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A DFT Study of the Amadori Rearrangement above a Phosphatidylethanolamine Surface: Comparison to Reactions in Aqueous Environment

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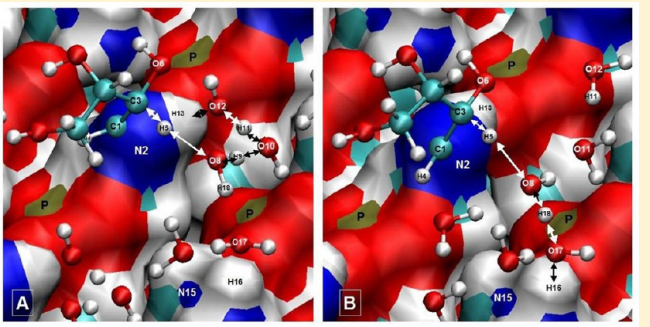
Supporting Information

ABSTRACT: Mechanisms for Amadori rearrangements from Schiff bases from reactions between D-erythrose and ethylamine, glycine, and phosphatidylethanolamine (PE) based on Dmol3/DFT calculations were realized. For the case of PE, calculations were done under periodic boundary conditions (PBCs), in an amine-phospholipid monolayer model with two molecules of PE by cell. In the three cases, the reaction started with a neutral Schiff base, having in the case of PE surface model a positive charged amine group belonging to the adjacent PE molecule. The catalytic role of PE surface components such as amine and phosphate groups is highlighted. All models included water molecules forming hydrogen bond networks, these networks were involved in the reactions by stabilizing reaction intermediates and transition states and performing as proton-transfer carriers, important in all steps of reactions. In all the studied reactions, they take place in two steps, namely, (1) formation of a 1,2-enaminol intermediate and (2) ketonization to the Amadori rearrangement product, having the last step clear lower values for relative free energies in the case of stationary points of the reaction on PE surface. An alternative pathway in the first step of Amadori rearrangement above PE surface, starting since positive charged Schiff base, was also evaluated, obtaining values for the free energy barrier similar to the step, starting from neutral Schiff base form. On the basis of our results, it is possible then to hypothesize that the cell membrane phospholipid surface environment modifies the kinetic behavior of some biological reactions, enhancing some of them through a catalyst effect.



INTRODUCTION

Amadori products are N-substituted 1-amino-1-deoxyketoses, representing an important class of intermediates in the nonenzimatic glycation (Maillard reaction). They are formed naturally in the initial phase of the nonenzimatic glycation by Amadori rearrangement of the corresponding Schiff bases, in equilibrium with their *N*-glycosylamines. Schiff bases are obtained previously by condensation of reducing sugars, mainly glucose, and free amino-containing moieties of several biomolecules such as proteins, nucleic acids, and phospholipids.[1,2] The importance of Amadori products stems from the fact that their formation as well as decomposition can be initiated under physiological conditions, appearing to be involved in the pathological effects of diabetes, Alzheimer's disease, and aging processes in general, through their transformation into a wide spectrum of compounds called advanced glycation end products (AGEs).[3,4] They are also formed during food processing and storage, being of great importance in the processing of foods for the production of aroma, taste, and color.[5,6]

While nonenzymatic protein glycation and its involvement in diabetic complications have been thoroughly investigated, less attention has been paid to glycation of phospholipids with free amino groups such as phosphatidylethanolamine (PE) and phosphatidylserine, which could be abnormally glycated under hyperglycemic conditions, contributing to the pathogenesis of diabetic complications, such as retinopathy, nephropathy, neuropathy, and atherosclerotic macrovascular disease.[2] Nonenzymatic glycation of cell membrane components such as PE has biomedical relevance since these membranous functional lipids are vital for the maintenance of cellular integrity and survival. Their glycation could result in deteriorating membrane structures, causing dysfunction into the cell.

The Amadori product from glucose and PE has been isolated, and its chemical structure has been investigated in detail.[7,8] Amadori products formed in the amino-carbonyl reaction yield reactive oxygen species (ROS) such as the superoxide anion, hydrogen peroxide, and the hydroxyl radical via autoxidation that is caused by one-electron transfer in the presence of metal

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ions.[9,10] Moreover, the Amadori moiety of PE, through its autoxidation, may also lead to the formation of ROS. The ROS are likely to cause peroxidation of unsaturated fatty acid residues in membrane lipids, which propagate free radical reactions and lead to products like phosphatidylcholine hydroperoxide, whose formation is closely involved in the pathophysiology of atherogenesis, diabetes, aging, and other conditions.[2,7,11]

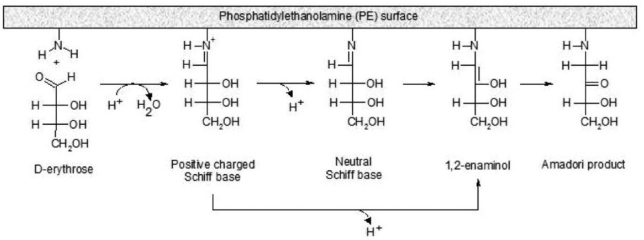
D-Erythrose is one of the two naturally occurring members of the aldotetroses. It is an intermediate in the biosynthesis of erythritol, a sugar alcohol, which naturally occurs in a variety of foods such as fruits, mushrooms, and fermented foods.[12,13] D-Erythrose has been considered to be a fast glycating sugar.[14,15] The formation of Amadori products from reaction between erythrose and lens crystalline proteins has been shown as being 10-fold faster than glucose,[16] what has been attributed to differences in the level of free aldehyde groups that are available for initial Schiff base formation due to its acyclic structure.

Despite of the Amadori rearrangement importance, there are some characteristics of this reaction such as its reversibility that continues to be a controversial issue,[17,18] lacking of theoretical studies describing these mechanisms at the atomic level, and deserving special attention of the possible differences for this reaction with different reactants or molecular environments. According to the most accepted mechanism for Amadori rearrangement, the initial reaction of a reducing sugar with an amino-container molecule gives the corresponding Schiff base (glycosylamine) that rearranges first to 1,2-enaminols via a sigmatropic shift followed by ketonization to the Amadori rearrangement product.[19] Previous experimental and theoretical studies by our group allowed Schiff base formation mechanisms for vitamin B_6 analogues and aminophospholipids to be elucidated.[20−28] However, this is the first time we modeled reaction mechanisms for obtaining Amadori products from Schiff bases. There is a density functional theory (DFT) study on the mechanism of the Amadori rearrangement reaction[29] and others about thermodynamic stability on intermediates for glycine nonenzimatic glycation reactions with glucose, ribose, and glyceraldehyde.[30−33]

The cell membrane surface is a special environment, and it can indeed interact with various kind of molecules, via electrostatic and hydrophobic interactions and/or hydrogen bonding. The cell membrane surface could also enhance the ability of protons to diffuse promptly along the membrane through hydrogen bonded networks of water molecules and charged or polar groups of phospholipids at the surface.[34] The reactivity of phospholipid components of cell membrane has been shown; examples of such reactions are the reactivity of Cl_2 and HOCl on PE,[35] changes due to oxidative stress,[36] and formation of Schiff bases with ketonic compounds.[20−22,37,38] There is also experimental and theoretical evidence that decomposition of H_2O_2 could be accelerated above the phospholipid membrane surface,[39−41] and it has also shown experimentally that phospholipid glycation is faster than protein glycation.[42] All they suggest, the catalytic potential of phospholipid surfaces participate in other reactions that occur on the cell membrane surface.

