THE JOURNAL OF PHYSICAL CHEMISTRY B
A JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/JPCB

Article

Binding of Dipeptides to Fatty Acid Membranes Explains Their Colocalization in Protocells but Does Not Select for Them Relative to Unjoined Amino Acids

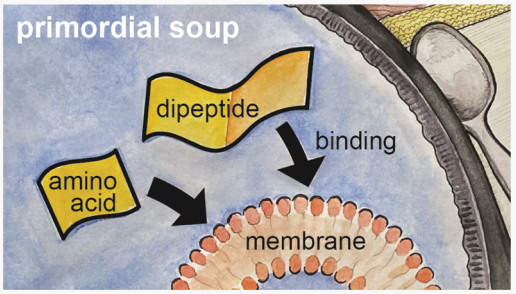
Mengjun Xue, Roy A. Black,* Zachary R. Cohen, Adrienne Roehrich, Gary P. Drobny, and Sarah L. Keller

Cite This: *J. Phys. Chem. B* 2021, 125, 7933−7939

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Dipeptides, which consist of two amino acids joined by a peptide bond, have been shown to have catalytic functions. This observation leads to fundamental questions relevant to the origin of life. How could peptides have become colocalized with the first protocells? Which structural features would have determined the association of amino acids and peptides with membranes? Could the association of dipeptides with protocell membranes have driven molecular evolution, favoring dipeptides over individual amino acids? Using pulsed-field gradient nuclear magnetic resonance, we find that several prebiotic amino acids and dipeptides bind to prebiotic membranes. For amino acids, the side chains and carboxylate contribute to the interaction. For dipeptides, the extent of binding is generally less than that of the constituent amino acids, implying that other mechanisms would be necessary to drive molecular evolution. Nevertheless, our results are consistent with a scheme in which the building blocks of the biological polymers colocalized with protocells prior to the emergence of RNA and proteins.



INTRODUCTION

The first protocells are commonly thought to have sequestered RNA, peptides (short chains of amino acids), and possibly proteins (longer chains of amino acids) in an aqueous space bounded by a membrane. Dipeptides, two amino acids joined by an amide bond, may have been among the first biological catalysts. Examples of dipeptide-catalyzed reactions relevant to the evolution of cells include the formation of sugars from glycolaldehyde[1] and the condensation of activated RNA monomers.[2] A dipeptide can also increase the rate of lipid incorporation into membranes at the expense of membranes lacking the dipeptide.[3]

The observation that very simple molecules like dipeptides can have catalytic functions leads to fundamental questions in the origin of life. How could peptides have become colocalized with the first protocells on the early Earth, and which structural features of peptides influence this association? Moreover, could the association of dipeptides with protocells have driven molecular evolution, favoring dipeptides over individual amino acids? Here, we quantify the association in terms of binding of dipeptides to the protocell's membrane. Specifically, we used pulsed-field gradient nuclear magnetic resonance (PFG-NMR) to determine (1) whether dipeptides bind to fatty acid membranes, (2) which structural moieties of amino acids and dipeptides are important for binding, and (3) how binding of dipeptides compares to the binding of individual amino acids.

Throughout, we use molecules that are prebiotically plausible. To form membranes, we use decanoic acid, a 10-carbon fatty acid which is found in meteorites[4] and is produced by various possibly prebiotic pathways.[5] Longer fatty acids produce more stable membranes but are less abundant in meteorites and therefore less plausible components of early membranes. The actual composition of the first membranes is unknown. In aqueous solutions over a pH range of ∼6.5 to 8, decanoic acid molecules self-assemble into membranes.[6] Membranes that encapsulate an aqueous solution are known as vesicles (Figure S1). Vesicles made from fatty acids can be tens of microns in diameter and allow the passage of small solutes across the membrane.[7]

A total of 10 of the amino acids found in modern proteins are regarded as prebiotically plausible.[8−12] Here, we investigate unmodified prebiotic amino acids rather than structures that have been modified to achieve specific chemical reactions. Several non-prebiotic amino acids and peptides have been previously shown to interact with fatty acid vesicles.[3,13−16]

Received: February 17, 2021
Revised: July 2, 2021
Published: July 20, 2021

Prebiotic peptides have been found on meteorites[17] and can be generated abiotically from (1) simple gases in Miller−Urey spark-discharge experiments,[18] (2) amino acids either at high temperature[19] or in the presence of prebiotic condensing agents,[20,21] and (3) aminonitriles.[22] Therefore, peptides would have been available on the early Earth, where they could plausibly have carried out some of the functions that were later assumed by proteins, including catalysis.

EXPERIMENTAL SECTION

Materials. Chemicals were used as purchased, without further purification. Sodium chloride (99.9% purity) was from Thermo Fisher Scientific (Waltham, MA), decanoic acid was from Nu-Chek Prep (Elysian, MN), and both D_2O and deuterated decanoic acid were from Cambridge Isotope Laboratories (Andover, MA). All other chemicals were from Sigma (St. Louis, MO) or Bachem (Torrance, CA). All chiral amino acids and dipeptides were in the L configuration except where indicated otherwise.

