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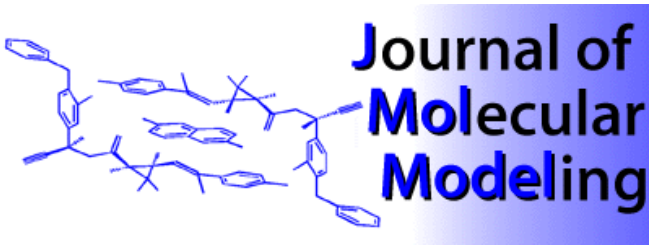

Short Communication

Extraction of Lipids from Phospholipid Membranes by Steered Molecular Dynamics

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Abstract

Steered molecular dynamics (SMD) simulations were employed to investigate the extraction step of the lipid head group cleavage reaction by human synovial phospholipase A_2 (PLA_2) by pulling a lipid molecule from a monolayer of dilauroyl-phosphatidyl-ethanolamin lipids into the active site of PLA_2 and into the aqueous phase. The results of the simulations were compared to draw inferences about the forces that stabilize the lipids in the membrane and to understand the mechanism of lipid extraction by PLA_2.

Keywords: Protein-membrane interactions, Phospholipase A_2

Introduction

Phospholipase A_2 (PLA_2) is a protein which complexes with membrane surfaces and catalyzes the hydrolysis reaction of the *sn*-2 ester bond of phospholipids [1–3]. One of the reaction products, arachidonic acid, is an important metabolic intermediate for producing eicosanoids, which are regulatory factors implicated in a wide range of physiological and pathological states [4]. The interfacial catalysis of PLA_2 involves four reaction steps: complex formation, scooting on the surface, lipid extraction from the membrane, and the hydrolysis reaction. In this paper we report the results of steered molecular dynamics (SMD) simulations [5–10] of the extraction of a lipid from a dilauroyl-phosphatidyl-ethanolamin (DLPE) membrane monolayer into the active site of PLA_2 as well as in the aqueous phase in the absence of the protein.

Methods

Formation of the complex of human synovial phospholipase A_2 with a DLPE membrane monolayer (see Fig. 1a) had been modeled previously for two structures of the enzyme with the membrane, i.e., the loosely and tightly bound complexes [11]. The structure of the DLPE monolayer in the absence of PLA_2 was obtained from the structure of the DLPE membrane bilayer modeled in [12] by deleting one lipid monolayer and the corresponding water molecules (see Fig. 1b).

The resulting system contained 101 lipid and about 4000 water molecules. The structures of PLA_2 complexes with the DLPE monolayer contained 101 lipid and about 3500 water molecules. The surface area per lipid in the DLPE monolayer was about 50 $Å^2$ as modeled in [12] in accordance with experimentally measured values [13] (see Refs. [11] and [12] and references therein). All three systems, comprising about 16,000 atoms each, were equilibrated in 50 ps simulations with coupling to a thermal bath at 300 K, followed by 50 ps

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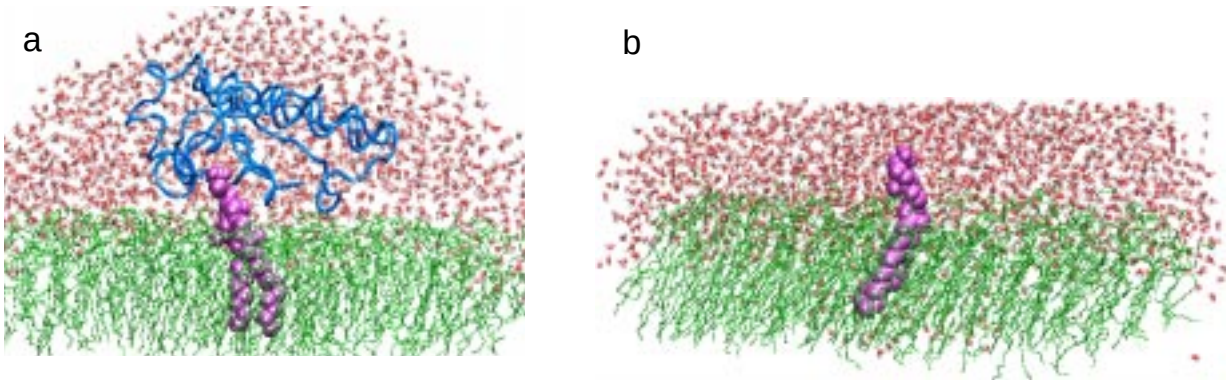