Therefore, the aim of this study is to obtain and analyze comparatively at the DFT level the mechanisms for Amadori rearrangement in Schiff bases from reactions between D-erythrose and ethylamine and glycine and PE, by means of calculating transition states, and describing energetic and geometric changes along the reaction coordinate. The mechanism could be extrapolated to the reaction having glucose or other reducing sugars instead of D-erythrose, contributing toward a better understanding of nonenzimatic glycation, matching conclusions that have been obtained experimentally. In this work, we also analyze comparatively the influence of the PE surface as a chemical environment on the reaction, modeling the reaction in Schiff bases from reaction between D-erythrose and PE (Scheme 1), in comparison to the reaction in an aqueous environment, having as starting reactants Schiff bases from D-erythrose/ethylamine and D-erythrose/glycine. This work also shows the role of water solvent molecules acting catalytically via a hydrogen transfer mechanism.

Scheme 1. Formation of Amadori Product from D-Erythrose and Phosphatidylethanolamine



METHODOLOGY

In order to make possible DFT calculus, PE surface model was designed from the crystal structure of 1,2-dilauroyl-DL-phosphatidylethanolamine.[43] The periodic boundary conditions made possible to obtain a surface model of a layer of phospholipids, useful for studying theoretically the reaction on an environment different to aqueous solvent. The models for ethylamine and glycine, due their more simple structure, were built without periodic boundary conditions but also included water molecules as explicit solvent and acetaldehyde. In all cases, an additional solvent environment was modeled via the conductor-like screening model (COSMO).[44−46]

The PE surface model was represented using a three-dimensionally periodic slab model. The supercell (Figure 1) contained two molecules of truncated PE, one of them as a neutral Schiff base from reaction between D-erythrose and PE, the other as a protonated PE, and six water molecules as explicit solvent in a hydrogen-bond network along the polar heads of phospholipids. Because we also evaluated an alternative path for the reaction, we modeled the same system, but having a protonated Schiff base from D-erythrose/PE and a neutral PE molecule as components of the molecular system. The designed models for the systems with Schiff bases from reaction between D-erythrose and ethylamine and glycine included the corresponding Schiff base and 21 water molecules. The purpose of including this number of water molecules in these molecular models was not exclusively to simulate a water solvation environment; rather, the water molecules were intended to act as reactive species facilitating several steps of studied reaction in the different models.

Additionally, in order to evaluate the possibility of an alternative pathway for Amadori rearrangement on PE surface, the structure of the positive charged Schiff base in the reaction between D-erythrose and PE was modeled; this form of Schiff base has been characterized previously as intermediate in the Schiff base formation above PE surface.[22] In this model, the partner PE molecule inside the cell has a neutral amine group, which participates as a proton acceptor in the step of formation of

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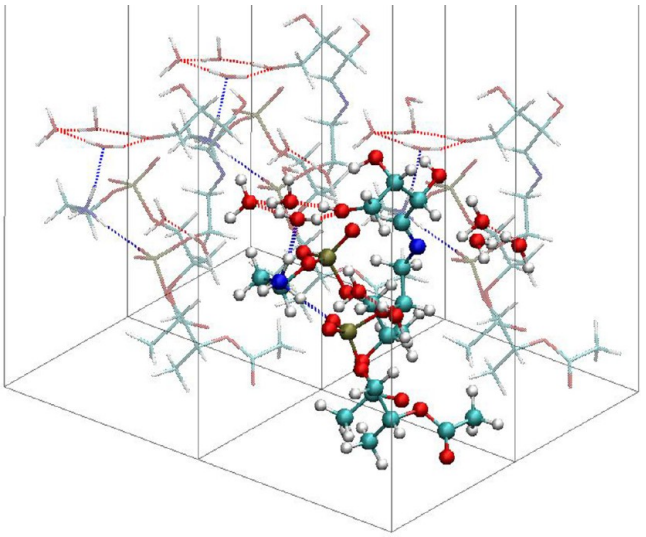


Figure 1. Section of four unit cells of the initial model for a phosphatidylethanolamine surface, including by cell a neutral Schiff base from reaction between phosphatidylethanolamine and D-erythrose, another phosphatidylethanolamine molecule, and the water hydrogen-bond network. The atoms in the representative cell are in balls and sticks. Atoms in neighboring cells are in sticks.

a 1,2-enaminol intermediate, that is the same product of the step starting since neutral PE−Schiff base.

