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Enantiomeric Segregation in the Gel Phase of Lipid Bilayers

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Supporting Information

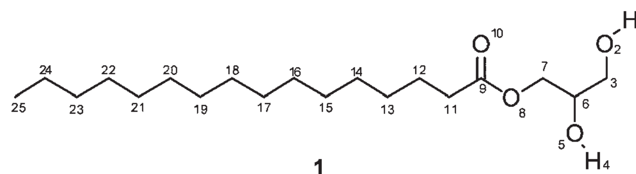
ABSTRACT: Enantiospecific interactions within a monoglyceride lipid bilayer are investigated using molecular dynamics simulations. Preferential homochiral interactions are observed in the gel phase, whereas no detectable enantiospecificity is seen in the liquid-crystal phase. On the basis of these results and available experimental data, a mechanism is proposed for the formation of the coagel phase of monoglycerides. Enantiomeric segregation in the gel phase is also discussed in terms of its possible implications for prebiological evolution and membrane raft function.

Lipid-based vesicles involving chemically simple lipids such as fatty acids or monoglycerides may have been present on the early Earth and contributed to prebiological chemical evolution.[1] Vesicles can form spontaneously, depending on the chemical structure of the lipid and on the lipid−water ratio, grow by incorporating additional amphiphilic molecules (and may eventually divide),[2] and encompass organic material. These three processes are accomplished without the need for enzymatic catalysis, nucleic acid-based information molecules, or energy expenditure.[1b,3] The consideration of vesicle participation during prebiological evolution has also been suggested as a hypothesis for the origin of biomolecular homochirality.[1b,4]

When a racemic mixture of a monoglyceride in water, at a composition appropriate for bilayer formation, is cooled below the melting temperature T_m, a phase transition from the liquid-crystal (LC) to the gel (GL) phase of the bilayer is observed.[5] The GL phase is metastable and, within hours to days, undergoes another phase transition to a coagel (CO) phase, namely a mixture of anhydrous lipid crystals and water.[6] Analysis of the CO crystals reveals that enantiomeric separation has occurred during this transition, leading to a structure involving alternating layers of the pure *R* and *S* enantiomers.[6e] Reheating the CO phase leads again to the racemic LC phase.

Considering that many contemporary biochemical reactions are catalyzed at the surface of lipid membranes, any process that could have physically or chemically promoted an enantiomeric enrichment of prebiotic bilayers involving chiral lipids could have indirectly contributed to the autoamplification of other chiral molecules. Enantiospecific interactions in lipid aggregates are not only interesting from the point of view of prebiological evolution; they may also influence the morphology of the aggregates[7] and control enantiodiscrimination toward chiral solutes.[8] Understanding those interactions might also help to explain the thermodynamic instability of the GL phase of monoglycerides and provide insight into the mechanism of the CO phase formation.

Recently, we have shown that an accurate determination of the melting temperature T_m of a lipid bilayer via molecular dynamics (MD) simulation is possible.[9] The system consisted of a racemic mixture of the monoglyceride glycerol-monopalmitate (**1**) in water, simulated at different hydration levels and temperatures.



In the present article, additional MD simulations are reported that were performed according to a similar methodology, involving 40 ns (2 fs time step) simulations of a 2×6×6 GMP bilayer patch under periodic boundary conditions and relying on a modified version of the GROMOS 53A5 force field described in ref 9, along with the SPC[10] water model. These simulations specifically investigate the possibility of enantiomeric segregation in the GMP−water system, at two hydration levels (full or half hydration) and at six different temperatures (from 318 to 338 K in steps of 4 K). In contrast to the previous study,[9] all simulations discussed here concern systems in the (meta)stable phase of the bilayer (GL or LC) at the selected hydration level and temperature. The key question that is addressed concerns the conditions (phase, hydration, temperature) under which enantiospecific interactions may become sufficiently strong so as to promote enantiomeric separation. The possibility of a differential interaction between identical (*R-R* and *S-S*) and opposite (*R-S*) enantiomers is investigated on the basis of the corresponding radial distribution functions $g_{RR}(r)$, $g_{SS}(r)$, and $g_{RS}(r)$ and the corresponding average numbers of hydrogen bonds n_{RR}, n_{SS}, and n_{RS}, displayed in Figures 1 and 2, respectively.