Solution Preparation. All samples were prepared in 50 mM sodium phosphate and 50 mM NaCl, with or without 50 mM deuterated decanoic acid, in D_2O at pD 7.0 and room temperature. Typically, these solutions were added to enough of a powder of a test compound (an amino acid or peptide) to yield a 10 mM solution. The compound was then ground briefly to facilitate dissolution, and the solution was vortexed twice for 3 s. The only exceptions were for glutamate and its dipeptide Glu−Glu, which are insoluble when added directly to a neutral aqueous solution. In these cases, a 1 M solution (in D_2O) of glutamic acid or Glu−Glu in 50 mM sodium phosphate and ∼750 mM NaOD was titered down to ∼pD 7, and the solution was then diluted 1:100 into a solution of 50 mM phosphate and 50 mM NaCl, with or without 50 mM deuterated decanoic acid. Throughout, we use "decanoic acid" to refer to mixtures of the protonated and unprotonated molecules. Decanoic acid forms vesicles in aqueous solutions over a pH range from roughly 6.5 to 8. These solutions contain a mixture of vesicles, micelles, and individual fatty acids (Figure S1).[6] To more closely mimic prebiotic conditions, we did not artificially constrain the size of vesicles by extruding them. Vesicles also form in solutions made with D_2O instead of H_2O, as seen Figure 1 of ref 24.

Pulsed-Field Gradient Nuclear Magnetic Resonance. Experiments were performed essentially as previously described[24] on an Avance 500 MHz NMR instrument (Bruker, San Jose, CA) with a Bruker 5 mm triple broadband inverse probe at 25 °C. The "stebpgp1s19" pulse sequence[24−27] was applied to determine each compound's translational diffusion coefficient (D), which was found via eq 1 below

$$\ln\left(\frac{I}{I_0}\right) = -\gamma^2 G^2 \delta^2 D_{\text{free}}\left(\Delta - \frac{\delta}{3}\right) \quad (1)$$

In eq 1, G is the gradient strength, which varied from 10 to 95% of the maximum strength (56.3 G/cm) for compounds in the presence of decanoic acid and from 10 to 60% of the maximum strength for compounds in the absence of decanoic acid. The variable I is the observed ^{1}H NMR peak intensity (the full peak area, determined from Bruker dynamic center software) corresponding to each value of G. I_0 is extracted from the Kärger model (as shown in the Supporting Information). In Figure 1, I_0 is the intensity at the initial G value that is measured, whereas in Figures S9−S15, I_0 is the intensity when G is 0. γ is the gyromagnetic ratio of ^{1}H (4257.64 Hz/G), δ is the length of the gradient pulse (set to 0.002 s), and Δ is the diffusion time (set to 0.3, 0.6, and 0.9 s, with uncertainties likely in the μs range). PFG-NMR experiments produced a series of one-dimensional ^{1}H spectra, which are shown in Figures S6−S8. For molecules that were free in solution, fast diffusion coefficients (D_{free}) were extracted by fitting the signal intensity decay with increasing gradient strengths to eq 1. NMR data were processed with TopSpin 4.0.8 software (Bruker), and NMR peak intensities were extracted using Bruker dynamics center software. Peak intensities were exported to OriginPro (OriginLab, Northampton, MA), which fit eq 1 and generated uncertainties of the fit.

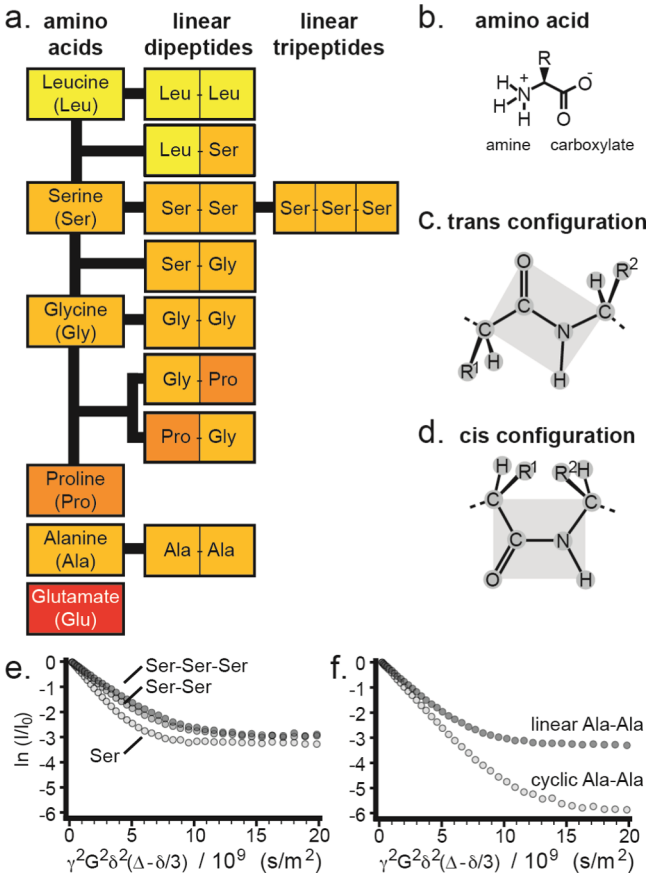


Figure 1. Amino acids, peptides, and typical PFG-NMR data. (a) Peptides contained combinations of five different amino acids. Glutamate is the conjugate base of glutamic acid. The color of each amino acid corresponds to the hydrophobicity scale of Wimley and White[23] (see Figure 2). (b) Each amino acid has an amine at one end and a carboxylate at the other end. (c) When assembled in a peptide, bulky side chains (labeled R^1 and R^2) tend to orient on opposite sides of the peptide in a trans configuration. The rectangular area shown in light gray, the peptide bond, is a rigid planar structure. (d) Bulky side chains are sterically hindered in the cis configuration. (e,f) Two slopes, corresponding to two diffusion coefficients, are shown in plots of the normalized intensity of an NMR spectral peak $[\ln(I/I_0)]$ vs the NMR parameter $[\gamma^2G^2\delta^2(\Delta - \delta/3)]$; each variable is defined in the Experimental Section. In (e), the compounds are serine as a single amino acid, a dipeptide, and a tripeptide, with NMR peaks analyzed at 3.77, 3.92, and 4.02 ppm, respectively. In (f), the compounds are a linear dipeptide and a cyclic dipeptide of alanine with NMR peaks analyzed at 1.47 and 1.39 ppm, respectively. All compounds were in a D_2O solution of 50 mM deuterated decanoic acid.