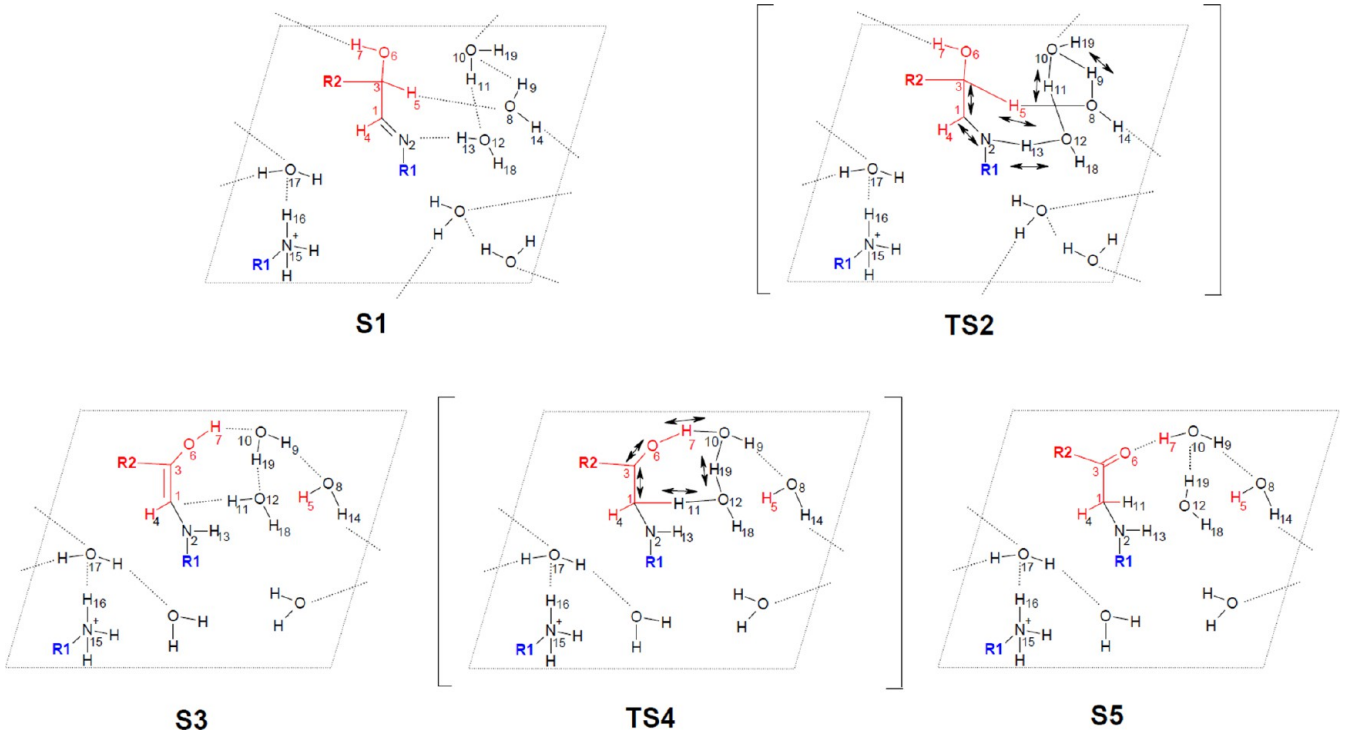
All of the calculations were performed in the frame of DFT with the DMol3 program package,[47−49] using double numerical with polarization (DNP) basis sets and Perdew−Burke−Ernzerhof (PBE) exchange-correlation functional.[50,51] The DNP numerical basis set is comparable to Gaussian 6-31G(d,p), and its accuracy for describing hydrogen-bond strengths has been tested.[52−54] The maximum number of numerical integration mesh points available in DMol3 was chosen for our computations, the threshold of density matrix convergence was set to 10^{-6}. A Fermi smearing of 0.005 hartree and a real-space cutoff of 4.5 Å were also used to improve the computational performance.

The initial models as reactants and the next models for stationary points generated during Schiff base formation in all the cases were modeled in Materials Visualizer and optimized using the conjugated gradient algorithm. Transition state (TS) searches were performed with the complete LST/QST method.[55] In this method, the linear synchronous transit (LST) maximization was performed, followed by an energy minimization in directions conjugating to the reaction pathway to obtain approximated TS. The approximated TS was used to perform quadratic synchronous transit (QST) maximization, and then another conjugated gradient minimization was performed. The cycle was repeated until a stationary point was located. The obtained TS was optimized via eigenvector following, searching for an energy maximum along one previous selected normal mode and a minimum along all other nodes, using the Newton−Raphson method. After this procedure, one transition state was found for each reaction step. Each TS structure was characterized by vibrational analysis with exactly one imaginary frequency.

RESULTS AND DISCUSSION

In order to study the Amadori rearrangement, we selected Schiff bases from reaction between D-erythrose and ethylamine and glycine and PE, like free amino-containing reagents; because of their differences in adjacent groups, they could let us evaluate the possible influence of these groups in the reaction. In the case of

Scheme 2. Mechanism of Amadori Rearrangement from Neutral PE/D-Erythrose Schiff Base, Using Periodic Boundary Conditions; Dotted Lines Represent Hydrogen Bonds, and Arrows Represent Changes in the Electronic Density and Proton Transfers