At full hydration, where the T_m value is about 324 K,[9] the radial distribution functions and hydrogen-bond numbers evidence partial segregation with preferential interaction for identical enantiomers at the two lowest temperatures only, i.e., when the bilayer is in the GL phase. The preferential interactions completely disappear at the four highest temperatures, i.e., when the bilayer is in the LC phase. At half-hydration, where the T_m value is about 332 K, enantiospecific interactions are important at the four lowest temperatures, their magnitude being amplified compared to the full-hydration situation, i.e., when the bilayer is in the GL phase. They disappear again at the two

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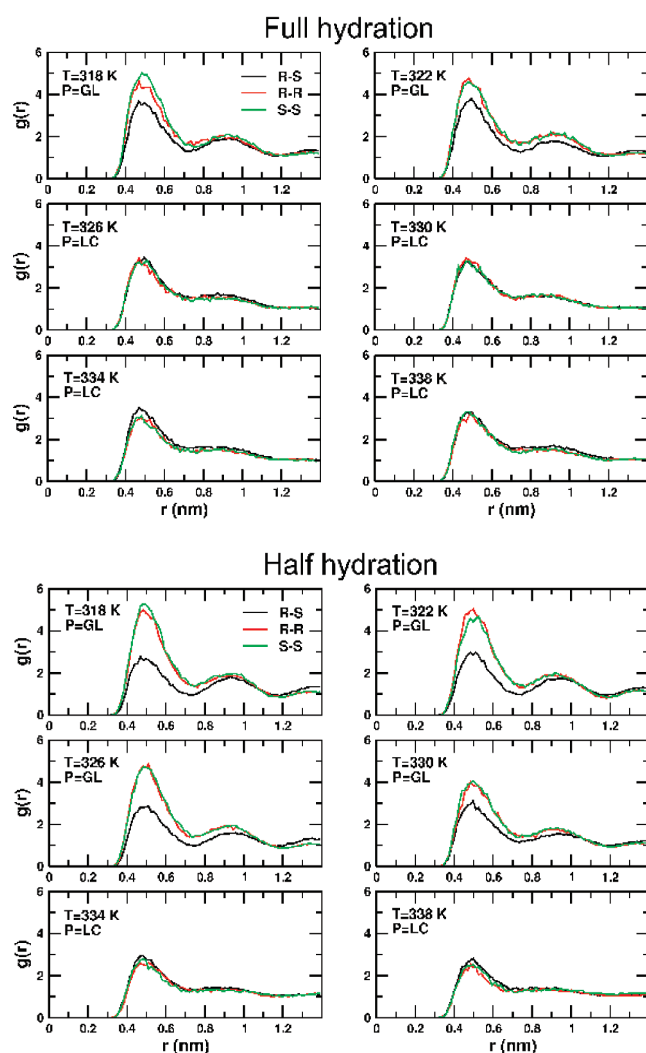

Figure 1. Radial distribution functions $g(r)$ between the central glycerol carbon atoms C2 (atom 6 in **1**) of identical (*R-R* and *S-S*) or opposite (*R-S*) enantiomers in simulations of GMP bilayers at full and half hydration and at different temperatures. Within each panel, T indicates the temperature and P the phase (GL or LC).

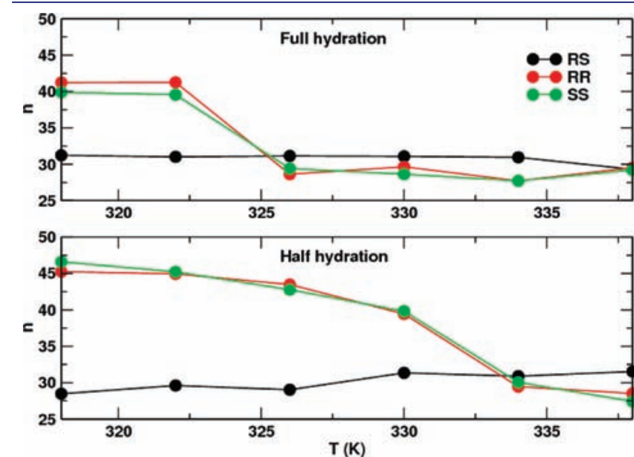

Figure 2. Average numbers of hydrogen bonds n between identical (*R-R* and *S-S*) or opposite (*R-S*) enantiomers in simulations of GMP bilayers at full or half hydration and at different temperatures T.

