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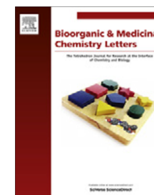

The hydrophobic substituent in aminophospholipids affects the formation kinetics of their Schiff bases

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ABSTRACT

Schiff bases (SBs) are the initial products of non-enzymatic glycation reactions, which are associated to some diabetes-related diseases. In this work, we used physiological pH and temperature conditions to study the formation kinetics of the SBs of 1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine (DPHE) and 1,2-dihexanoyl-*sn*-glycero-3-phospho-L-serine (DHPS) with various glycating compounds and with pyridoxal 5'-phosphate (an effective glycation inhibitor). Based on the obtained results, the hydrophobic environment simultaneously decreases the nucleophilic character of the amino group (k_1) and increases its pK_a, thereby increasing the formation rate of SB (k_{obs}). Therefore, the presence of hydrophobic chains in aminophospholipids facilitates the formation and stabilization of SBs, and also, in a biological environment, their glycation. Additionally, the results confirm the inhibitory action of B_6 vitamers on aminophospholipid glycation.

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The non-enzymatic glycation of aminophospholipids starts with the reaction of their amino groups with carbonyl groups in reducing sugars to form an initial Schiff base (SB). In fact, the SBs of the target aminophospholipids phosphatidylethanolamine (PE) and phosphatidylserine (PS) have been detected.[1,2] Subsequently, the SB formed rearranges into an Amadori compound that undergoes a heterogeneous series of reactions leading to advanced glycation end-products (AGEs). These compounds cause structural and functional changes in lipid membranes,[3] and their accumulation has been related to various clinical conditions and complications related to ageing, diabetes, cancer and Alzheimer's disease.[4,5]

The diseases arising from lipid glycation can be prevented by using effective inhibitors. Thus, amino compounds such as aminoguanidine and pyridoxamine are known to competitively inhibit glycation by scavenging carbonyl and dicarbonyl compounds in substances such as glucose, glyoxal or 3-deoxyglucose, and reducing their concentration as a result.[6] Otherwise the carbonyl group in pyridoxal 5'-phosphate (PLP) forms a protective SB with amino groups in phospholipids.[7,8] Thus, the compound PE-PLP has been detected in human red cells,[8,9] and the levels in Amadori–PE compounds in diabetic rats have been found to be reduced on administration of a PLP dietary supplement.[10,11]

In a recent work, we examined the influence of the nature of the polar head of aminophospholipids on the formation kinetics of their SBs. Specifically, we studied the reactivity of *O*-phosphorylethanolamine (PEA) and *O*-phospho-DL-serine (PSer) towards reducing sugars and PLP. We found the equilibrium constant of the reactions of PLP with PEA and PSer to be smaller than those for the reactions with glycine and serine, a result which we ascribed to the phosphate group in the aminophosphates. Also, the presence of a carboxyl group in α with the amino group in PSer decreased the formation constant of the SB with respect to PEA.[12,13] Finally, the kinetic constants of formation of the SBs of PLP with PEA and PSer were four orders of magnitude greater than those for reducing sugars, which confirmed the potential of PLP as a competitive inhibitor of glycation in aminophosphates.[12]

Unlike naturally occurring aminophospholipids, PEA and PSer lack the glycerol group bonded to the phosphate group in the former, which is esterified by fatty acids. It would therefore be interesting to extend our previous studies to models more closely mimicking natural phospholipids. In this work, we examined the reactivity of 1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine (DPHE) and 1,2-dihexanoyl-*sn*-glycero-3-phospho-L-serine (DHPS) with the glycants glucose, arabinose and acetol in addition to PLP. The target compounds are two low-molecular weight aminophospholipids previously used by other authors as PE and PS models in various biological systems.[14–16] These compounds are more readily

Abbreviations: DHPE, 1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine; DHPS, 1,2-dihexanoyl-*sn*-glycero-3-phospho-L-serine; PE, phosphatidylethanolamine; PEA, *O*-phosphorylethanolamine; PS, phosphatidylserine; PSer, *O*-phospho-DL-serine; PLP, pyridoxal 5′-phosphate; PM, pyridoxamine; SB, Schiff base.

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soluble[17] than lipids in cell membranes and hence easier to handle in non-micellar systems.

Scheme 1 shows the kinetic mechanism proposed for the reactions of short-chain aminophospholipids with various carbonyl compounds in the presence of sodium cyanoborohydride. The nucleophilic attack of the amino group on the carbonyl group yields a SB that is reduced by $NaCNBH_3$ at neutral pH.[18] This leads to the irreversible formation of a secondary amine (a reduced Schiff base, redSB) and facilitates indirect monitoring of the kinetic formation of the SB.

In addition, the reactive form of each reactant is in equilibrium with a non-reactive form. Constant K_3 defines the equilibrium between the carbonyl and cyclic forms in glucose and arabinose or that between the carbonyl and hydrated form in acetol. Table 1 lists K_3 values obtained from various sources.[19,20] Constant K_4 represents the acid–base equilibrium of the amino group in the aminophospholipids, whereas all other ionic groups are in their deprotonated form at physiological pH (where pK_a is 1.40 for the phosphate group and 2.65 for the carboxyl group).[21] pK_a for the amino group in DHPE and DHPS was determined by potentiometric titration at 37 °C and found to be 8.8 ± 0.1 for the former compound and 8.6 ± 0.1 for the latter. These pK_a values differ by more than one unit from those for the corresponding aminophosphates,[12] which is consistent with previous results of other authors and has been ascribed to an increased molecular hydrophobicity.[22]