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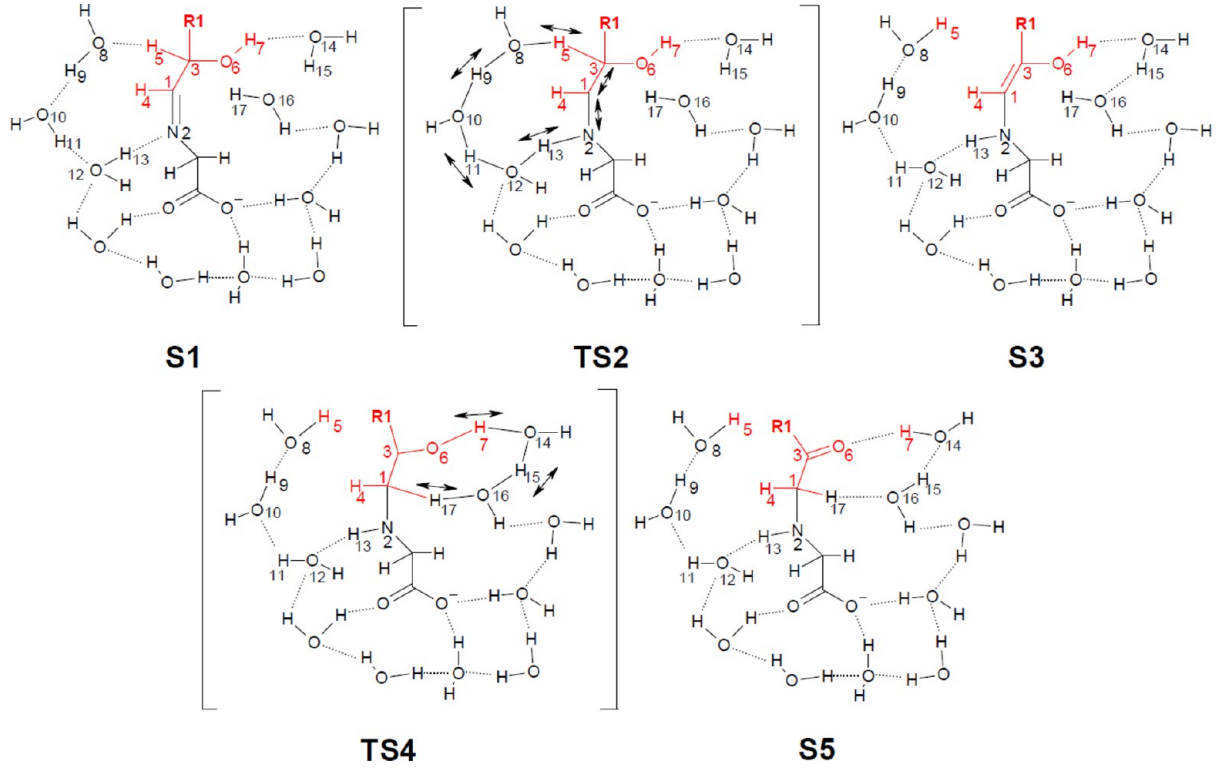
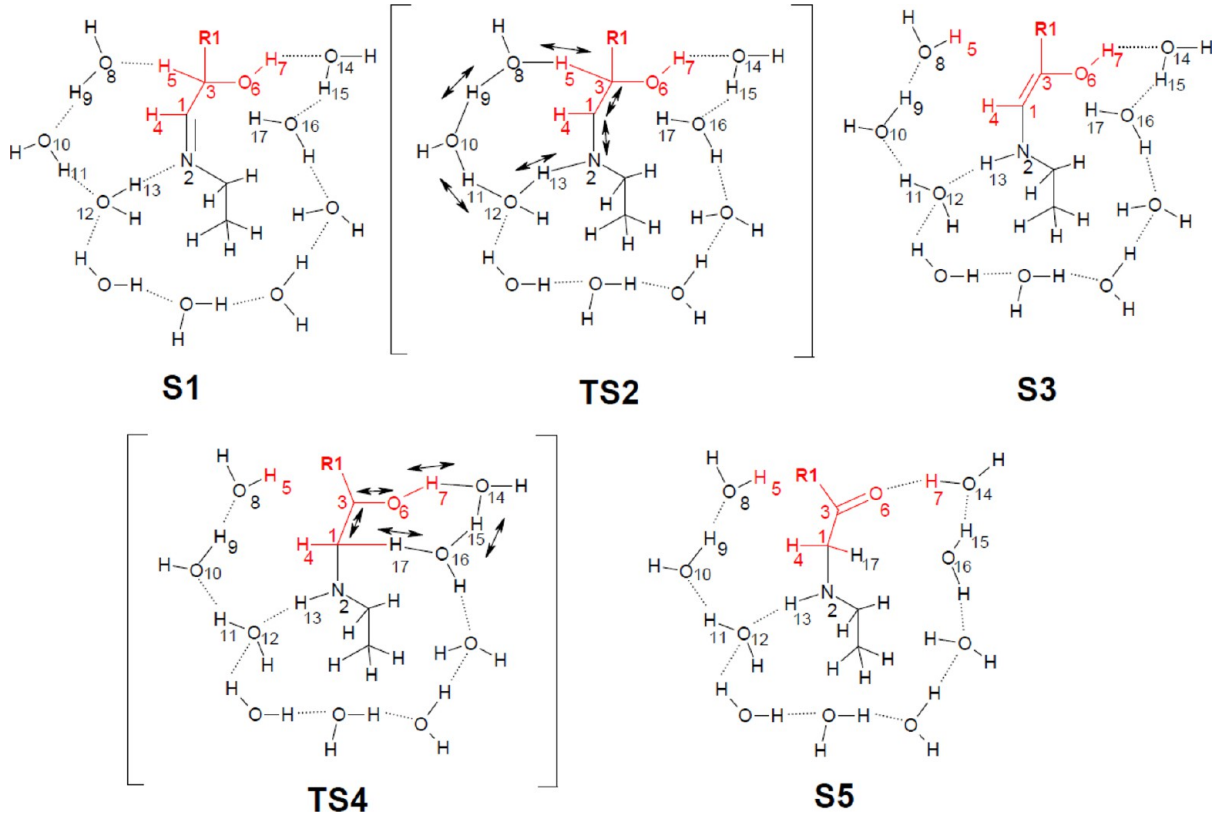
Scheme 3. Mechanism of Amadori Rearrangement from Glycine/D-Erythrose Schiff Base; Dotted Lines Represent Hydrogen Bonds and Arrows Represent Changes in the Electronic Density and Proton Transfers



Scheme 4. Mechanism of Amadori Rearrangement from Ethylamine/D-Erythrose Schiff Base; Dotted Lines Represent Hydrogen Bonds and Arrows Represent Changes in the Electronic Density and Proton Transfers



PE, it is possible to evaluate additionally the influence in the reaction of an environment different to aqueous solvent. PE is one of the main lipid components of the biological membranes, and its Amadori product with glucose has been isolated and

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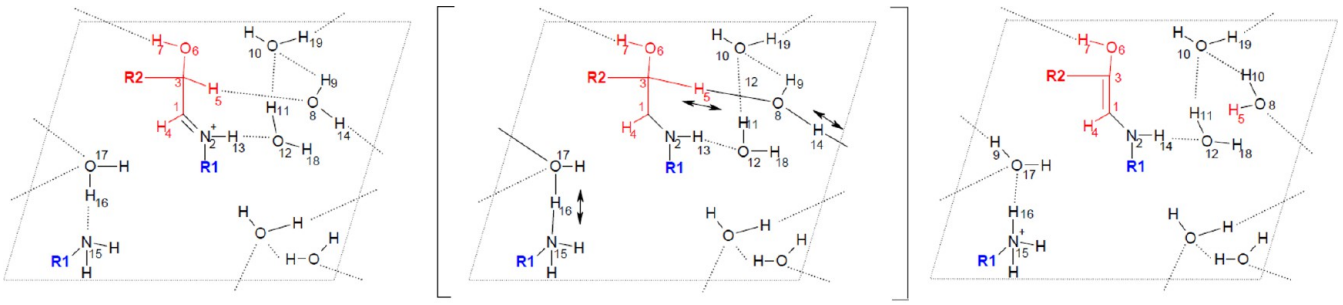
Scheme 5. Mechanism of Amadori Rearrangement from Positive Charged Schiff Base of PE/D-Erythrose, Using Periodic Boundary Conditions; Dotted Lines Represent Hydrogen Bonds and Arrows Represent Changes in the Electronic Density and Proton Transfers



studied experimentally.[8,56,57] Several experimental works have studied the reaction between D-erythrose, a tetrose reducing sugar, and amine-container biomolecules.[14−16] Because of its size, its Schiff bases were chosen and modeled as appropriate reagents for studying the Amadori rearrangement.

Schemes 2−4 show the atoms directly involved in the reactions. In the three cases, the Amadori rearrangement essentially involve two steps, namely, formation of a 1,2-enaminol intermediate (structures 1−3 for ethylamine, glycine, and PE) and its ketonization to the Amadori rearrangement product (structures 3−5 for the three cases). Scheme 5 shows the pathway from the charged Schiff base on PE surface. The 1,2-enaminol product of this alternative step is the same of the reaction starting since neutral Schiff base. Table 1 lists the ΔG values for each structure involved in the process, and Figure 2 shows the comparative free energy profile. We offer structural data (atoms distances) for each intermediate and transition state in the Supporting Information.