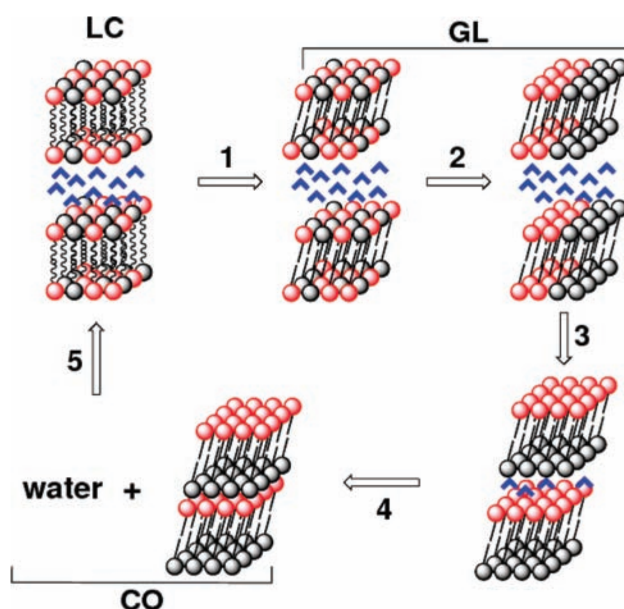

Figure 3. Suggested phase-transition mechanism between liquid-crystal (LC), gel (GL), and coagel (CO) phases, based on simulation and experiment in the context of the GMP—water system.

highest temperatures, i.e., when the bilayer is in the LC phase. These results suggest that (i) enantiomeric segregation is significant only in the GL phase and essentially absent in the LC phase and (ii) the thermodynamic instability of the GL phase and the driving force for its slow conversion to the CO phase are probably related to enantiomeric separation.

A possible phase-transition mechanism compatible with these observations is illustrated in Figure 3. Cooling below T_m promotes a fast transition to a racemic GL phase (step 1), followed by slow enantiomeric separation (step 2), bilayer aggregation (step 3), and dehydration into the CO phase (step 4). Finally, reheating of the CO phase above T_m leads back to the racemic LC phase (step 5). Intralayer segregation by lateral diffusion is generally viewed[6d,e] as the main mechanism for the enantiomeric separation in step 2. The alternative interlayer mechanism via flip-flop events, none of which were observed in the present simulations, is probably associated with longer time scales. The extent of correlations between the locations of the resulting enantiomerically pure patches across the two leaflets of a bilayer will be investigated in future simulations involving larger bilayer patches. This correlation is probably weak prior to aggregation, considering that it is only mediated by tail—tail interactions. It is further suggested here that the patches of pure enantiomers within the individual layers could serve as nucleation seeds for the subsequent aggregation in step 3. Note that, in contrast to the four other steps of Figure 3, step 3 is at present not directly supported by experimental or/and simulation results and only represents an intuitively reasonable connection in the proposed phase-transition mechanism.

When formed in aqueous solutions, the CO phase always involves crystals of alternating *R* and *S* layers.[6d,e] However, enantiomerically pure *R* and *S* crystals can also be formed in nonaqueous solvents.[11] It is therefore unclear which of the two crystal forms is thermodynamically more stable. Consequently, if simulation and experimental results both support preferential intralayer (lateral) interactions between identical enantiomers,

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the differential nature of the headgroup—headgroup interactions between bilayers within a crystal remains unclear. The fact that chiral recognition is enhanced in the GL phase may also have biological and evolutionary implications in the context of membrane rafts, which are known to exhibit GL-phase microdomains.[12] The presence of these microdomains may potentially facilitate both chemical and chiral recognition, compared to the dominant LC component of the membrane. In other words, the role of rafts might be connected not only to a high density of functional proteins but also to a facilitation of chiral recognition. As a final remark, although the correlation between phase transition and enantiomeric segregation in simple lipids is compatible with a lipid-based scenario for the origin of homochirality in prebiotic evolution, by no means does it provide a confirmation for such a scenario. The lipid-based hypothesis is itself only one possible hypothesis; alternative scenarios involve proteins, nucleic acids, or carbohydrates as primary components.[13]

ASSOCIATED CONTENT

Supporting Information. Methodological details and additional analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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