The kinetic study of the reactions depicted in Scheme 1 was conducted at pD 7.4 and at 37 °C, using two-dimensional NMR spectra (^{1}H, ^{13}C-HMQC) in those cases where the one-dimensional (^{1}H) spectra exhibited overlap between the signals of reactants and products. As an example, Figure 1 shows the temporal variation of the two-dimensional spectrum for the DHPE–glucose reaction mixture. As can be seen, the $H_2C(1)$–C(1) signal for DHPE (3.26, 42.60 ppm) decreased simultaneously with the appearance of a new signal at 3.37, 50.62 ppm corresponding to redSB. The $H_2C(2)$–C(2) signal for DHPE was also shifted by effect of the formation of redSB and the compound exhibited two new signals at 65 ppm assigned to H_2C–C in the glucidic substituent (R^1) in redSB (see Supplementary data). The signals at 70–80 ppm corresponded to the excess of glucose in the medium.

Temporal variation of the intensity of the HC(1)–C(1) crossing peaks for the aminophospholipids was used to determine the concentration of DHPE and DHPS in each reaction under *pseudo* first-order conditions. The variation fitted Eq. (1), which allowed the respective observed constants, k_{obs}, to be determined:[23,24]

$$\ln\frac{[\mathrm{R-NH_2}]_t}{[\mathrm{R-NH_2}]_0} = -k_{obs}[\mathrm{R^1-CO-R^2}]_T t \qquad (1)$$

where [R–NH_2] denotes the initial aminophospholipid concentration and after a time t, and [R^1–CO–R^2] the total concentration of glycating carbonyl compounds (see Supplementary data). k_{obs} was applied to the equilibria of Scheme 1 to determine the microscopic formation constant of the SBs, k_1, using the following equation, which was established in previous work:[12]

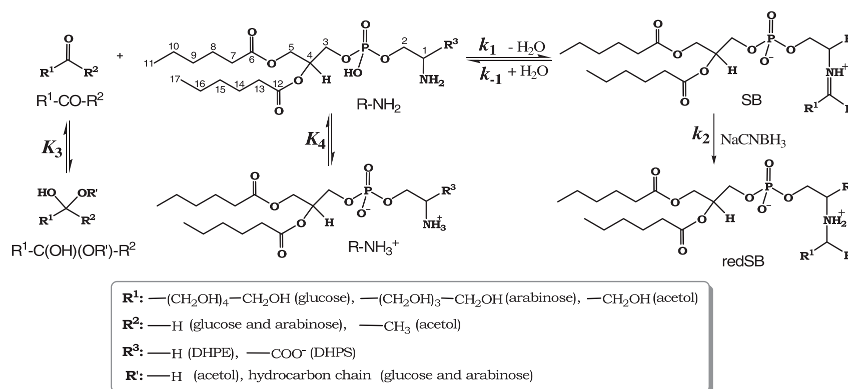
$$k_{obs} = \frac{k_1 K_3 K_4}{[\mathrm{H_3O^+}] + K_4 + [\mathrm{H_3O^+}]K_3 + K_3 K_4} \qquad (2)$$

As expected, k_1 changed in parallel to the electrophilicity of the carbonyl compounds (Table 1).[12,18,25] Also, the constant was smaller for DHPS than for DHPE, which was also the case with PSer and PEA.[12] Based on the k_1 values obtained, the presence of a carboxyl group in α with the amino group decreases the reactivity of aminophospholipids. Also, the k_1 values for DHPE and DHPS are 3–7 times smaller than those for PEA and PSer,[12] which suggests that removing a negative charge from the phosphate group and introducing a hydrophobic group reduce the nucleophilic character of the amino group.

The observed rate constant, k_{obs}, is a measure of reactivity under physiological conditions. k_{obs} for DHPE and DHPS was four and eight times greater, respectively, than it was for the aminophosphates.[12] This was essentially the result of their amino groups having a smaller pK_a value and hence an increased proportion of free amino groups at pH 7.4 (viz. 3.8% in DHPE and 5.9% in DHPS vs 0.14% in PEA and 0.20% in PSer). As noted earlier, pK_a is directly related to molecular hydrophobicity,[22] which is increased by the presence of hydrocarbon chains bonded to the phosphate group in the studied aminophospholipids in relation to PEA and PSer. However, the environmental conditions of lipid membranes may influence pK_a[26,27] and alter the efficiency of these reactions in vivo.

Data of Table 1 suggest that both the efficiency and intrinsic reactivity of DHPE in the first glycation stage exceed those of DHPS. This is consistent with previous results of Hazen et al.,[1] who showed the formation of a reduced SB to be easier in PE than in PS. However, the subsequent formation of Amadori compounds and AGEs is governed by other factors.[28] Interestingly, Fountain et al.[29] detected AGEs from PE and PS (*N*-carboxymethyl–PE and *N*-carboxymethyl–PS) both in vivo and in vitro. Also, formation of the PE–Amadori complex in the absence of a PS–Amadori adduct has been demonstrated in diabetic patients.[8,30,31]

The effect of reducing sugars on the polar head of aminophospholipids is biologically significant because it can alter their recognition and molecular interactions.[32] The adverse effects of the resulting changes can be avoided by using an effective inhibitor for the reactions involved in the non-enzymatic glycation of aminophospholipids. PM is one such inhibitor by virtue of its ability to scavenge glycating carbonyl compounds.[33] The kinetic constants for the reactions of PM with the carbonyl compounds studied here were determined in a previous work.[18] k_1 and k_{obs} were 3–14 times greater than those for DHPE and DHPS. The



Scheme 1.

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