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Article in Colloids and surfaces B: Biointerfaces · October 2019
DOI: 10.1016/j.colsurfb.2019.110539

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Interactions of a short hyaluronan chain with a phospholipid membrane

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\textbf{ARTICLE INFO}

Keywords:
Hyaluronic acid
Phospholipids
Hydrogen bonds
Molecular dynamics simulations

\textbf{ABSTRACT}

Hyaluronic acid and phospholipids are two components that are present in the synovial fluid, and both are implicated as important facilitators of joint lubrication. In this work we aim to clarify how hyaluronic acid interacts with a phospholipid bilayer through their molecular interactions at the bilayer surface. To this end we performed molecular dynamics simulations of one hyaluronic acid molecule at a phospholipid bilayer in aqueous solution. The simulations were carried out for two aqueous solutions of equal concentrations, containing either NaCl or CaCl\(_2\). We analyzed hydrogen bonds, hydrophobic contacts and cation mediated bridges to clarify how hyaluronic acid binds to a phospholipid bilayer. The analysis shows that calcium ions promote longer lasting bonds between the species as they create calcium ion bridges between the carboxylate group of hyaluronic acid and the phosphate group of the phospholipid. This type of additional bonding does not significantly influence the total number of contact created, but rather stabilizes the contact. The presented results can facilitate understanding of the role of hyaluronic acid and phospholipid interactions in terms of lubrication of articular cartilage.

1. Introduction

Hyaluronic acid (HA), Fig. 1, is a physiologically ubiquitous biomacromolecule which has received high interest in recent decades. It is a linear polysaccharide comprised of alternating units of D-glucuronic acid and N-acetyl-D-glucosamine. It is in some cases found directly at the surface of phospholipid membranes [1]. The chains can be very long, reaching tens of thousands of units or millions of Daltons in weight. The pKa of the glucuronic acid is about 3 [2] and the pH of the synovial fluid is typically 7.6–7.8 [3]. Thus, in the joint area HA exists in anionic hyaluronan form. Their semi-rigidity and affinity for physical cross-linking gives HA its major physiological properties; for instance, gel-like networks of HA increase the viscosity in vitreous humor, provided structurally rigid in complexes with aggrecan (alongside collagen) in cartilage, contribute to lubrication in synovial fluid (SF), and modulate migration in the extracellular matrix [4]. It has also been adopted for various therapeutic and commercial purposes related to joints and skin [4].

The lubricative contributions of HA are of particular interest in biochemistry and biophysics. It’s anomalous viscoelastic properties in bulk have long been known [5]. Notably, it shows a transition from an elastic gel to a viscous liquid when placed under load. However, it is still not well understood how HA works in accord with other molecules to produce exceptionally low friction on the weight bearing surfaces of joint cartilage.

Early electron microscopy studies revealed multiple stratified phospholipid bilayer structures on such surfaces [6]. Further studies have shown a significantly thick (1500 nm) outermost layer comprised of phospholipids, proteins, proteoglycans, and notably free from the collagen in deeper layers [7,8]. Such a composition suggests the importance of interplay between phospholipids and biomacromolecules for mediating mechanical properties within joints, and the biomechanically conspicuous HA molecule is a natural choice for study.

In the larger context, longer chains of HA interact with, at minimum, lubricin and brush-like aggrecan to facilitate lubrication [9]. A lack of a convincing explanation for its performance, however, has spurred investigation into these molecules more basic interactions. Kreutzer et al. have reported HA locates between bilayers of DPMC [10]

\textbf{Abbreviations:} DPPC, dipalmitoylphosphatidylcholine; GCU, D-glucuronic acid; HA, hyaluronic acid; H–bonds, hydrogen bonds; NAG, N-acetyl-D-glucosamine; PL, phospholipid; RDF, radial density function; SF, synovial fluid

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https://doi.org/10.1016/j.colsurfb.2019.110539
Received 24 July 2019; Received in revised form 9 September 2019; Accepted 29 September 2019
Available online 01 October 2019
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and others have shown HA adsorbs to DPPC vesicles [11], but we also note a study showing no interactions between high molecular weight HA and phospholipids [12]. Recently we described the interaction of phospholipid molecules with chains of HA in confined geometry [13], however interactions between HA and phospholipid bilayers need further attention. In this study we use molecular dynamics simulations to examine the interaction between one HA molecule with such a phospholipid surface (Fig. 2).

