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Thermodynamic and kinetic characterization of hydroxyethylamine β-secretase-1 inhibitors

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ABSTRACT

Alzheimer's disease (AD) is a devastating neurodegenerative disease affecting millions of people. β-Secretase-1 (BACE-1), an enzyme involved in the processing of the amyloid precursor protein (APP) to form Aβ, is a well validated target for AD. Herein, the authors characterize 10 randomly selected hydroxyethylamine (HEA) BACE-1 inhibitors in terms of their association and dissociation rate constants and thermodynamics of binding using surface plasmon resonance (SPR). Rate constants of association (k_a) measured at 25 °C ranged from a low of 2.42×10^4 M^{-1} s^{-1} to the highest value of 8.3×10^5 M^{-1} s^{-1}. Rate constants of dissociation (k_d) ranged from 1.09×10^{-4} s^{-1} (corresponding to a residence time of close to three hours), to the fastest of 0.028 s^{-1}. Three compounds were selected for further thermodynamic analysis where it was shown that equilibrium binding was enthalpy driven while unfavorable entropy of binding was observed. Structural analysis revealed that upon ligand binding, the BACE-1flap folds down over the bound ligand causing an induced fit. The maximal difference between alpha carbon positions in the open and closed conformations of the flap was over 5 Å. Thus the negative entropy of binding determined using SPR analysis was consistent with an induced fit observed by structural analysis.

1. Introduction

AD is a chronic and progressive neurodegenerative disorder characterized by irreversible loss of memory and cognitive decline leading to death. It is estimated that over 20 million people worldwide have the disease. Two defining hallmarks of AD are the presence of senile plaques consisting of insoluble amyloid β (Aβ) and neurofibrillary tangles mostly composed of hyperphosphorylated tau [1]. Biochemical and genetic data suggest that the pathogenesis of AD is associated with the accumulation of Aβ1–40 and Aβ1–42 known as the amyloid hypothesis [2]. Aβ1–40 and Aβ1–42 are formed by the sequential cleavage of APP by BACE-1 and γ-secretase [2,3].

BACE-1 is a type I integral membrane glycoprotein consisting of a 434 amino acid ectodomain, a single transmembrane domain of 22 amino acids and a short cytoplasmic tail. It is an aspartyl protease with an active site containing two conserved aspartic acid residues at Asp32 and Asp228 [4]. From a drug discovery perspective, BACE-1 is a validated target for AD and as such there has been significant interest in BACE-1 from the pharmaceutical industry. Specifically, the hydroxyethylene transition state isostere was identified as a scaffold which was optimized to yield potent compounds with cellular permeability and activity [5]. A series of HEA-based inhibitors were reported to have high potency and cell activity [6,7]. However, many of these peptidomimetic inhibitors suffered from poor metabolic stability or other pharmacokinetic (PK) issues [8]. Structure guided drug design approaches initiated by fragment screening have yielded more selective and tractable BACE-1 inhibitors, yet it remains to be determined how this new wave of inhibitors will perform in terms of efficacy and toxicity in clinical phase testing [9,10].

Most traditional medicinal chemistry efforts use simple IC_{50} measurements to drive their structure activity relationships (SAR) during lead optimization campaigns. Although this paradigm has been successful, a number of recent reviews have suggested the importance of determining the rate constants of association (k_a) and dissociation (k_d) for compounds as relevant parameters for optimization [11,12]. A fast k_a, or slow k_d of a compound from its receptor can under some circumstances mitigate poor PK properties of a compound or provide selectivity towards off-target effects [13,14]. Herein, we investigate the kinetics and thermodynamic properties of a series of HEA BACE-1 inhibitors.

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2. Materials and methods

2.1. Materials

Series S CM5 chips, amine coupling kit, HBS-N Buffer (10 mM HEPES pH 7.4, 150 mM NaCl), acetate buffers (10 mM acetate, pH 4.5 and pH 5.0) and 10% (v v^{-1}) Tween-20 solution were purchased from GE Healthcare (Piscataway, NJ). Recombinant β-secretase enzyme (BACE-1) was obtained from Proteos Inc. (Kalamazoo, MI). Cathepsin-D, neutravidin and DMSO were from Sigma Aldrich (Rockville, MD). EZ-NHS-LC-LC-Biotin and dialysis cassettes were purchased from Thermo-Fisher Scientific (Rockford, IL). Filter plates and vacuum device were obtained from Millipore Corporation (Billerica, MA) and 96- or 384 well plates from Greiner Bio-One Inc. (Monroe, NC).

2.2. SPR based assays and binding kinetics of BACE-1

BACE-1 was immobilized on a CM5 sensor chip using standard amine coupling procedure at a concentration of 20 μg mL^{-1} (in 10 mM acetic acid, pH 4.5) to a level of 1500–2000 response units (RU). Typically, the 4 flow cells of the sensor chips were used as follows: flow cell 1 served as a reference cell was activated and deactivated, flow cell 2,3,4 contained BACE-1 at different immobilization levels.

