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Sphingosine 1-phosphate pK_a and binding constants: Intramolecular and intermolecular influences☆

Mor M. Naor [a], Michelle D. Walker [b], James R. Van Brocklyn [c], Gabor Tigyi [b], Abby L. Parrill [a,*]

[a] *Department of Chemistry and Computational Research on Materials Institute, The University of Memphis, Memphis, TN 38152, United States*
[b] *Department of Physiology and University of Tennessee Cancer Institute, University of Tennessee Health Science Center, Memphis, TN 38163, United States*
[c] *Division of Neuropathology, Department of Pathology, The Ohio State University, Columbus, OH 43210, United States*

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Abstract

The dissociation constant for an ionizable ligand binding to a receptor is dependent on its charge and therefore on its environmentally-influenced pK_a value. The pK_a values of sphingosine 1-phosphate (S1P) were studied computationally in the context of the wild type $S1P_1$ receptor and the following mutants: E3.29Q, E3.29A, and K5.38A. Calculated pK_a values indicate that S1P binds to $S1P_1$ and its site mutants with a total charge of −1, including a +1 charge on the ammonium group and a −2 charge on the phosphate group. The dissociation constant of S1P binding to these receptors was studied as well. The models of wild type and mutant proteins originated from an active receptor model that was developed previously. We used *ab initio* RHF/6-31+G(d) to optimize our models in aqueous solution, where the solvation energy derivatives are represented by conductor-like polarizable continuum model (C-PCM) and integral equation formalism polarizable continuum model (IEF-PCM). Calculation of the dissociation constant for each mutant was determined by reference to the experimental dissociation constant of the wild type receptor. The computed dissociation constants of the E3.29Q and E3.29A mutants are three to five orders of magnitude higher than those for the wild type receptor and K5.38A mutant, indicating vital contacts between the S1P phosphate group and the carboxylate group of E3.29. Computational dissociation constants for K5.38A, E3.29A, and E3.29Q mutants were compared with experimentally determined binding and activation data. No measurable binding of S1P to the E3.29A and E3.29Q mutants was observed, supporting the critical contacts observed computationally. These results validate the quantitative accuracy of the model.

Keywords: GPCR; pK_a; Sphingosine 1-phosphate; $S1P_1$; Agonist

1. Introduction

Sphingosine 1-phosphate (S1P) is a bioactive lipid with broad biological effects. In the last decade, S1P was found to act as an agonist of a G protein-coupled receptor (GPCR), EDG-1/$S1P_1$ [1]. This led to the discovery and classification of additional S1P-responsive GPCR in the endothelial differentiation gene (EDG) family, EDG-3/$S1P_3$ [2], EDG-5/$S1P_2$ [2,3], EDG-6/$S1P_4$ [4,5] and EDG-8/$S1P_5$ [6,7] with 40–50% sequence identity [8]. S1P receptors regulate endothelial cell migration both positively ($S1P_1$ and $S1P_3$) and negatively ($S1P_2$) [9,10]. S1P receptors are important for enhancement of cell survival, cell proliferation, regulation of the actin-based cytoskeleton affecting cell shape, adherence, chemotaxis, and the activation of Cl^- and Ca^{2+} ion conductances [11–13]. The $S1P_1$ receptor is the target of a novel immunosuppressive agent in phase III clinical trials to treat transplant rejection [14] and is the focus of ongoing efforts in multiple laboratories to identify novel agonists with similar therapeutic promise [15–24].

Abbreviations: S1P, sphingosine 1-phosphate; IEF-PCM, C-PCM conductor-like polarization continuum model; GPCR, G protein-coupled receptor; EDG, endothelial differentiation gene; DFT, density functional theory; DMEM, Dulbecco's modified minimal essential medium; RH7777, rat hepatoma 7777; HEK, human embryonic kidney; BSA, bovine serum albumin; FBS, fetal bovine serum; SDS, sodium dodecyl sulfate; EDTA, ethylene diamine tetra-acetic acid

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* Corresponding author. Tel.: +1 901 678 2638; fax: +1 901 678 3447.
E-mail address: aparrill@memphis.edu (A.L. Parrill).

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GPCR exhibit conformational equilibrium between active and inactive conformations [25,26]. In the simplest model of ligand influence on GPCR equilibria, ligands can bind to and stabilize the active conformation (agonist), the inactive conformation (inverse agonist) or can bind to both conformations without preference (neutral antagonist). We have previously reported models of active ($S1P_1$, $S1P_4$, LPA_{1-3}) and inactive (LPA_{1-3}) conformations of EDG family members in complex with both agonists and antagonists [27–33]. These previous studies have largely focused on validating qualitative structure-based predictions regarding relative binding affinities and roles of amino acids in binding. The present study focuses on the validation of the active conformation of the $S1P_1$ receptor as a quantitatively accurate tool to examine agonist binding. However, the charge on the S1P phosphate group in the receptor binding site is ambiguous due to the overlap of the second pK_a value with the biological pH range. As binding affinity depends strongly on the charge of the S1P phosphate group, the environmental dependence of the phosphate group pK_a must be computed before binding affinities can be addressed. Accurate pK_a and binding affinity computation requires a model that includes coulombic interactions, hydrophobic interactions, and hydrogen bond interactions between the ligand and the receptor as well as intramolecular interactions of these types within the ligand.