https://doi.org/10.1021/acs.jpcb.1c01485
J. Phys. Chem. B 2021, 125, 7933−7939

We used WATERGATE solvent suppression to increase the signal-to-noise ratio. We set the transmitter frequency at 4.7 ppm, d_1 = 15 s, and d_{19} = 0.0002 s; any peaks at frequencies 1/(2 × 0.0002 s) = 2500 Hz away from the transmitter were suppressed. This procedure did not distort our measurements: we showed previously[24] that the diffusion coefficient of adenine measured with WATERGATE is 6.58 ± 0.01 × 10^{-10} m^2/s, and without it, the diffusion coefficient is 6.66 ± 0.03 × 10^{-10} m^2/s.

Calculation of Percent Bound (f_{bound}). The percent of each amino acid and peptide that bound to decanoic acid vesicles was calculated as previously described:[24] the appearance of two slopes in PFG-NMR plots of $\ln(I/I_0)$ versus G^2 indicates slow exchange between two populations of a compound. One population is free in solution and diffuses quickly. The other is bound to fatty acid vesicles. We used eqs 2 and 3 below (the "Simplified Kärger model") to calculate the fast and slow diffusion coefficients (D_{free} and D_{bound}) and the percent bound (f_{bound}). Graphs in the main text refer to f_{bound} at Δ = 0.3 s.

$$\frac{I}{I_0} = f_{free}\exp\left[-\gamma^2G^2\delta^2D_{free}\left(\Delta - \frac{\delta}{3}\right)\right] + f_{bound}\exp\left[-\gamma^2G^2\delta^2D_{bound}\left(\Delta - \frac{\delta}{3}\right)\right] \quad (2)$$

$$f_{free} + f_{bound} = 1 \quad (3)$$

For some (but not all) compounds, diffusion coefficients, the fraction bound, and residence times can be calculated from the full Kärger model using equations shown in the Supporting Information.

Diffusion coefficients D_{bound} can be so small (on the order of 10^{-12} m^2/s) that the fit can yield non-physical, negative values (Table S1), which indicates that the true value of D_{bound} is better represented as $\leq 10^{-11}$ m^2/s. For experiments that were repeated, the uncertainty in f_{bound} is the error of the mean of the independent experiments. The largest magnitude of these uncertainties corresponds to 9.4% of the mean value (Table S1). For experiments that were not repeated, the experimental uncertainty is assumed to be 9.4% of the mean value. Values of f_{bound} in this paper cannot be directly compared to previously published values[28] of f_{bound} because of differences in experimental procedures: for example, amino acid concentrations and the instrument used differ in the two papers. Raw NMR data are found in Table S2. Diffusion coefficients of amino acids and peptides in solutions without decanoic acid are shown in Table S3.

The presence of a second, shallow slope in plots of $\ln(I/I_0)$ versus G^2 is due to binding rather than to encapsulation of amino acids (and peptides) because the vesicles are sufficiently large that diffusion of encapsulated compounds would not be significantly lower than diffusion of free compounds.[24,28] Moreover, we observe (both here and in previous work[24]) order-of-magnitude differences in the diffusion constants of many different small molecules, which would not be the case if the shallow slopes arose due merely to encapsulation of those molecules in vesicles. In separate controls, we have shown that amino acids (specifically leucine, glycine, and serine) diffuse freely (i.e., show a single, steep slope in plots of $\ln(I/I_0)$ vs G^2) in solutions that lack decanoic acid.[28] Any decrease in diffusion rate due to partial deuteration would be too small to detect in our system.

As further controls, we confirmed that binding of an amino acid varies with trends we would predict with changes in decanoic acid concentration and solution pH. First, we determined the extent of alanine binding in 20 mM decanoic acid—slightly above the critical vesicle concentration—instead of 50 mM. As with 50 mM decanoic acid, we observed two slopes in the plot of the normalized intensity of the spectral peak $[\ln(I/I_0)]$ vs $[\gamma^2G^2\delta^2(\Delta - \delta/3)]$, but the percent bound was only 0.4% versus the 4.8% seen with the higher decanoic concentration. Next, we measured binding at pH 8—above the range in which vesicles form. As expected, we observed no second slope that would have indicated binding of alanine, and the diffusion constant of unbound alanine was only slightly slower than that seen with no decanoic acid present. Data from these control experiments are shown in Figures S12 and S13 and Table S4. We also tested the validity of the PFG-NMR-based binding results by repeating three key comparisons using a filtration procedure.[28] As found with PFG-NMR, in all three cases, the dipeptide bound less than or about the same as the unjoined amino acid (Table S5).

RESULTS AND DISCUSSION

Could prebiotic membranes have plausibly played a role in increasing the complexity of molecules in protocells by selecting for peptides relative to unjoined amino acids? Our experiments address fundamental questions of (1) whether prebiotic dipeptides bind to prebiotic membranes, (2) if so, whether they bind more strongly than their constituent amino acids, and (3) which chemical groups of amino acids and dipeptides might be responsible for the binding.

