Entropy-driven homochiral self-sorting of a dynamic library†

Joan Atcher,^a Jordi Bujons^a and Ignacio Alfonso^{a,*}

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A dynamic mixture of stereoisomeric macrocycles derived from glutamic acid displayed a homochiral self-selection when increasing the acetonitrile content of the aqueous mixed medium. The homochiral self-sorting required the anionic form of the side chains and increased at higher temperature, implying an entropic origin. Conformational analysis (NMR and MD simulations) allowed us to explain the observed behaviour. The results show that entropy can play a role in the homochiral self-sorting in adaptive bio-inspired chemical systems.

Natural chemical systems are homochiral, since the chiral biomolecules mainly exist in living beings as single enantiomeric forms (i. e. L-amino acids and D-carbohydrates). The actual origin of the homochirality is still a matter of debate,¹ but its observed preservation must imply the accurate transmission of the stereochemical information through an efficient homochiral self-selection of the implicated species.² Despite the fundamental importance of homochirality in biomolecular systems, a definitive physicochemical explanation for the process remains elusive.³ Previous theoretical studies have suggested that the homochirality in natural systems has an entropic nature,⁴ although this hypothesis has not been experimentally proved so far. Only recently, Moore⁵ and Mastalerz⁶ have considered entropy for explaining homochiral self-sorting in relatively rigid molecules. Fascinated by this idea, we decided to investigate how the chiral information could be transmitted in simple dynamic chemical systems under equilibrium (Dynamic Combinatorial Libraries).⁷ DCLs have the advantage of being adaptive and capable of proof-reading and self-correction processes, thus being responsive to external stimuli.⁸ However, although they comprise a very good benchmark model for unravelling structural and chemical information,⁹

studies focused on the expression of chiral information within DCLs are still scarce.¹⁰ Accordingly, we synthesized enantiomeric building blocks (BBs, **1**) derived from a natural amino acid (Glu) and bearing thiol functionalities for establishing dynamic covalent disulphide chemistry in aqueous media (Scheme 1a).¹¹ For an easier analysis of the mixtures, we isotopically labelled (deuterium) the BB derived from the unnatural D-Glu. Considering previous results,¹² the combination of these BBs would mainly render macrocyclic dimers (**1₂**) as a mixture of all the possible stereochemical combinations (Scheme 1b).

$$(c) \quad 2 (R,S)\text{-}1_2\text{-}d_4 \xrightleftharpoons{K_{HS}} (S,S)\text{-}1_2 + (R,R)\text{-}1_2\text{-}d_8$$

$$K_{HS} = \frac{[(S,S)\text{-}1_2] \cdot [(R,R)\text{-}1_2\text{-}d_8]}{[(R,S)\text{-}1_2\text{-}d_4]^2} \quad (\text{Eq. 1})$$

Scheme 1. (a) Chemical structures of the building blocks. (b) Dynamic equilibrium between homo- and heterochiral macrocycles. (c) Definition of the homochiral selection exchange constant (K_{HS}).

^a Department of Biological Chemistry and Molecular Modelling, Institute of Advance Chemistry of Catalonia (IQAC-CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain. E-mail: ignacio.alfonso@iqac.csic.es

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Within this dynamic mixture, the dithiol BBs can form one heterochiral *meso* macrocycle and two homochiral stereoisomers. If the stereochemistry of the molecules does not affect the stability of the disulphide bond, one would expect a statistical distribution where the concentration of the heterochiral macrocycle should double the one of each homochiral dimer. For characterizing the dynamic mixture, we used the thermodynamic exchange constant defined as the equilibrium constant between hetero- and homodimers (K_{HS} in Eq. 1, Scheme 1c). In the absence of selection (i. e. at the statistical distribution) the value of K_{HS} is 0.25.

We initially studied the system (0.5 mM in each BB) in buffered water at pH 8.0, where the carboxylic groups of the Glu side chains are deprotonated. Fortunately, we were able to separate all the possible stereoisomers of the major dimeric macrocycles by chiral-HPLC (Fig. 1a-c). The identity of the species formed in the library was confirmed by UPLC-MS, which allowed the unambiguous assignment of the different stereoisomers thanks to the deuterium labelling. Moreover, reversibility tests were carried out at selected representative conditions to ensure that the systems reached the equilibrium composition.¹³

Fig. 1. Chiral-HPLC traces (a-c) and plot of the K_{HS} values (d) obtained from an oxidized library of (S)-1 and (R)-1-*d*₄ (0.5 mM each in aqueous acetonitrile, pH 8, 22 °C) in media with different polarity (% of acetonitrile). PB means phosphate buffer and G means guanidinium chloride.