Table 1. Relative Free Energies (kcal mol^{-1}) for the Structures of the Studied Reaction Paths

structure	ethylamine	glycine	phosphatydilethanolamine
S1	0.00	0.00	0.00
TS2	16.87	12.82	16.78
S3	1.68	0.08	−7.10
TS4	17.25	16.90	5.43
S5	−12.59	−7.45	−8.02

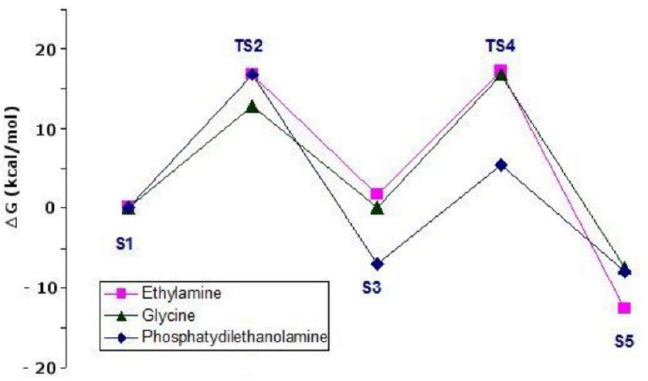


Figure 2. Comparative free energy profile for the Amadori rearrangement from Schiff bases of D-erythrose and ethylamine (pink line), glycine (black line), and phosphatidylethanolamine (blue line).

Formation of a 1,2-Enaminol Intermediate. The starting points of this intramolecular rearrangement for the three cases were neutral Schiff bases (S1 in Schemes 2−4), what can further react through an imine−enamine tautomerism mechanism, rearranging to an enaminol intermediate. In this step, nitrogen atom of Schiff bases as a Bronsted base do a nucleophilic attack on a proton joined to a carbon atom (C3) attached to the OH group, realizing a proton transfer from C3 to N2 atom through a chain of three water molecules, and displacing concertedly the double bond between N2 and C1 to C1 and C3 (S1 to S3 through TS2 in Schemes 2−4). When a proton (H5) is released from C3, the electrons from the C3−H5 bond form a π bond between C3 and C1 carbon atoms. In the case of PE surface, the periodic boundary conditions allow O6, O8, and O17 to form hydrogen bonds with atoms of neighboring cells.

Above PE surface, when the reaction proceeds from the neutral Schiff base, the proton transfer from C3 to N2 is carried out through a hydrogen bond chain of three water molecules (TS2, Scheme 2), making possible the protonation of N2 atom. In the case of the reaction from the positive charged Schiff base, the proton transfer proceeds via TS2′ from C3 to N15 atoms, through a hydrogen bond chain of two water molecules; the periodic boundary conditions make possible to model the proton (H14) transfer from one face of the unit cell cleaving a bond with O8 atom, on the opposite face to form a bond with O17 atom (Scheme 5).

The relative free energy barriers for this step, had values of 16.87, 12.82, and 16.78 kcal mol^{-1} for ethylamine, glycine, and PE surface, respectively. The lower value for free energy barrier in the reaction from Schiff base of glycine could be attributed to the presence of a carboxylate group, which stabilizes the hydrogen bond network around, influences the partial charge stability of its adjacent atoms, and facilitates the proton transfer through the three water molecules. The role of amino acid carboxyl group in the catalysis of the Amadori rearrangement has been shown in other works; when the carboxyl group was absent (e.g., in aliphatic or aromatic amines), the glycosylamine, a ring closure product of Schiff base, is more stable and, in many cases, has been isolated.[19]

According to other theoretical works,[58,59] the rate of converting the imine to enamine depends on how easy it is for an α carbon in the imine to deprotonate, and that is influenced directly by its substituents, which could contributed to lower the basicity of the α carbon during the reaction. In the studied molecular systems, this α carbon corresponds to C3 (Schemes 2−4). In the three systems, this atom has an electronegative hydroxyl group (O6−H7), which could render inductively

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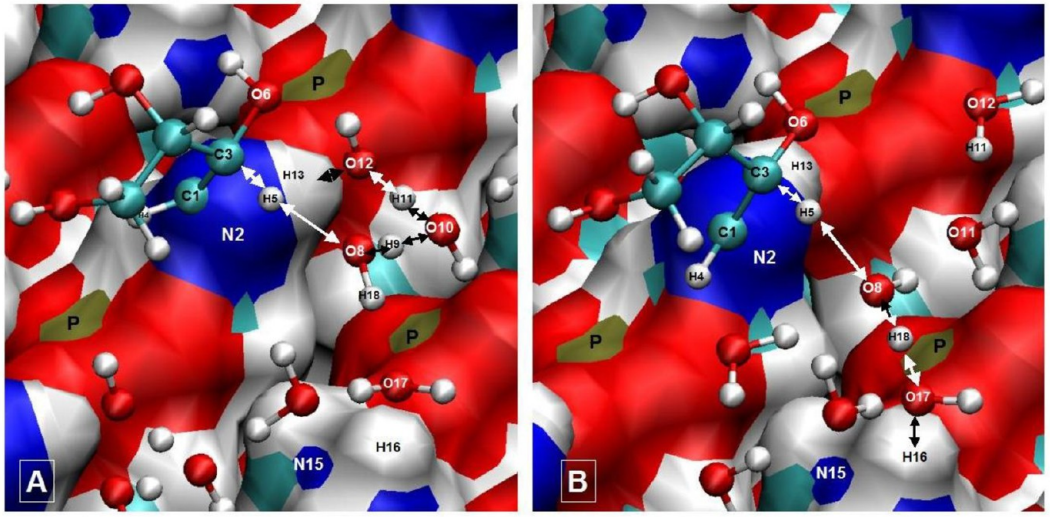

Figure 3. Sights of phosphatidylethanolamine surface showing (A) the transition state (TS2) of formation of a 1,2-enaminol intermediate from neutral Schiff base and (B) the transition state (TS2′) of formation of a 1,2-enaminol intermediate from positive charged Schiff base. Arrows represent proton transfers.

electron-withdrawing during the deprotonation of the adjacent carbon atom, dispersing its negative charge, facilitating more this deprotonation (TS2 in Schemes 2−4). In the conversion from Schiff bases to enaminol, the hydroxyl group has a conversion from weak electron-withdrawing when it is attached to an sp^3 carbon (S1 structures) to electron releasing group due to the resonance effect when it is attached to an sp^2 carbon (S3 structures). The hydroxyl group also participates in the next keto−enol tautomerism step but as an electron releasing group to produce the carbonyl group of Amadori products.