Kinetics and affinity experiments were performed at 25 °C using Biacore T200 instrument by initial injection of 20 buffer blanks to equilibrate the surfaces followed by the injection of analyte solutions at different concentrations in 2-fold dilutions over all flow cells. The running buffer for the binding experiments was PBS, pH 7.4 with 0.005% Tween-20 and 4% DMSO with a flow rate of 50 μL min^{-1}. Running buffer samples containing 2–6% DMSO were also injected to create a DMSO calibration plot.

The time-dependent binding curves were monitored simultaneously. Affinity constants were determined using the Biacore T200 evaluation software V.1 by curve fitting using a 1:1 binding model. Association and dissociation curves were fitted globally. The rate of complex formation during analyte injection was calculated according to the Eq. (1):

$$dR/dt = k_a C(R_{max} - R) - k_d R \quad (1:1 \text{ interaction}) \tag{1}$$

where R is the SPR signal in response units (RU), C is the concentration of analyte, R_{max} is the maximum analyte binding capacity in RU, dR/dt is the rate of SPR signal change. To determine the association constant k_a between analyte and protein the early binding phase was used. The dissociation phase k_d was measured using the rate of decline in RU after the injection stop when free running buffer is flowing over the surface. Data were simultaneously fitted by the software and the dissociation constant K_D calculated using Eq. (2).

$$K_D = k_d/k_a \tag{2}$$

2.3. Immobilization of neutravidin and binding kinetics of Cathepsin-D using SPR

Neutravidin was immobilized on a CM5 sensor chip using standard amine coupling procedure at a concentration of 200 μg mL^{-1} (in 10 mM acetic acid, pH 5.0) to a level of about 15,000 RU on two flow cells (Fc1 and 2).

2.4. Biotinylation of Cathepsin D (CAT-D)

CAT-D (0.5 mg mL^{-1}) was biotinylated in PBS using 1:0.5 M ratio of enzyme to EZ-link Sulfo-NHS-LC-LC-Biotin by incubating the enzyme with biotin at room temperature for 30 min. At the end of 30 min, the reaction mixture was transferred to ice. Unreacted biotin was removed by overnight dialysis in PBS. Biotinylated CAT-D was captured on neutravidin surface on Fc2 to a level of 3000–4000 RU. A Kinetic and affinity experiment on CAT-D surface was carried out similar to BACE-1 kinetic experiments as described above.

2.5. Determination of thermodynamic parameters by SPR

Temperature dependent binding interactions of different analytes with BACE-1 were carried out at 12, 18, 24 and 30 °C. At each temperature, 3 buffer blanks were first injected to equilibrate the surface. Analyte solutions were prepared in 2-fold dilutions in running buffer (PBS, pH 7.4 with 0.005% Tween-20 and 4% DMSO). To regenerate the surface, a blank injection was included between each analyte injection. The surface activity of BACE-1 during the course of the experiment was monitored by short injection of a control sample at each temperature. Van't Hoff analysis was used to estimate the enthalpy (ΔH^0) and entropy (ΔS^0) of binding by a linear regression analysis from K_D values obtained at different temperatures using the following Eq. (3):

$$\ln(K_D) = -\Delta H^0/(RT) + \Delta S^0/R \tag{3}$$

where R is the gas constant and T is the absolute temperature in Kelvin. Eyring analysis was used to determine the thermodynamic parameters (ΔH_{on}, ΔH_{off}, ΔS_{on} and ΔS_{off}) from the rate constants at different temperatures according to the following equations in a similar manner as the van't Hoff analysis:

$$\ln(k_{on} C\hbar/k_b T_\kappa) = -\Delta H_{on}/(RT) + \Delta S_{on}/R \tag{4}$$

$$\ln(k_{off}\hbar/k_b T\kappa) = -\Delta H_{off}/(RT) + \Delta S_{off}/R \tag{5}$$

where k_b is the Boltzman constant, ħ is Planck's constant, C is the state of the solvent and κ is the transmission coefficients which were both set to 1.

2.6. Crystallization and X-ray structure determination

BACE-1 protein crystallization and X-ray structure determination was carried out as described previously [9]. Molecular graphics images were created with the PyMOL Molecular Graphics Systems, Version 1.5.0.5 Schrödinger, LLC.

3. Results

3.1. Affinity, association and dissociation constant determinations by SPR

An affinity constant, K_D, is the ratio of the dissociation rate constant (k_d) and the association rate constant (k_a). Compounds with similar affinities may have markedly different k_a and k_d which comprise that affinity measurement. In drug discovery programs it can often be advantageous for a drug to have a slow k_d, manifested as a long residency time on the receptor (residency time = $1/k_d$), which may mitigate PK liabilities. Hence, we have randomly selected 10 of our proprietary BACE-1 HEA inhibitors of low, medium, and high potency and performed a kinetic analysis of these compounds using SPR with the Biacore T200 instrument.

In Table 1, we provide affinity K_D, k_a, and k_d measurements for HEA containing compounds 1–10. Association rate constant k_a values ranged from a low of 2.42×10^4 M^{-1} s^{-1} for Compound 6 to the highest value of 8.3×10^5 M^{-1} s^{-1} for Compound 2 (Table 1). For the k_d of HEA containing compounds, values ranged from slowest for Compound 1 of 1.09×10^{-4} s^{-1} to fastest for Compound 10 of 0.028 s^{-1} (Table 1).