We constructed a panel of dipeptides from a group of amino acids that are all prebiotically feasible.[8−11] These amino acids have a range of side-chain structures and hydrophobicities (Figures 1, S2−S5, and 2). Leucine has a hydrophobic side

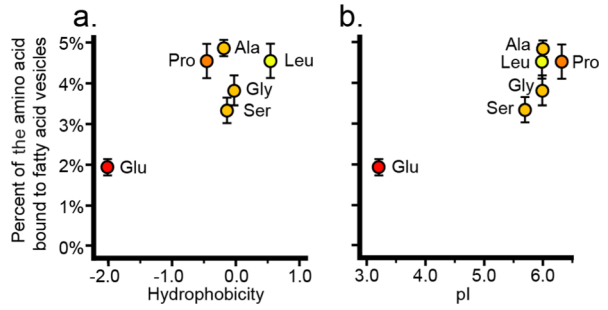


Figure 2. Percent of amino acid bound to decanoic acid vesicles vs the amino acid's (a) hydrophobicity (the free energy of transferring a peptide containing the amino acid from the surface of a lipid bilayer to bulk water, in kcal/mol)[23] and (b) pI of an individual amino acid.[30] Symbols are color-coded from least hydrophobic (red, Glu) to most hydrophobic (yellow, Leu).

chain, whereas glycine has no side chain. Proline is of interest because it imparts a bend in protein structures. Serine increases the number of lamellae in decanoic acid vesicles.[28] Glutamate is negatively charged.

Using PFG-NMR, we found that every amino acid and dipeptide in our panel binds to decanoic acid membranes. Specifically, for every compound, NMR peak intensities decay with two exponential functions. When these intensities are plotted as in Figures 1e,f and S9−S11, two slopes appear. The steep slope represents molecules that diffuse freely (and

https://doi.org/10.1021/acs.jpcb.1c01485
J. Phys. Chem. B 2021, 125, 7933−7939

quickly) in solution. The shallow slope represents molecules that diffuse more slowly because they bind to the surface of decanoic acid vesicles, as explained in the Experimental Section.[24,29]

We used the percent of each compound that binds to the membrane (Table S1) as an indicator of the strength of each compound's interaction with the membrane. Among all the amino acids tested, glutamate is an outlier (Figures 2 and 3). It

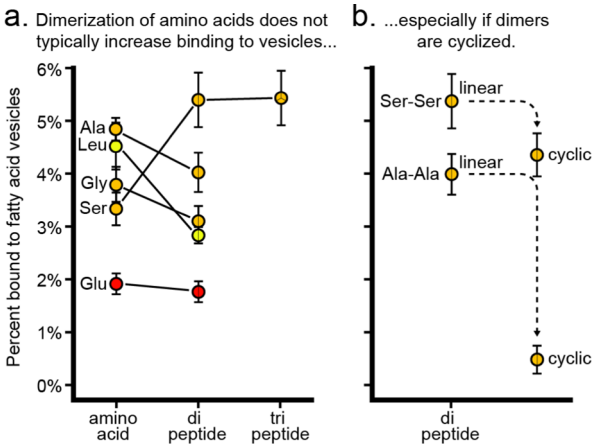


Figure 3. (a) Percent of amino acids, homo-dipeptides, and homo-tripeptides bound to decanoic acid vesicles. (b) Percent of alanine dipeptide that binds decreases significantly when the linear form is converted to the cyclic form (specifically, the diketopiperazine of alanine). Colors correspond to the hydrophobicity scale in Figure 2.

is negatively charged, and it binds the least to the decanoic acid membranes, which also presumably carry negative charges. The negative charge on glutamate's side chain is reflected in its low hydrophobicity[23] and low isoelectric point (pI, Figure 2). All other amino acids are more hydrophobic than glutamate, and all bind better. The trend in binding strength seen in Figure 3 is similar to that seen in our previously published filtration-based study: here, alanine and leucine showed ~3% depletion from the filtrate, while serine and glycine showed ~1.5−2% depletion (after subtraction of the background values).

A correlation has been previously reported between hydrophobicity and permeation of amino acids through fatty acid membranes.[31]

It is difficult to extrapolate from binding data for individual amino acids to predict the strength of binding of dipeptides to membranes. Only one of the five homo-dipeptides binds more strongly than their constituent amino acids (Figures 3 and 4). Hydrophobicity alone does not predict the binding of peptides, especially given that dipeptides tend to be more hydrophobic than individual amino acids.

Steric hindrance may explain some of the data. The most hydrophilic amino acid in our set is glutamate. The doubly negatively charged dipeptide of Glu−Glu might have been expected to bind very poorly to membranes, but it binds as good as the glutamate monomer. In a Glu−Glu dipeptide, the two side chains are sterically hindered from residing on the same side of the peptide (the cis configuration in Figures 1 and S4). Therefore, if a Glu−Glu peptide binds at a membrane interface, at least one of the hydrophilic side chains must be exposed to the aqueous solvent, a favorable interaction. Similarly, our most hydrophobic amino acid is Leu. If a Leu−Leu peptide binds at a membrane interface, one