How molecules adsorb to surfaces has been extensively studied both experimentally and theoretically (see [14,15,37] for example). It has been observed that a large affinity between the polymer and the surface leads to an overall thinner adsorbed layer [16], which is expected to affect the response to load and shear. Additionally, recent studies on long chain hyaluronic acid have shown that it does not adhere to membranes for certain preparations [10]. Perhaps more pertinent are the findings from Wieland et al. showing decreasing effect on DPPC Langmuir layers with increasing HA molecular weight [17]. This is presumably related to the difficulty of shorter chains folding back on themselves and forming intramolecular as opposed to intermolecular bonds. Investigation of dynamic effects for such long chain networks are beyond the scope of all-atom molecular dynamics. Instead, we examine the adsorption of a relatively short chain HA to a phospholipid bilayer in contact with aqueous solutions containing either sodium or calcium ions.

HA interacts, e.g. with phospholipids, through its constituent polar (oxygen and nitrogen) and non-polar (hydrocarbon) parts. The relative radial distributions of these oxygen molecules are presented to examine the binding tendencies between the molecules. Hydrogen bonds (H–bonds), hydrophobic contacts and calcium ion bridges are analyzed to characterize the adsorption. As the importance of hydration for lubrication has been proposed [18,19], and the radial density distribution for the oxygen atoms in water has been included. There is also considerable research showing ion specific (Hoffmeister) effects on bilayers alone [20–23]. In light of these, we are also careful to contrast the results obtained in presence of sodium and calcium ions.

2. Methods

All atom molecular dynamics simulations were performed using the AMBER03 force field [24] to evaluate interactions between one HA molecule and a dipalmitoylphosphatidylcholine, DPPC, bilayer. We used the AMBER03 potential function as in our previous work [25]. The HA structure was downloaded from PubChem [26] and modified to obtain longer chains by using the YASARA Structure Software. The final molecular mass of HA considered in this study was 3 kDa. The DPPC bilayer was taken from [27]. This DPPC bilayer contains 72 lipids and it was then multiplied by 4 to obtain a larger structure which would allow a large contact area between HA and the bilayer without forcing the HA to bend. The bilayer was simulated for 5 ns to allow the 4 bilayer parts to assemble into one continuous structure. After this the HA molecule was placed next to the bilayer without influencing the bilayer structure. Periodic boundary conditions were applied to create an “infinite” bilayer. The TIP3P water model was used [28]. For the isobaric-isothermal ensemble, all atom simulations were performed under the same conditions: temperature 310 K (physiological), pH = 7.0. The time step was set to 2 fs. Two situations were studied: i) HA at the bilayer in contact with an aqueous solution of NaCl, ii) HA at the bilayer in contact with CaCl2 aqueous solution. The simulation box contained water, one HA molecule, a DPPC bilayer, 56 cations and the corresponding number of anions to achieve electroneutrality. Simulations were carried out for 40 ns to collect the data from the equilibrium state of the system, which was reached after approximately 20 ns. The Berendsen barostat [29] and thermostat with a relaxation time of 1 fs were
used to maintain constant pressure and temperature.

From the molecular dynamics simulations we evaluate the number of H–bonds formed. These contacts are presented in form of contact maps showing how often a particular pair forms an H–bond. Moreover,
as water plays an important role for interactions in aqueous media we also present the water distribution near both molecules of interest. The distribution of calcium and sodium ions from the HA and DPPC molecules are also presented to clarify their role in the interactions.

2.1. Radial distribution function

The radial distribution function (or RDF), \( g(r) \), is an example of a pair correlation function, which describes how, on average, the atoms in a system are radially packed around each other.

\[
    g(r) = \frac{n(r)}{(\rho 4\pi r^2 dr)} \\
\]

In which \( n(r) \) is the mean number of atoms in a shell of width \( \Delta r \) at distance \( r \) and \( \rho \) is the mean atom density. The method is not restricted to one atom - all atoms in the system can be considered, leading to an improved determination of the RDF as an average over many atoms.

2.2. Hydrogen bond identification

We utilized the YASARA definition of a hydrogen bond, where it is regarded to be formed when the hydrogen bond energy is greater than 6.25 kJ/mol, which is 25% of the optimum value of 25 kJ/mol [30]. The following formula yields the bond energy in kJ/mol as a function of the Hydrogen-Acceptor distance and two scaling factors:

\[
    E_{HB} = 25 \times \frac{2.6 - \max(Dist_{HA} - 2.1, 0)}{0.5} \times \text{Scale}_{HA} \times \text{Scale}_{HA-X} \\
\]

where the first scaling factor depends on the angle formed by Donor-Hydrogen-Acceptor, and the second scaling factor is derived from the angle formed by Hydrogen-Acceptor-X, where the X stands for the atom covalently bound to the acceptor.