Figure 1. *(a) Extraction of the lipid (purple spheres) from the DLPE monolayer (green lines) by protein phospholipase A_2 (blue tube) solvated in water (red). (b) Extraction of a lipid (purple spheres) from the DLPE monolayer (green lines) into the aqueous phase. This figure was produced using the program VMD [14], URL: http://www.ks.uiuc.edu/Research/vmd/.*

of free dynamics. Further simulations were performed without temperature coupling.

SMD [6] has been employed to simulate the extraction of a lipid molecule from a DLPE monolayer. In SMD simulations a macromolecular system is subject to user-defined external forces chosen to induce reactions on a nanosecond time scale. In the simulations reported here, external forces were applied to the amino nitrogen atom of the head group of the lipid by harmonically restraining this atom to a restraint point which was moved perpendicular to the surface of the membrane with a constant velocity [5, 9,10]. The lipid, thus, was pulled out from the membrane into the aqueous phase or into the active site of PLA_2. The absolute value of the external force acting on the lipid is given by $F(x,t) = K(vt - x)$, where x is the displacement of the restrained atom, v = 0.014 Å/ps, is the chosen velocity of the restraint point and K = 10 kcal/mol·Å^2 is the chosen harmonic restraint force constant.

The simulations were carried out using the program NAMD [15] with version 19 of the CHARMM [16] force field. Repulsive harmonic boundary conditions were imposed on the systems to avoid the escape of water molecules. In addition, oxygen atoms of the water molecules in the 2 Å outer layer of the system were harmonically restrained to their initial positions. In all simulations we assumed a dielectric constant ε = 1 and a cut-off of Coulomb forces with a switching function starting at 9.5 Å and reaching zero at a distance of 11.5 Å. All atoms, except for nonpolar hydrogens,