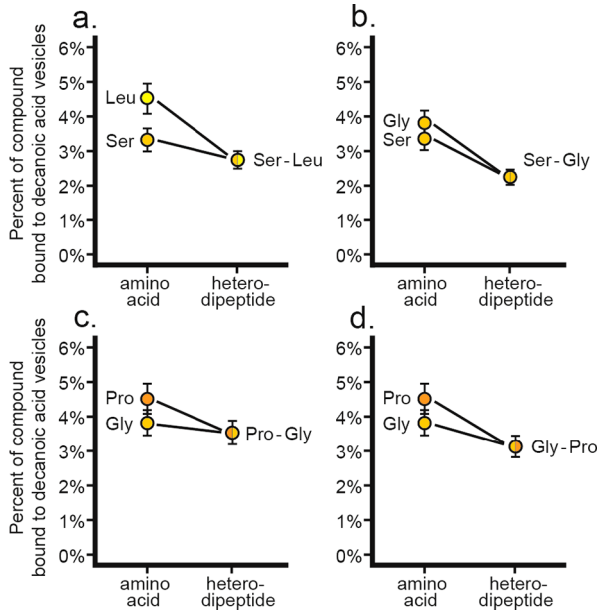


Figure 4. Percent binding to decanoic acid vesicles for the hetero-dipeptides of (a) Ser−Leu, (b) Ser−Gly, (c) Pro−Gly, and (d) Gly−Pro. (c,d) differ only in the order of the amino acids, and the resulting dipeptides have equivalent binding to the decanoic acid membranes, within experimental uncertainty. Colors correspond to the hydrophobicity scale in Figure 2.

hydrophobic side chain can be buried in the membrane's hydrophobic interior, but the other must be exposed to the aqueous solvent, an unfavorable interaction. We find that the dipeptide Leu−Leu binds weakly to decanoic acid membranes, roughly half as good as the Leu monomer. However, steric hindrance fails to explain other parts of Figures 3 and 4. Glycine has no side chain, so we might expect Gly−Gly to bind better than glycine alone if overall hydrophobicity were beneficial, but it does not. Similarly, we might expect a heterodimer of glycine and a hydrophobic amino acid to bind better than its constituent amino acids, but Pro−Gly and Gly−Pro defy this prediction.

The only case in which we observed that oligomerization increased binding was for serine: the dipeptide Ser−Ser (and the tripeptide Ser−Ser−Ser) binds better than serine alone. This exception is not explained by hydrophobicity because serine is as hydrophobic as alanine and glycine, according to the scale by Wimley and White[23] in Figure 2. One possible explanation is that the hydroxyl group on serine's side chain hydrogen-bonds with the aqueous solvent. However, this favorable interaction should result in a greater (or at least equal) binding of Ser−Leu or Ser−Gly compared to the individual amino acids Leu and Gly, which is not observed (Figure 4). The different outcome with Ser−Ser could be due to hydrogen bonding by the other serine hydroxyl group to a decanoic acid carboxylate.

Because hydrophobicity, steric considerations, and hydrogen bonding do not fully explain why it is common for dimers to bind to vesicles less well than amino acids do, we considered the amine, the carboxylate, and the peptide bond of dimers. In one set of experiments, we measured the binding of cyclic peptides, which possess neither an amine nor a carboxylate (Figure S5). Cyclization causes the binding of Ser−Ser to decrease and of Ala−Ala to plummet (Figure 3b), suggesting that one or both ends of a linear peptide play a role in its

https://doi.org/10.1021/acs.jpcb.1c01485
J. Phys. Chem. B 2021, 125, 7933−7939

binding to membranes. In a second set of experiments, we tested the structural variants of Ala−Ala shown in Figure S5. In the first variant, the amine at one end of the molecule was altered through the addition of an acetyl group. In the second variant, the carboxyl group at the other end of the molecule was altered through amidation (the replacement of one oxygen with NH_2). Neither modification significantly reduced the percent of Ala−Ala bound to the membrane (Figure 5b),

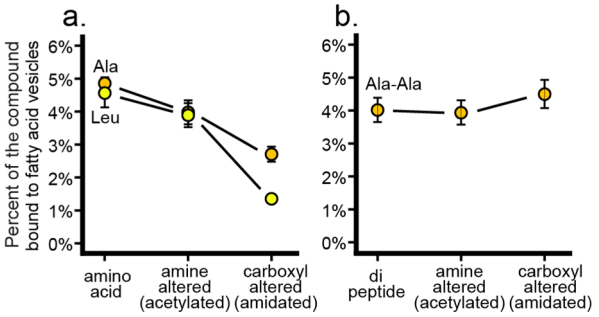


Figure 5. Percent binding to decanoic acid vesicles for (a) amino acids Leu and Ala, (b) dipeptide Ala−Ala, and their structural variants. In the first variant, an acetyl group was added to the amine. In the second variant, one of the oxygen atoms of the carboxylate group was replaced with NH_2. Colors correspond to the hydrophobicity scale in Figure 2.

suggesting an opposite conclusion that neither end of a linear peptide, on its own, plays a significant role in its binding to membranes. This result is perplexing given that amidation of the carboxyl group of single amino acids (Ala and Leu) causes a drastic reduction in the percent bound (Figure 5a). The role of the peptide bond is similarly unclear; it is not apparent why this bond would hinder binding of a dimer relative to an amino acid. We could speculate that the altered charge distribution, particularly the increased spacing between the amine and the carboxylate, contributes. In summary, the mechanism by which dipeptides bind to fatty acid membranes remains an open question.

To verify the consistency of values for percent binding, we performed two additional sets of experiments. Because we do not expect the chirality of an amino acid or dipeptide to affect its binding to fatty acids (which are achiral), we tested D-alanine and D,L-Ala−Ala and compared our results with those for alanine and Ala−Ala. As anticipated, we observed no significant difference between the stereoisomers in the percent bound to membranes (Figure 6a). Because the structures of α-amino-isobutyric acid (AIBA) and γ-amino-butyric acid (GABA) are similar to those of the amino acids alanine and glycine (and, to a lesser extent, leucine), we expect these compounds to exhibit similar binding to decanoic acid vesicles, which they do, within experimental uncertainty (Figure 6b).