Both scaling factors change linearly from 0 to 1 as follows

\[
    S(\alpha) = \frac{\dot{\alpha}_2 - \alpha}{\dot{\alpha}_2 - \dot{\alpha}_1} \\
\]

Where \( \alpha \) is an angle between \( \dot{\alpha}_1 \) and \( \dot{\alpha}_2 \). If \( \alpha < \dot{\alpha}_1 \) or \( \alpha > \dot{\alpha}_2 \) scaling factors take the following values:

\[
    \text{Scale}_{HA} = \begin{cases} 
    0 & \text{in range 0} \leq \dot{\alpha}_1 = 100 \text{ degrees} \\
    1 & \text{in range } \dot{\alpha}_2 = 165 \text{ to } 180 \text{ degrees} 
    \end{cases} \\
\]

if X is a heavy atom, the second scaling factor is

\[
    \text{Scale}_{HA-X} = \begin{cases} 
    0 & \text{in range 0} \leq \dot{\alpha}_1 = 85 \text{ degrees} \\
    1 & \text{in range } \dot{\alpha}_2 = 95 \text{ to } 180 \text{ degrees} 
    \end{cases} \\
\]

if X is a hydrogen, slightly smaller angles are allowed, and the scaling factor then is

\[
    \text{Scale}_{HA-X} = \begin{cases} 
    0 & \text{in range 0} \leq \dot{\alpha}_1 = 75 \text{ degrees} \\
    1 & \text{in range } \dot{\alpha}_2 = 85 \text{ to } 180 \text{ degrees} 
    \end{cases} \\
\]

2.3. Hydrophobic contacts

In aqueous solution, non-polar groups of atoms tend to aggregate, since this reduces the number of water molecules that must participate in an ordered (and entropically unfavorable) dynamic cage structure at the interface. This water-mediated ‘hydrophobic effect’ can be interpreted as an attractive ‘hydrophobic interaction’ between non-polar groups. The hydrophobic interaction strength between hydrophobic atoms is calculated as follows. Hydrophobic atoms are identified and assigned an atom type from the groups depicted as type 1 for carbon atoms with three or more bound hydrogen atoms (\(-\text{CH}_3\)) and type 2 for carbon atoms with two hydrogen (\(-\text{CH}_2\)) (Fig. 3).

The values of \( d_{\text{min}}, d_{\text{opt}}, \) and \( d_{\text{max}} \) for all types of interacting
hydrophobic atoms are provided in Table 1. The specific contribution to the hydrophobic interaction for any pair, \( h_{ij} \), depends on the interatomic distance, \( d_{ij} \), as follows:

\[
h_{ij} = \begin{cases} 
\frac{d_{ij} - d_{\text{min}}}{d_{\text{opt}} - d_{\text{min}}} & \text{if } d_{ij} \in (d_{\text{min}}, d_{\text{opt}}) \\
1 & \text{if } d_{ij} = d_{\text{opt}} \\
\frac{d_{\text{max}} - d_{ij}}{d_{\text{max}} - d_{\text{opt}}} & \text{if } d_{ij} \in (d_{\text{opt}}, d_{\text{max}})
\end{cases}
\]

3. Results and discussion

The initial and final structure of the studied molecular assemblies are presented as simulation snapshots in Fig. 4, and \( \text{H} \)-bonds are illustrated in Fig. 5. In both cases HA was adsorbed at the membrane surface throughout the simulation. All the distribution results are calculated over the plateau region i.e. between 20 and 40 ns. Fig. 6 shows how all HA oxygens and the nitrogen structure water around them. We note that atoms O5 and O6 in the GCU moiety and O8 and O10 in the NAG moiety attract the most water molecules (note that the RDF shows a radial distribution and averages over each \( \Delta r \) chunk, see Eq (1)), but HA excludes water in some angles due to Born repulsion (excluded volume effect). Clearly, the excluded volume effect is important since the oxygen atoms most distant from the sugar ring (2 bonds) also have most water around them (O5, O6, O10 and O11). We note that O11 also suffers shielding from a methyl group, reducing the RDF around this atom class even though it is far from the main chain. The O5 and O6 in the carboxylate group has the highest RDF, and the first peak in the RDF is located at the smallest distance. Thus, it is clear that this group interacts particularly efficiently with water.
3.1. Direct interactions between HA and DPPC

The number of hydrophobic contacts between HA and DPPC is shown in Fig. 7, and this quantity does not change systematically after 20 ns of simulation. We note that there is around 30–40% more hydrophobic contacts between HA and the DPPC bilayer in presence calcium ions as compared to a solution with sodium ions. This is also true for the number of hydrogen bonds contacts presented in Fig. 8. Thus, it is clear that calcium ions promote close contact between HA and DPPC bilayers, consistent with the experimental finding that the presence of calcium ions enhances the effect of HA on Langmuir layers of DPPC [17]. Fig. 9 shows the hydrogen bond map between HA and DPPC, and almost identical distributions are obtained in solutions containing sodium ions and calcium ions.