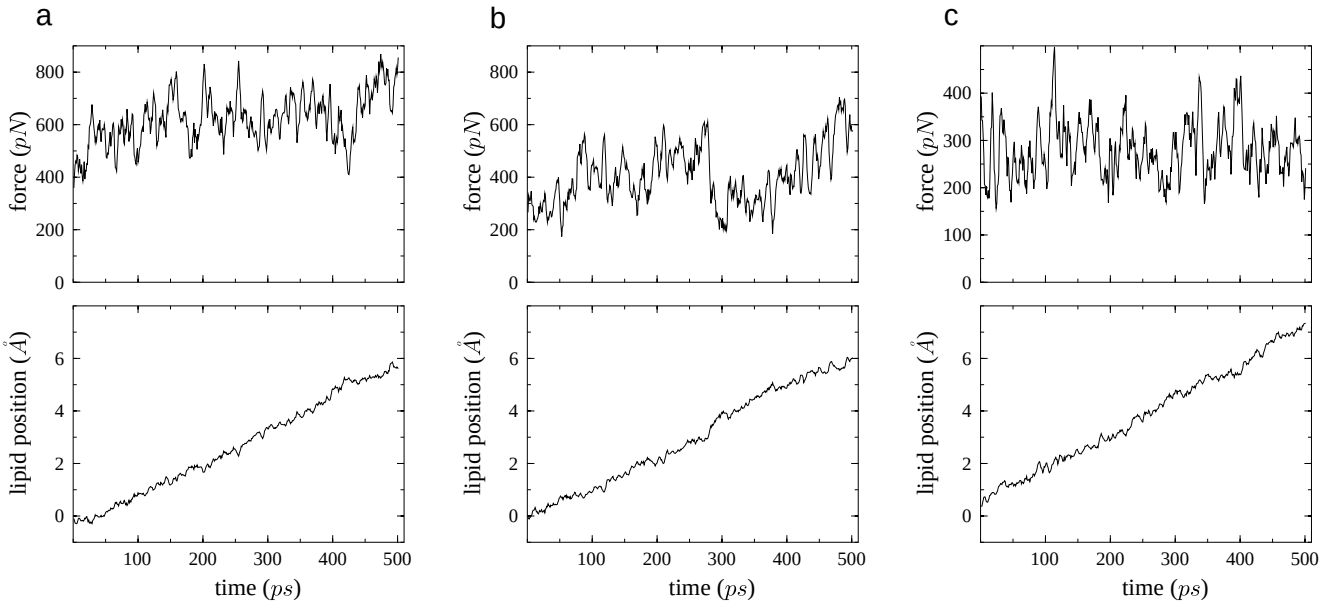

Figure 2. *Time averaged forces acting on the extracted lipid and positions of the lipid as functions of time in the simulations of (a) tightly bound and (b) loosely bound complexes of PLA_2 with the membrane, as well as for (c) a DLPE monolayer.*

were described explicitly. An integration time step of 1 fs was employed.

Results and Discussion

The forces as well as the positions of the lipid head group as functions of time are presented in Fig. 2. Due to the stiff restraint, the head group of the lipid was not allowed to fluctuate substantially and its motion essentially followed that of the restraint point, i.e., the head group moved with a constant velocity. The forces required to induce the extraction of the lipid exhibited large fluctuations of the order of 300 pN, as expected when a stiff restraint is employed [6-7]. The simulations showed that forces of 400–600 pN were required to break the hydrogen bonds between the phosphate and amino groups of the extracted lipid and the corresponding groups of the neighboring lipids. Breaking of these bonds was accompanied by an abrupt decrease of the force applied to the extracted lipid at 400 ps for the tightly bound complex (see Fig. 2a), and at 280 ps for the loosely bound complex (see Fig. 2b). In the absence of PLA_2, however, the lipid head groups were well solvated and did not form hydrogen bonds with each other. The average force applied to the lipid in the simulations of the DLPE monolayer in the absence of PLA_2 was nearly constant, i.e., of the order of 300 pN as shown in Fig. 2c.

The temperature of the systems increased by 2° during the simulation. Test runs (data not shown) indicated that the qualitative picture does not change if the external force is applied to a different atom of the lipid head group.

The forces required to displace the lipid into the binding pocket of PLA_2 for the loosely and tightly bound complexes were similar, within the range of force fluctuations. These forces were larger than those required to displace the lipid from the membrane into the aqueous phase. The differences in the measured forces were partially due to the fact that the steric hindrance experienced by the lipid on its way out of the membrane into the aqueous phase was less than that for the movement into the active site of PLA_2. The presence of hydrophobic residues surrounding the entrance of the active site of PLA_2 induced formation of hydrogen bond networks between the head groups of the lipids, resulting in stronger interactions of the lipid with the membrane than those in the absence of PLA_2. These results do not agree with the previously proposed hypothesis of destabilization of the lipids by PLA_2 [11]. Such a disagreement may have resulted from (i) an imperfect choice of the pulling direction for the lipid extraction into the enzyme, or (ii) insufficient sampling, i.e., short (500 ps) times of simulations. The short sampling times also made it impossible so far to perform further quantitative comparison of the forces measured in the simulations of the tightly and loosely bound complexes of PLA_2 with the membrane.

In spite of the described difficulties, our results show that SMD simulations of lipid extraction in protein-membrane complexes are already feasible. However, in order to draw conclusions about the qualitative mechanism of heterogeneous catalysis through phospholipase A_2, e.g., desolvation of lipid head groups and steering through the active site, and to determine quantitative energy profiles governing the binding of lipids by the enzyme, extensive SMD simulations on longer time scales are required.

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