All the results above focus on binding of compounds to decanoic acid membranes based on the presence of a second, shallow slope in the decay of NMR spectra. On its own, the steep slope also contains information, namely, the diffusion coefficient for the free movement of each compound that we tested. Here, we verify that these coefficients are consistent with physical expectations, which provides an internal check that our analysis is correct.

In Figure 1e, the steepness of the slope for freely diffusing serine is higher than that for diserine, which, in turn, is higher than that for triserine. This result follows the well-known result

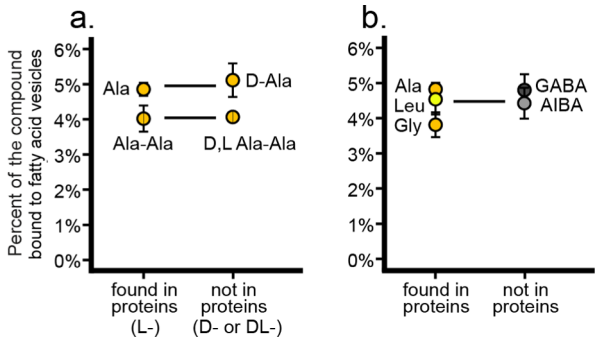


Figure 6. Percent binding to decanoic acid vesicles for (a) D-Ala, the stereoisomer of Ala, and D,L-Ala−Ala, a heterochiral version of Ala−Ala, and (b) two amino acids that are not found in proteins, AIBA and GABA. Colors correspond to the hydrophobicity scale in Figure 2.

that large objects diffuse more slowly in solution.[32,33] We quantify this relationship by taking ratios of diffusion coefficients, shown in Table 1. Because ratios are unaffected

Table 1. Ratios of Fast Diffusion Coefficients for Five Amino Acids and One Dipeptide[a]

	ratios of fast diffusion coefficients from four data sources			
	this work[a,c]	Cornell[28,a,d]	Longsworth[34,b,e]	Ma[35,b]
Gly/diglycine	1.35 ± 0.03		1.33	
Gly/Ala	1.20 ± 0.02		1.16	1.15[f], 1.17[g]
Gly/Pro	1.22 ± 0.02		1.20	
Gly/Ser	1.20 ± 0.02	1.21	1.20	1.20[f]
Gly/Leu	1.44 ± 0.03	1.47		
Ala/Ser	1.00 ± 0.02		1.03	1.05[f]

[a]Coefficients were determined by (a) PFG-NMR or (b) interferometric methods. The solute concentrations were (c) 10 mM, (d) 50 mM, (e) between 0.577 and 0.676 g solute per 100 g of solution, (f) about 0.1 M, and (g) about 0.3 M. Uncertainties in the first column are taken to be 2%, the standard error of the mean of fast diffusion values for the two Leu experiments in Table S1.

by the viscosity of the solution, they can be compared across experimental studies measured at different concentrations by different methods. We find excellent agreement between ratios of diffusion coefficients from Table S1, from Cornell et al., from Longsworth, and from Ma et al.[28,34,35]

We also considered whether dipeptides affect the structure of decanoic vesicles differently than unjoined amino acids do. We found no significant difference in the structures observed with Ser−Ser versus serine, Leu−Leu versus leucine, or Ala−Ala versus alanine (Figure S14). Cyclic Ala−Ala caused less multilamellarity than Ala−Ala and alanine. This difference does not appear to explain the lesser binding observed with the cyclic dipeptide, however, because we observed no correlation between lamellarity and binding in other comparisons (e.g., leucine vs serine). We further tested whether differences we observe in binding strength could be due to effects on vesicle formation by comparing the critical vesicle concentration in the presence of serine versus Ser−Ser and in the presence of Ala−Ala versus cyclic-Ala−Ala. In both sets, the critical vesicle concentrations were indistinguishable (Figure S15). Of course, fluorescence microscopy and measurements of critical vesicle concentration may overlook some subtle changes in the vesicle structure.

https://doi.org/10.1021/acs.jpcb.1c01485
J. Phys. Chem. B 2021, 125, 7933−7939

CONCLUSIONS

We find that unmodified dipeptides constructed from prebiotic amino acids do bind to prebiotic membranes. This binding supports a scheme for the development of early protocells in which membranes concentrated the building blocks of biological polymers (such as amino acids and short peptides) from the environment.[36] The strength of a dipeptide's binding to fatty acid vesicles is difficult to predict, even though the binding of individual amino acids follows expectations based on charge and hydrophobicity. Except for Ser−Ser, all the dipeptides tested bound less well than (or, at best, the same as) the constituent amino acids. As a result, membrane association on its own is not sufficient to drive molecular evolution from amino acids to dipeptides, en route to peptides long enough to have biological functions. Mechanisms by which a membrane could have driven selection for dipeptides over individual amino acids must therefore entail consequences of the binding, such as an increase in vesicle stability or rate of growth.[3]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.1c01485.