Clearly, the number of hydrophobic contacts between the HA molecule and the DPPC layer is significantly larger than the number of hydrogen bonds formed. Even though the energy of an H–bond is larger than that of a hydrophobic contact one has to consider that in order to form HA-DPPC H–bonds one has to break H–bonds with water. Thus, one cannot draw the conclusion that H–bond formation drives the adsorption. Rather, a view emerges that hydrophobic contacts are of paramount importance for the adsorption, while the role of H–bond formation by HA and DPPC is more akin to compensation for H–bonds lost with water due to adsorption. It is also clear that not all oxygen atoms in HA form H–bonds with the DPPC bilayer, but such bonds are preferentially formed by the hydroxyl oxygens HA-O1, HA-O2 and HA-O10, with some H–bonds also involving HA-O8 (hydroxyl group), HA-O3 (ether group) and HA-N (amide group). Thus, as expected, the hydroxyl oxygens that can act as donors in the hydrogen bond participate in most direct H–bonds. For DPPC most H–bonds to HA are formed by DPPC-O3 and DPPC-O4, which belong to the phosphate group. However, we also observe some H–bonds with DPPC-O6 and DPPC-O7.
DPPC-O8, located next to the acyl chains. This demonstrates that HA penetrates the head group region of the DPPC bilayer. Fig. 10 shows that the presence of calcium ions promote more HA-PL contacts (defined as the atoms of HA found at a distance ≤ 0.4 nm from atom DPPC-O3). A plot for DPPC-O4 shows similar tendencies. We note in particular that oxygen type DPPC-O3/O4 is closer to atom HA-O1, HA-O5 and HA-O6 (of the carboxylate group) in the calcium ion containing solution. This is an indication of formation of calcium ion bridges between the phosphate group of DPPC and the carboxylate group of HA, as discussed further below. In conclusion, we note that direct H-bonds are formed between HA and DPPC and that specific H-bonds between hydroxyl oxygen atoms of HA and the oxygen atoms in the phosphate group of DPPC are predominant. The presence of calcium ions brings the HA closer to the DPPC bilayer promoting formation of more contacts.

3.2. Role of water

Since there is a change in position of atom HA-O6 near the DPPC bilayer surface in presence of calcium ions we decided to look at how HA and PL atoms bind water depending on the presence of sodium and calcium ions. Figs. 11 and 12 show that in presence of calcium ions there is a smaller number (around 30%) of H-bonds formed between oxygen atoms of HA/DPPC and water as compared to sodium ions. However, the loss for the GCU moiety was ~35% as compared to ~25% for the NAG moiety. This further suggests that calcium ions promote closer contact between the oxygen atoms in the carboxylic moiety of GCU and the bilayer, and as a consequence the number of contacts with water decreases.

The contact between water and the nitrogen atom and the two tail oxygens (DPPC-O5 and DPPC-O7) in DPPC are, as expected, very limited due to shielding by the non-polar groups, see Fig. 4. Most water is found close to the oxygen atoms DPPC-O3 and DPPC-O4 in the phosphate group, and the type of ion present in solution does not change the water distribution in the head group region much.

3.3. Role of ions

Considering the data presented in Fig. 10 it seems plausible that calcium ions create cation bridges between HA-O5/6 and DPPC-O3 and HA-O4 atoms. Indeed, the snapshot shown in Fig. 13 shows the presence of such ion bridges. Figs. 14 and 15 show that calcium ions accumulate closely only to the above mentioned atoms. It appears that Ca2+ ions are accumulated close to the phosphate groups and whereby attracts HA-O5/6 atoms in the carboxylate group. This will contribute to more energetically favorable interactions between HA and DPPC, and likely promote stabilization of the HA/DPPC self-assembly structure.

4. Conclusion

Our simulations show that a short molecule of HA binds to the phospholipid bilayer, unmediated by proteins, in salt solutions. These results cannot necessarily be applied to physiological conditions with many HA molecules of much greater molecular weight. They do, however, highlight the importance of ion specificity in such systems. Additionally, they may aid in the development of coarse grained...
HA binds to the bilayer surface. The presence of calcium ions has a significant effect on the orientation of HA at the bilayer surface. The authors of [35] also show for the similarly zwitterionic DMPC bilayer the striking increase in ion uptake close to the phosphate group for Ca2+ compared to Na+ ions, as shown in Figs. 7 and 8, as well as the change in distribution distances reported in Fig. 10.

The resulting additional electric potential could help explain the dramatic (40–50%) increase in total hydrophobic contacts and H-bonds in presence of Ca2+ ions, compared to Na+ ions, as shown in Figs. 7 and 8, and 11, and 12).

Acknowledgement

P.B. wish to acknowledge a financial support from German Academic Exchange Service (DAAD) for financing his stay at Friedrich-Alexander-Universität Erlangen-Nürnberg 1.02-1.05.2019 (Grant no 57381327). This work was supported by internal grants: UTP BN-10/19 of the Institute of Mathematics and Physics of the UTP University of Science and Technology in Bydgoszcz.

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