We considered a neutral Schiff base as the starting point for the Amadori rearrangement in the first three studied cases. Other authors taking account that Schiff bases of sugars as glucose could also stay in equilibrium between as the open-chain imino form and cyclic glycosylamines,[60] considering the last as the starting point. These glycosylamines previously showed that the formation of 1,2-enaminol intermediate should be converted in their corresponding Schiff bases, but in their positive charged forms.[19] In the case of the studied molecular systems, there are not these conversions due to the acyclic nature of D-erythrose. However, according to the mechanism obtained by us for neutral Schiff base formation on PE surface,[22] the pathway for reaction between D-erythrose and PE could have the positive charged Schiff base as the previous intermediate to the final product, and thus, the formation of the 1,2-enaminol intermediate could proceed directly from this positive charged form, bypassing the pathway of the neutral form.

In order to evaluate the positive charged form of the Schiff base as the starting point of the rearrangement, a model of PE surface having this Schiff base form was designed. In this model, the partner PE molecule had a neutral amine group, which participated in the formation of the 1,2-enaminol, as proton acceptor, facilitating the removal of a proton from carbon atom (C3) and its transfer through two water molecules (S1′ to S3′ through TS2′ in Scheme 5 and Figure 3B). The relative free energy barrier for this step had a value of 17.50 kcal mol^{-1}, not so different from the value of the same step considering a neutral Schiff base as reactant. According to this result, components of the PE surface could have a catalytic effect on the studied reaction, not only stabilizing intermediates and transition states of the pathway but also assisting it directly, as proton donors and acceptors. This path also makes possible to regenerate the catalytic acidic charged amine group of the PE molecule partner, generated in the previous Schiff base formation on PE surface,[22] and considering PE-Amadori formation as final product of the reaction between PE and a reducing aldehydic compounds. PE differs from other phospholipids such as phosphatidylcholine in which its primary amine group becomes deprotonated at a pH of about 8,[61,62] having a pK_a value of 9.6 ± 0.1 in PE vesicles.[63] This suggests that it is possible having in physiological conditions a certain fraction of PE molecules with neutral amine groups that could participate as proton acceptors during 1,2-enaminol formation froma positively charged Schiff base from D-erythrose/PE.

PE is known to be isoelectric over a wide pH range, as indicated by the pH dependence of several physicochemical properties.[64] Previous experimental studies[65,66] have shown that the amino protons on the outside vesicle surface exchange rapidly with water protons at pH >5; the rate of proton exchange of the amino protons of PE increases with increasing pH, being the dominant exchange process, an intrasurface reaction in which NH_2 groups react via water with NH_3^+ groups on the outer surface. In these works, it was hypothesized that proton transfer may involve several water molecules so that the distance between nitrogen atoms may be as large as 9−10 Å during the proton transfer.[67] In the periodic models of our work, these distances between nitrogen atoms from PE-molecule and Schiff base from D-erythrose/PE have values around 7 Å.

The hydrogen bond network also has an influence in the reactivity of the amino group of PE; experimental studies have shown a decrease in this reactivity when the composition of self-organized monolayers used in these studies was passed from the pure PE to PE/phosphatidylcholine mixtures,[64,68] what has been explained by a resulting decrease in intermolecular ammonium−phosphate interactions between contiguous PE molecules due to the interdispersion of PE with phosphatidylcholine molecules. This intermolecular interaction between PE charged amino group and phosphate groups is also evidenced in the modeled PE surfaces, being two of them by cell, in the model including neutral Schiff base from D-erythrose/PE (S1 structure, Figure 1). In the

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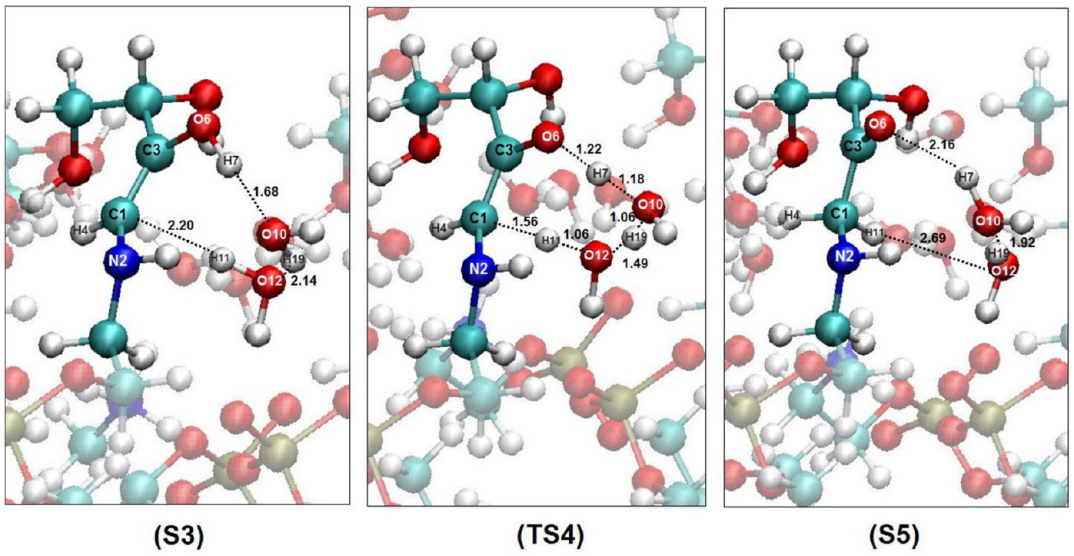

Figure 4. Mechanism of Amadori rearrangement from neutral PE/D-erythrose Schiff base: (S3) 1,2-enaminol intermediate; (TS4) transition state; (S5) Amadori product.