Interestingly, the analysis of the equilibrated mixture showed a moderate preference toward the homochiral species, since $K_{HS} = 0.36 \pm 0.01$ (PB in Fig. 1d). This means that the homochiral species are slightly more stable than the *meso*-heterochiral one. We hypothesized that the intramolecular polar attractive (H-bonding) and repulsive (electrostatic) interactions could be playing a role in the process. Accordingly, the K_{HS} value decreased to 0.30 ± 0.01 in the presence of 1 M guanidinium chloride (PB + G in Fig. 1d), a well-known chaotropic agent. Then, we decided to systematically study the effect of the polarity of the medium on the relative stability of the species. To do that, we generated the dynamic library with increasing

proportions of acetonitrile (AN) in the reaction medium, adding tetrabutylammonium hydroxide (pH 8) to ensure the full deprotonation of the carboxylic groups and the presence of enough thiolate for establishing a fast disulphide exchange. Interestingly, the homochiral self-selection (K_{HS} value) increased as the polarity of the medium decreased, especially above the azeotropic composition. Remarkably, at 92% AN the K_{HS} value is tenfold the theoretical one for the absence of chiral selection. These results imply a stronger homochiral self-selection in less polar media.

For analysing the effect of other environmental stimuli, we considered the energetic difference favouring the homochiral selection ($\Delta\Delta G_i$) as the stabilization energy compared with the statistical proportion, which can be calculated with Eq. 2:

$$\Delta\Delta G_i = \Delta G_i - \Delta G_{stat} = -RT \ln(K_{HS} / 0.25) \quad (\text{Eq. 2})$$

Since the side chains of the obtained macrocycles contain carboxylic acid residues, the effect of the protonation degree of the library members was evaluated by carrying out the DCLs in different aqueous buffers (pH 2.5-7.5) at an intermediate proportion of AN (56%). The homochiral selectivity gradually increased as the pH went from 2.5 to 7.5, reaching a plateau at pH 6.5-7.5 (Fig. S6). This observation implies that the tetra-anionic species are the most stereoselective ones. On the other hand, the DCLs showed identical composition when generated from the BBs at 0.5-2.0 mM, ruling out intermolecular effects within this concentration range (ESI[†]).

We also used the temperature as stimulus by preparing the dynamic mixtures at 2, 22 and 45 °C.† The corresponding $-\Delta\Delta G_i$ values versus the AN mole fraction (χ_{AN}) are plotted in Fig. 2a. The homochiral selection dependence on the polarity of the medium was similar for the three studied temperature values: the selectivity increased with the χ_{AN} . On the other hand, the homochiral selectivity also increased with the temperature. The corresponding van't Hoff analysis (Fig. 2b) showed that the energy associated to the homochiral selection ($\Delta\Delta G_i$) arises from positive enthalpies ($\Delta\Delta H_i$) and much larger positive entropies (negative $-T\Delta\Delta S_i$) at all the solvent mixtures. This means that the homochiral selection in this system is enthalpically disfavoured but entropically favoured, suggesting that the homochiral species might be less ordered than the heterochiral one under all tested conditions.^{§14}

The comparison of the ¹H NMR signals of the homochiral compounds with those of the heterochiral species rendered interesting results (Fig. S13[†]). First of all, the spectra showed a quaternary symmetry for the two isomers, implying that both are highly flexible in solution with a rapid exchange between different conformers in the NMR timescale. Besides, the two stereoisomers showed different chemical shifts for positions away from the chiral centres, meaning that the average conformations of the two macrocycles must be different enough to produce clearly different environments for all the NMR signals of the macrocyclic frame. More interestingly, although the amide NH connected to the aromatic ring appeared overlapped for the two isomers, a downfield shift of

Fig. 2 (a) Plot of the homochiral selectivity energy ($-\Delta\Delta G_i$) as a function of the acetonitrile mole fraction (χ_{AN}) at three different temperatures. (b) Enthalpic ($\Delta\Delta H_i$, blue) and entropic ($-T\Delta\Delta S_i$, red) contributions to the homochiral selectivity energy ($\Delta\Delta G_i$) in different solvent compositions (percentage of AN in water, PB means phosphate buffer in water).

