecular spatial requirements enhancing chiral selectivity find example in transcription experiments in synthetic supramolecular systems and are related to tacticity control in helical crowded synthetic vinyl polymers (Wilson et al. 2005; Nakano et al. 1992; Guerra et al. 2003).

One study on chiroselective self-assembly of nucleotides drew the interesting conclusion that high degrees of oligomerization preclude, for statistical reasons, a mirror image relationship between the d and l chains, a phenomenon also realized in synthetic polymers (Bolli et al 1997; Green and Garetz 1984). In this manner the all d and all l heterostructural entities could evolve along different prebiotic paths, a variation of Kuhn's competition, and play a role in the origin of homochirality (Kuhn 1972; Bolli et al. 1997).

Wald came to an interesting view of the competition he saw as necessary to arrive at the homochiral ensemble in life today, which we learn about in a conversation he had with Albert Einstein in 1952, several years before he published his views (Wald 1957, 1988). Einstein asked: "*Why do you think the natural amino acids are all left-handed?"* Einstein went on to say: "*I have wondered for years how the electron came out to be negative. Negative and positive are perfectly symmetrical principles in physics, so why is the electron negative? All I could think of was, the negative electron won in the fight."* Wald reports that he answered: *"That is exactly what I think of those left-handed amino acids—they won in the fight."*

An apt aphorism occurs: Two equal runners, one tripped. And it makes most sense, based on the discussion above, if the "runners" were polymers and their aggregated states, capable of primordial life's functions.

Nevertheless, there is a large body of work stimulated by the possibility that the origin of homochirality in life was seeded by small molecules with even exceptionally low enantiomeric excesses, which could be amplified (Frank 1953; Sandars 2003). Many beautiful stereochemical experiments and interesting ideas have been driven by interest in this area and fundamental ideas on chirality have been advanced as a consequence (Siegel 1998; Weissbuch et al. 2005). There is no way to know for certain if, or how, the equal runners idea may be combined with these views (Frank 1953; Sandars 2003; Siegel 1998; Weissbuch et al. 2005; Noorduin et al. 2008; Klussmann et al. 2006; Soai et al. 1995; Kawasaki et al. 2009; Barabás et al 2008; Welch 2001; Pizzarello et al. 2008; Mason 1983, 1988; Tranter 1987; Lahav et al. 2006) an uncertainty, which has been expressed in more general terms for the origin of life itself by Eschenmoser: *"The origin of life cannot be discovered, as other things in science, it can only be "re-invented"*(Eschenmoser 2008). Indeed life and homochirality may have evolved from chemical entities, no longer present, but which played an early role (Eschenmoser 1999).

In the view taken by the literature focused on here, homochirality in the individual primordial oligomer/aggregate states and early organisms is essential for the efficiency sought by life's functions. However, a racemic state of this homochirality may be efficient in the early stages compared to heterochirality but inefficient as life evolved, therefore driving life to the single homochiral state as we live with now, that is, driving the competition between the two equal runners.

The question of the origin of the homochirality in life becomes part of the question of the origin and evolution of life (Eschenmoser 1999, 2007; Calvin 1969), which was destined to produce the homochirality we live with today to satisfy the necessity for

efficiency but without any investment in the beginning for one side of the mirror or the other. In Wald's and Kuhn's view, this came later.

One exceptionally critical experiment, impossible at this time, would then be to discover life elsewhere than our planet allowing a test of Wald's view as he expressed it:

> *"If the choice of optical isomers is as arbitrary as proposed one should expect that a survey of life throughout the universe would reveal approximately equal numbers of planetary populations in which the choice of metabolically connected series of dissymmetric molecules came out L or D-; roughly equal numbers in which life is based upon L- and upon D-amino acids, and similarly, for the other molecules"* (Wald 1957).

A perhaps more hopeful experiment has been proposed by the possibility that Precambian sediments might contain evidence of early life forms of the chirality that lost out in the hypothesized competition (Kuhn and Waser 1983b).

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