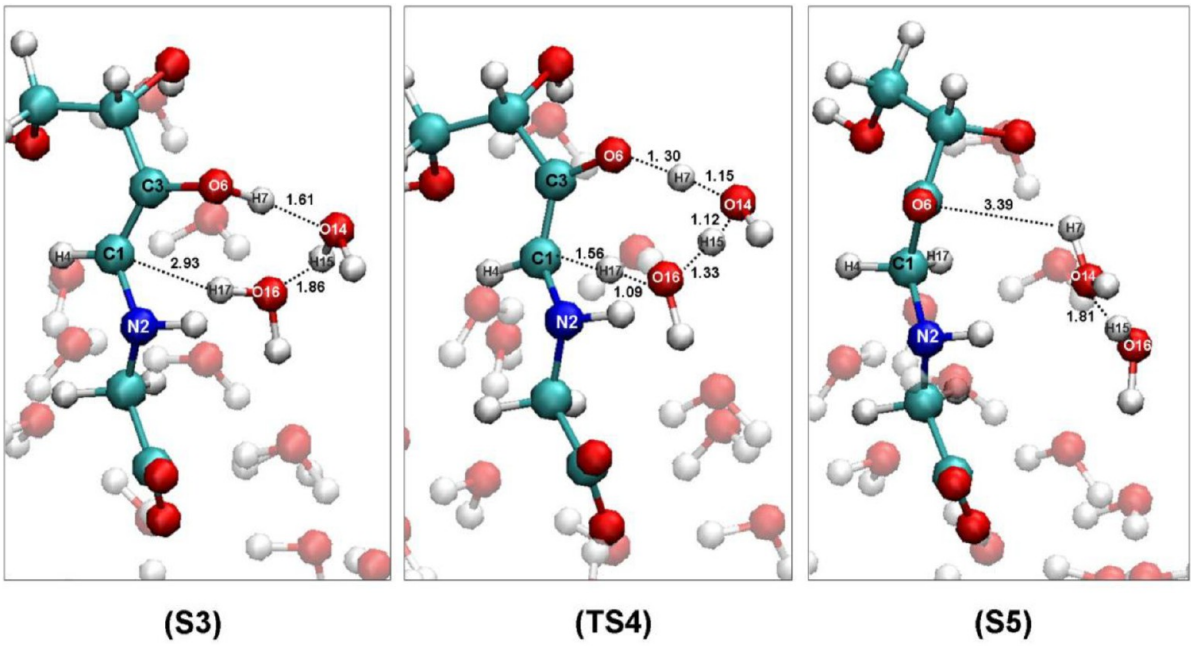

Figure 5. Mechanism of Amadori rearrangement from glycine/D-erythrose Schiff base: (S3) 1,2-enaminol intermediate; (TS4) transition state; (S5) Amadori product.

case PE suface model including a positive charged Schiff base from D-erythrose/PE, there are also two interactions by cell, but in this case, the intermolecular interactions with phosphate groups are established, one with neutral amine group of the accessory PE molecule partner and the other with the positive charged Schiff base group. These interactions are shown comparatively on PE surfaces in Figure 3, where it is shown the transition states of the reaction with the neutral form of the Schiff base (Figure 3A) and with positive charged form of the Schiff base (Figure 3B) as starting points of the rearrangement. On the surface is evidenced the electrostatic interactions of oxygen atoms from phosphate groups with hydrogen atoms from amine or Schiff base groups, the interactions between water molecules and also proton transfers.

Ketonization. The next step in the reaction is the ketonization of the 1,2-enaminol intermediates to their *N*-(1-deoxyketos-1-yl)-amine derivatives or Amadori products, which involves the concerted protonation of the double bond between C1 and C3 atoms and the removal of the enolic proton (H7 atom) from O6. Two water molecules through a hydrogen bond wire make possible the proton transfer, acting at the same time as an acid catalyst and a general base (S3 to S5 through TS4 in Schemes 2−4 and Figures 4−6). The evolution of this step could be evidenced through the changes of the bond distances; not only in the 1,2-enaminol intermediate but also in the chain of water molecules that mediates the proton transfer. This step additionally causes the formation of a carbonyl group between C3 and O6 atoms.

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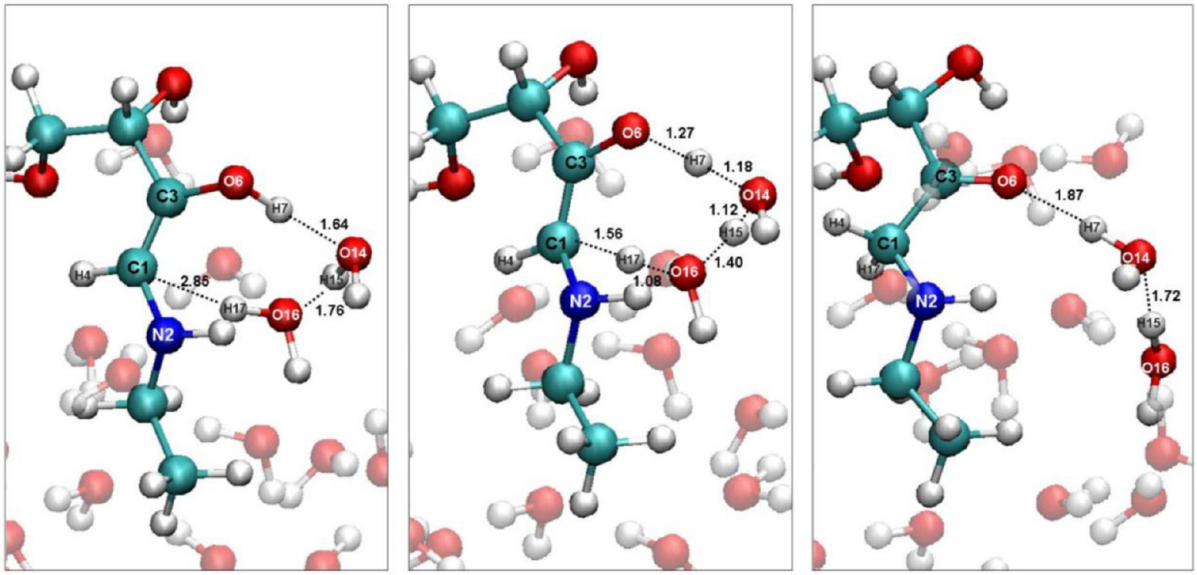

Figure 6. Mechanism of Amadori rearrangement from ethylamine/D-erythrose Schiff base: (S3) 1,2-enaminol intermediate; (TS4) transition state; (S5) Amadori product.

In the three studied reactions, water has a significant catalytic effect because it can act both as a proton donor and as a proton acceptor and thus mediates the intramolecular proton transfer that leads to the formation of Amadori product from 1,2-enaminol, supplying furthermore a bridge for proton relay. The catalytic effect of explicit water molecules on the keto−enol tautomerism through a water-chain mechanism has been shown in keto−enol tautomerism in pyruvate, acetone, acetylacetone, and vinyl alcohol.[69−71] The theoretical work of Cucinotta et al.[70] about acetone tautomerism showed a drastic reduction of energy barrier (20 kcal mol^{-1}) for this reaction when the system included 28 water molecules that participated collectively in the reaction through a Grotthuss-like diffusion mechanism for the proton.