Equations of the Kärger model; structures of decanoic acid, decanoate, a micelle, and a vesicle; structures of the amino acids investigated; structures of modified amino acids investigated; structures of linear dipeptides investigated; structures of cyclic dipeptides, modified dipeptides, and a tripeptide; ^{1}H NMR spectra of 10 mM amino acids in decanoic acid vesicle solutions; ^{1}H NMR spectra of 10 mM linear dipeptides and tripeptides in decanoic acid solutions; ^{1}H NMR spectra of 10 mM cyclic dipeptides in decanoic acid solutions; echo decay curves {plots of $[\ln(I/I_0)]$ vs $\gamma^2G^2\delta^2(\Delta - \delta/3)$} from ^{1}H NMR spectra of 10 mM amino acids in decanoic acid solutions; echo decay curves {plots of $[\ln(I/I_0)]$ vs $\gamma^2G^2\delta^2(\Delta - \delta/3)$} from ^{1}H NMR spectra of 10 mM linear dipeptides and tripeptides in decanoic acid solutions; echo decay curves {plots of $[\ln(I/I_0)]$ vs $\gamma^2G^2\delta^2(\Delta - \delta/3)$} from ^{1}H NMR spectra of 10 mM cyclic dipeptides in decanoic acid solutions; echo decay curves with alanine at pH 8; echo decay curve with alanine in 20 mM decanoic acid; fluorescence micrographs of vesicles with various compounds; plots to determine critical vesicle concentrations; percent binding for amino acids and peptides; raw and analyzed NMR data; diffusion coefficients of amino acids and peptides in solutions without decanoic acid; PFG-NMR results at altered decanoic acid concentrations and at altered pH; and binding based on filtration of vesicles (PDF)

AUTHOR INFORMATION

Corresponding Author

Roy A. Black − *Department of Chemistry, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0002-3914-5747; Phone: 206-713-4603; Email: blackr5@uw.edu

Authors

Mengjun Xue − *Department of Chemistry, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0003-4832-4226

Zachary R. Cohen − *Department of Chemistry, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0002-1257-623X

Adrienne Roehrich − *Department of Chemistry, University of Washington, Seattle, Washington 98195, United States*

Gary P. Drobny − *Department of Chemistry, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0002-7293-1897

Sarah L. Keller − *Department of Chemistry, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0001-5919-0185

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jpcb.1c01485

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The Keller and Drobny laboratories were supported by NASA grant NNX17AK86G (Exobiology). G.P.D. acknowledges support from NSF MCB-1715123 and NIH RO1-GM109417. S.L.K. acknowledges support from NSF MCB-1402059 and MCB-1925731.

REFERENCES

(1) Pizzarello, S.; Weber, A. L. Stereoselective Syntheses of Pentose Sugars under Realistic Prebiotic Conditions. *Orig. Life Evol. Biosph.* **2010**, *40*, 3−10.

(2) Wieczorek, R.; Dörr, M.; Chotera, A.; Luisi, P. L.; Monnard, P.-A. Formation of RNA Phosphodiester Bond by Histidine-Containing Dipeptides. *ChemBioChem* **2013**, *14*, 217−223.

(3) Adamala, K.; Szostak, J. W. Competition between Model Protocells Driven by an Encapsulated Catalyst. *Nat. Chem.* **2013**, *5*, 495−501.

(4) Lawless, J. G.; Yuen, G. U. Quantification of Monocarboxylic Acids in the Murchison Carbonaceous Meteorite. *Nature* **1979**, *282*, 396−398.

(5) McCollom, T. M. Miller-Urey and Beyond: What Have We Learned About Prebiotic Organic Synthesis Reactions in the Past 60 Years? *Annu. Rev. Earth Planet. Sci.* **2013**, *41*, 207−229.

(6) Namani, T.; Walde, P. From Decanoate Micelles to Decanoic Acid/Dodecylbenzene-sulfonate Vesicles. *Langmuir* **2005**, *21*, 6210−6219.

(7) Chen, I. A.; Walde, P. From Self-Assembled Vesicles to Protocells. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a002170.

(8) Longo, L. M.; Blaber, M. Protein Design at the Interface of the Pre-Biotic and Biotic Worlds. *Arch. Biochem. Biophys.* **2012**, *526*, 16−21.

(9) Doi, N.; Kakukawa, K.; Oishi, Y.; Yanagawa, H. High Solubility of Random-Sequence Proteins Consisting of Five Kinds of Primitive Amino Acids. *Protein Eng. Des. Sel.* **2005**, *18*, 279−284.

(10) McDonald, G. D.; Storrie-Lombardi, M. C. Biochemical Constraints in a Protobiotic Earth Devoid of Basic Amino Acids: The "Baa(-) World". *Astrobiology* **2010**, *10*, 989−1000.

(11) Cleaves, H. J., II The Origin of the Biologically Coded Amino Acids. *J. Theor. Biol.* **2010**, *263*, 490−498.

(12) Patel, B. H.; Percivalle, C.; Ritson, D. J.; Duffy, C. D.; Sutherland, J. D. Common origins of RNA, protein, and lipid precursors in a cyanosulfidic protometabolism. *Nat. Chem.* **2015**, *7*, 301−307.

(13) Murillo-Sánchez, S.; Beaufils, D.; González Mañas, J. M.; Pascal, R.; Ruiz-Mirazo, K. Fatty Acids' Double Role in the Prebiotic