The pK_a of receptor-bound S1P was determined using the method Li and Jensen [34] applied to determine amino acid sidechain pK_a values. This method extends from initial theoretical models by Tanford and Kirkwood that treated all ionizable sidechains as points on an impenetrable spherical protein surface [35], by Shire et al. who incorporated static solvent accessibility terms to compensate for the assumption of a smooth boundary between the exterior and interior of the protein [36], by Warshel who described the importance of electrostatic solvation differences due to both permanent and induced protein dipoles [37], and by Bashford and Karplus who eliminated the need to estimate intrinsic pK_a corrections [38]. Since our protein structure is a computational model, we validated its structure by calculating dissociation constants for a series of receptor mutants and compared the computed binding affinities to experimental results. Accurate binding affinity results validate both the computed pK_a values and the use of homology models of EDG receptors for quantitative studies of agonist binding. In this paper, we present the dissociation constant calculation approach, and the pK_a values and binding constants of S1P in the wild type $S1P_1$ receptor and its mutants.

2. Methodology

2.1. Theoretical basis

2.1.1. pK_a calculation

Following the method developed by Li and Jensen [34] for carboxyl pK_a values, the pK_a of the phosphate group in S1P when it is bound to a receptor, R·HS1P, is related to the standard free energy change, ΔG, of the proton exchange reaction with a reference molecule, Ref.:

$$\mathrm{R \cdot HS1P + Ref.^- \rightleftharpoons R \cdot S1P^- + Ref.H} \quad (1)$$

By the equation

$$\mathrm{p}K_a = 5.66 + \frac{\Delta G}{1.36} = 5.66 + \frac{[G(\mathrm{R \cdot S1P^-}) - G(\mathrm{R \cdot HS1P})] - [G(\mathrm{Ref.^-}) - G(\mathrm{Ref.H})]}{1.36} \quad (2)$$

The value 5.66 is the experimental pK_a value of the reference molecule *O*-phosphoethanolamine [39], at 298 K. This pK_a value describes the transition between ionization states of 0 and −1. The value 1.36 is 2.303RT at the same temperature in kilocalories per mole. The free energy of a molecule X, $G(X)$, is given by the sum of ground state electronic energy E_{ele} and the solvation energy G_{sol}.

$$G = E_{elec} + G_{sol} \quad (3)$$

Free energies were calculated using structures optimized at the RHF/6-31(+)G* level including solvation energy using a new geometry optimization algorithm, developed by Li and Jensen [34]. Solvation energy and energy derivatives at each optimization step were calculated using the conductor-like polarizable continuum model (C-PCM) [40,41] and integral equation formalism polarizable continuum model (IEF-PCM) [42], using GAMESS [43] parameters: IEF = −10, and RET = 100, to prevent use of additional spheres. The iterative solvent was water. Diffuse functions (L shell) were added to the heavy atoms on the negatively charged groups (one carbon, four oxygen, and one phosphorus atom of the phosphate group and two carbon and two oxygen atoms of the glutamic acid sidechain) under consideration. The United Atom Hartree Fock (UAHF) [44] radii determined with Gaussian98 [45] were used to define the molecular cavities. The energies were calculated using the quantum mechanics program GAMESS [43]. The

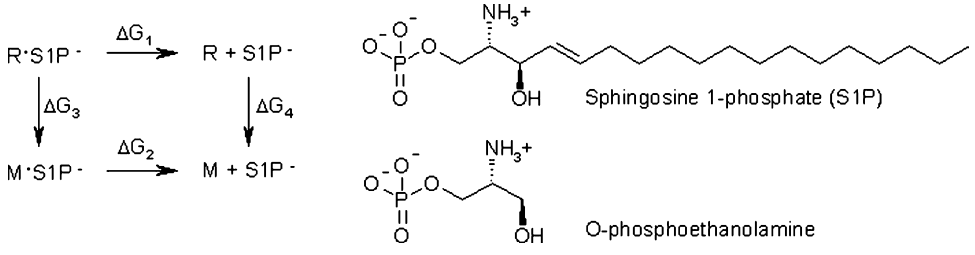

Fig. 1. Thermodynamic cycle for binding affinity of $S1P^-$ to a mutant (M) relative to the reference receptor (R).