the peptidic amide NH (the one coming from the Glu amino acid) was obtained for the heterochiral molecule. This observation suggested that this NH is implicated in stronger H-bonding interactions in the case of the heterochiral macrocycle. In order to gain insight into the conformational differences between the two species, molecular modelling studies were performed. Since the systems showed to be flexible in solution, we carried out long molecular dynamic simulations of the fully ionized heterochiral and one homochiral dimers plus the corresponding TBA counterions in explicit acetonitrile solvent, at 300 K (10 x 250 ns independent simulations, meaning 2.5 μ s of total simulation time). These simulations produced 50000 conformations for each macrocycle that were carefully analysed in order to establish their structural differences.

Inspection of the MD simulations results (see details and movies in the ESI[†]) showed that the heterochiral macrocycle adopts a more efficiently folded conformation, stabilized by intramolecular interactions during longer periods of time.

Actually, the analysis of the H-bonding patterns during the simulations showed that the heterochiral species is able to establish a larger number of intramolecular H-bonding interactions than the homochiral one (average from 2.5 μ s total simulation time: 6.5 ± 0.9 vs. 5.3 ± 0.9 , Fig. S17 and S20 in ESI[†]). This trend correlates with the experimental observations by NMR and suggests that the heterochiral species must be enthalpically favoured, in agreement with the data shown in Fig. 2b.

The accessible surface area (ASA) of the homo/hetero macrocycles during the simulations was also evaluated (Fig. S19 and S22, ESI[†]). The two molecular systems (including the TBA counterions) showed negligible differences in their respective ASAs indicating that, on average, a similar number of solvent molecules would be surrounding both isomers and, consequently, there would be no significant differences in their solvation entropies.

Additionally, in order to study the corresponding conformational properties, we performed a clustering analysis of the full set of conformations for each dimer using different methods (Fig. S24-S26, ESI[†]). Interestingly, all clustering calculations showed a larger number of energetically accessible conformations for the homochiral macrocycles, implying an entropic contribution associated to their higher flexibility. We used the CENCALC software¹⁵ to get a quantitative estimation of the conformational entropy of both isomers and the results showed that under the conditions of our simulations $T\Delta\Delta S = 7.9$ kJ mol⁻¹ favouring the homochiral dimer, in an excellent agreement with the experimental values for the closest conditions (92% AN, 22 °C, Fig. 2b). Altogether, these results suggest that the conformational entropy would be the main contributor to the total entropy difference observed between both macrocycles. This is more evident at high acetonitrile concentrations. A reasonable explanation of this solvent dependence is that the conformational properties of these molecules must be related to their ability to fold by intramolecular H-bonding. We hypothesized that the more efficient folding observed for the heterochiral macrocycle must be related to its higher H-bonding abilities (as observed by NMR and MD simulations). A less competitive medium favours more intramolecular H-bonding interactions in the heterochiral than in the homochiral macrocycle, thus increasing their differences in conformational flexibility and entropically favouring the homochiral species.

In conclusion, we described a dynamic library of amino acid derived macrocycles that is able to express an entropy-driven homochiral self-sorting in aqueous solution. The homochiral selection has been traditionally associated to order-increasing processes such as crystallization¹⁶ or self-assembling.¹⁷ By contrast, our minimalistic dynamic chemical system represents a remarkable experimental example for the illustration of the importance of entropy in the homochiral self-sorting of flexible molecules in solution. As the universe expands by increasing its entropy,¹⁸ our observations support the concept that homochirality can be the natural evolution of certain molecular systems,¹⁹ with an implication in the understanding of the prebiotic world.²⁰

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Notes and references

‡ Higher temperatures produced over-oxidation of the disulphide groups, precluding the analysis.

§ Moore and co-workers (ref. 5) have recently justified the homochiral selection in a macrocyclization reaction due to the higher symmetry of the homochiral species. In our system, the upper-limit estimation of the entropic contribution to $\Delta\Delta G_i$ due to the molecular symmetry is $T\Delta\Delta S_i = 3.4 \text{ kJ}\cdot\text{mol}^{-1}$ (at 22 °C, Fig. S15 in the ESI[†]) favouring the homochiral macrocycle (ref. 14). This value is lower than the experimental observations, implying that there must be another contribution to the entropic difference.

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