Formation of a Hydrophobic Dipeptide. *Chem. Sci.* **2016**, *7*, 3406−3413.
(14) Kamat, N. P.; Tobé, S.; Hill, I. T.; Szostak, J. W. Electrostatic Localization of RNA to Protocell Membranes by Cationic Hydrophobic Peptides. *Angew. Chem., Int. Ed.* **2015**, *127*, 11901−11905.
(15) Blocher, M.; Liu, D.; Walde, P.; Luisi, P. L. Liposome-Assisted Selective Polycondensation of Alpha-Amino Acids and Peptides. *Macromolecules* **1999**, *32*, 7332−7334.
(16) Mayer, C.; Schreiber, U.; Dávila, M.; Schmitz, O.; Bronja, A.; Meyer, M.; Klein, J.; Meckelmann, S. Molecular Evolution in a Peptide-Vesicle System. *Life* **2018**, *8*, 16.
(17) Shimoyama, A.; Ogasawara, R. Dipeptides and Diketopiperazines in the Yamato-791198 and Murchison Carbonaceous Chondrites. *Orig. Life Evol. Biosph.* **2002**, *32*, 165−179.
(18) Parker, E. T.; Zhou, M.; Burton, A. S.; Glavin, D. P.; Dworkin, J. P.; Krishnamurthy, R.; Fernández, F. M.; Bada, J. L. A Plausible Simultaneous Synthesis of Amino Acids and Simple Peptides on the Primordial Earth. *Angew. Chem.* **2014**, *126*, 8270−8274.
(19) Rodriguez-Garcia, M.; Surman, A. J.; Cooper, G. J. T.; Suárez-Marina, I.; Hosni, Z.; Lee, M. P.; Cronin, L. Formation of Oligopeptides in High Yield under Simple Programmable Conditions. *Nat. Commun.* **2015**, *6*, 8385−8392.
(20) Frenkel-Pinter, M.; Samanta, M.; Ashkenasy, G.; Leman, L. J. Prebiotic Peptides: Molecular Hubs in the Origin of Life. *Chem. Rev.* **2020**, *120*, 4707−4765.
(21) Forsythe, J. G.; Yu, S.-S.; Mamajanov, I.; Grover, M. A.; Krishnamurthy, R.; Fernández, F. M.; Hud, N. V. Ester-Mediated Amide Bond Formation Driven by Wet-Dry Cycles: A Possible Path to Polypeptides on the Prebiotic Earth. *Angew. Chem., Int. Ed.* **2015**, *54*, 9871−9875.
(22) Canavelli, P.; Islam, S.; Powner, M. W. Peptide Ligation by Chemoselective Aminonitrile Coupling in Water. *Nature* **2019**, *571*, 546−549.
(23) Wimley, W. C.; White, S. H. Experimentally Determined Hydrophobicity Scale for Proteins at Membrane Interfaces. *Nat. Struct. Biol.* **1996**, *3*, 842−848.
(24) Xue, M.; Black, R. A.; Cornell, C. E.; Drobny, G. P.; Keller, S. L. A Step toward Molecular Evolution of RNA: Ribose Binds to Prebiotic Fatty Acid Membranes, and Nucleosides Bind Better Than Individual Bases Do. *ChemBioChem* **2020**, *21*, 2764−2767.
(25) Stejskal, E. O.; Tanner, J. E. Spin Diffusion Measurements: Spin Echoes in the Presence of a Time-Dependent Field Gradient. *J. Chem. Phys.* **1965**, *42*, 288−292.
(26) Tanner, J. E. Use of the Stimulated Echo in NMR Diffusion Studies. *J. Chem. Phys.* **1970**, *52*, 2523−2526.
(27) Cohen, Y.; Avram, L.; Frish, L. Diffusion NMR Spectroscopy in Supramolecular and Combinatorial Chemistry: An Old Parameter−New Insights. *Angew. Chem., Int. Ed.* **2005**, *44*, 520−554.
(28) Cornell, C. E.; Black, R. A.; Xue, M.; Litz, H. E.; Ramsay, A.; Gordon, M.; Mileant, A.; Cohen, Z. R.; Williams, J. A.; Lee, K. K.; Drobny, G. P.; Keller, S. L. Prebiotic Amino Acids Bind to and Stabilize Prebiotic Fatty Acid Membranes. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 17239−17244.
(29) Zhang, W.; Haman, K. J.; Metzger, J. M.; Hackel, B. J.; Bates, F. S.; Lodge, T. P. Quantifying Binding of Ethylene Oxide-Propylene Oxide Block Copolymers with Lipid Bilayers. *Langmuir* **2017**, *33*, 12624−12634.
(30) Weast, R. C. *CRC Handbook of Chemistry and Physics*; CRC Press: Boca Raton, FL, 1987.
(31) Kwiatkowski, W.; Bomba, R.; Afanasyev, P.; Boehringer, D.; Riek, R.; Greenwald, J. Prebiotic Peptide Synthesis and Spontaneous Amyloid Formation inside a Proto-Cellular Compartment. *Angew. Chem., Int. Ed.* **2021**, *60*, 5561.
(32) Einstein, A. Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. *Ann. Phys.* **1905**, *322*, 549−560.
(33) Sutherland, W. LXXV. A dynamical theory of diffusion for non-electrolytes and the molecular mass of albumin. *Phil. Mag.* **1905**, *9*, 781−785.
(34) Longsworth, L. G. Diffusion Measurements at 25° of Aqueous Solutions of Amino Acids, Peptides, and Sugars. *J. Am. Chem. Soc.* **1953**, *75*, 5705−5709.
(35) Ma, Y.; Zhu, C.; Ma, P.; Yu, K. T. Studies on the Diffusion Coefficients of Amino Acids in Aqueous Solutions. *J. Chem. Eng. Data* **2005**, *50*, 1192−1196.
(36) Black, R.; Blosser, M. A Self-Assembled Aggregate Composed of a Fatty Acid Membrane and the Building Blocks of Biological Polymers Provides a First Step in the Emergence of Protocells. *Life* **2016**, *6*, 33.

https://doi.org/10.1021/acs.jpcb.1c01485
J. Phys. Chem. B 2021, 125, 7933−7939