The structural and energetic information of intermediate S3 of all studied reactions correspond to 1,2-enaminol intermediates, having water molecules in a conformation belonging to the ketonization step of the reaction. According to our results, the influenced PE surface in the reaction is translated in lower free energy values for S3 and TS4 in comparison, which were modeled in pure aqueous environment (Figure 2). 1,2-Enaminol intermediates (S3) are members of a group of compounds called amino-reductones, most of them especially enaminol compounds, are unstable reaction intermediates and are difficult to isolate, being their structural characteristics are still unclear.[72] However, in the case of 1,2-enaminol from PE/D-erythrose, its stability is lower than its Schiff base and similar to its Amadori product,; it could be attributed to the formation of stable hydrogen bonds with water molecules that connect polar groups of this intermediate and phosphate groups around. In comparison to its Schiff base, the N2 atom from PE/D-erythrose 1,2-enaminol is protonated, stabilizing better these interactions. As is possible to see in Figure 3, almost all the PE surface is occupied by atoms from amine and phosphate groups, while the hydrocarbon chains are driven toward inside in order to reduce hydrocarbon−water contact.

PE phosphate groups have very low pK_a values such as 0.5,[64] having a low possibility to be a proton acceptor at physiological values of pH. However, it is known to adsorb strongly water molecules and interact with adjacent amine groups through strong hydrogen bonds on the biological membrane surfaces, which can greatly influence the biological surface chemistry. Despite the lower hydration of PE (∼9−10 waters/phospholipids), in comparison to phosphatidylcholine (∼23 waters/phospholipids),[73,74] in PE, water molecules can create hydrogen-bonded bridges that connect positively charged NH_3 groups with negatively charged oxygens attached to phosphorus, which determine its packing, polarization, and the surface free energy of the interphase. These water bridges serve as a glue that keeps membrane surfaces together.[74] In our models, these bridges also connect reactive groups of stationary points with the polar and charged groups of the PE surface, stabilizing all the molecular systems. Because of the periodic nature of the models, this connection is extended along all the PE surface cell by cell (Figures 1 and 3).

The influence of the PE surface is clearer in the case of transition state TS4 in comparison to the transition states from glycine/D-erythrose and ethylamine/D-erythrose molecular systems, having an approximate relative free energy value of 11 kcal mol^{-1} lower than the others (Figure 2). This neighboring catalyst effect above PE surface is done through the different charged and polar groups of the PE surface. One of the two water molecules (H11−O12−H18) that participate in the proton transfer interacts directly with the C1 reactive atom from PE/D-erythrose 1,2-enaminol, and it also has a hydrogen bond with a phosphate group of the other PE molecule. The phosphate group through this interaction is able to polarize the water molecule, favoring the release of a proton to the C1 atom. This clear difference with the other studied reactions was not determined in the before reaction step because the phosphate group in that case establishes a hydrogen bond with a water molecule that does not interact directly with any of the reactive atoms of the Schiff base from PE/D-erythrose. This influence of the phospholipid surface

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on the studied reaction could also explain experimental evidence that the kinetics of lipid glycation is little faster than that of protein glycation.[42] The effects of surfaces on this kind of reaction have been evidenced in a theorethical study about the adsorption and tautomerization reactions of acetone on acidic zeolites, where, because of the acidity of the zeolite and the framework confinement effect, the tautomerization of acetone proceeded through a much lower activation barrier than in the isolated gas phase or in the presence of water molecules alone.[75]

Summarizing, phosphate and amine groups from PE could play three roles above the phospholipids surface: (i) accumulation of H_2O on the surface, resulting in increased local concentrations; (ii) polarization of the water bonds as a result of an interaction with the charged groups, for example, the phopshate-bound water molecules tend to orient their dipoles with their positive ends pointing toward the negative phosphates, resulting in a net orientational polarization. This polarization could facilitate the role of solvation water molecules as bridges in the proton exchange between donor and acceptor protons in the reaction; (iii) passive catalytic effect through a charge stabilization of different intermediate structures of reaction, due to direct electrostatic interactions with the positive charged groups generated in the different steps of the reaction. Additionally, amine groups could participate as donors or acceptors in the proton transfers.

In the nonenzymatic glycosylation of hemoglobin, the Amadori rearrangement takes a long time (days) in comparison with Schiff base formation (hours).[18] According to the work of Oak et al., obtaining Amadori-PEs from glucose and dioleoyl PE takes 15 days,[76] The formation of imines or Schiff base derivatives is considered reversible,[77] whereas the Amadori rearrangements are generally regarded as irreversible, although it is still unclear why this should be so.[78,79] By analyzing the values for free energies of the stationary points of the pathways for Schiff bases from glycine/D-erythrose and ethylamine/D-erythrose, this irreversibility could be attributed to the greater stability of the keto tautomer (Amadori product) in the equilibrium mixture with its enol form, what makes more difficult its conversion to its 1,2-enaminol, having higher free energy barriers in the direction Amadori product → 1,2-enaminol, with values of 24.34 and 29.84 kcal mol^{-1} in the case of Amadori products from glycine/D-erythrose and ethylamine/D-erythrose, respectively (Table 1 and Figure 2). This feature has been also verified in other molecular systems such as acetone.[80,81] This could be explained by the orientation of the CH_2 group (H4−C1−H11) in the Amadori product, which disfavors formation of the C−C π bond of 1,2-enaminol (structure S5 in Figure 4). In the case of PE, the free energy barriers for this tautomerism in both directions have similar values, being in the direction 1,2-enaminol → Amadori product, 12.53 kcal mol^{-1}, and in the contrary direction, 13.44 kcal mol^{-1} (Table 1 and Figure 2). This similar free energy barrier is also attributed to the influence of the PE surface, stabilizing the 1,2-enaminol intermediate and the TS4 transition state, how we explained in previous paragraphs.

CONCLUDING REMARKS

In summary, we have carried out a series of DFT calculus in order to obtain mechanisms for Amadori rearrangements from neutral Schiff bases, products of previous reactions between D-erythrose and ethylamine, glycine, and PE. In all the studied reactions, they take place in two steps, namely, (1) formation of a 1,2-enaminol intermediate by imine−enamine tautomerism and (2) ketonization to the Amadori rearrangement product, having the last step clear lower values for relative free energies in the case of stationary points from reaction above the PE surface. The most important outcome of our study is highlighting the catalytic role of the PE surface on the Amadori rearrangement reaction, which proceeds above it. This catalytic effect is realized through PE surface components as amine groups and phosphate groups, which might enhance reaction forming hydrogen bonds with water molecules of the hydrogen bond network and facilitate the water molecule accumulation in the proximity of the PE surface; amine groups could participate additionally as donors or acceptors in the proton transfers. This study also gives a detailed picture through theoretical calculus of the intermolecular mechanism of the Amadori rearrangement having water molecules and an active role facilitating proton transfer along hydrogen-bonded chains in all the studied molecular systems. An alternative pathway in the first step of Amadori rearrangement above the PE surface, starting from positive charged Schiff base, was also evaluated, obtaining values for the free energy barrier similar to the step starting from the neutral Schiff base form.

ASSOCIATED CONTENT

Supporting Information

Tables with structural information of different intermediates in studied reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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