



Effects of serum albumin on SPR-measured affinity of small molecule inhibitors binding to nerve growth factor



Allison E. Kennedy^{a,b}, Rahul Vohra^c, John A. Scott^d, Gregory M. Ross^{b,*}

^a Laurentian University, Biomolecular Sciences, Sudbury, ON, Canada

^b Northern Ontario School of Medicine, Sudbury, ON, Canada

^c Sussex Research Laboratories Inc., Ottawa, ON, Canada

^d Laurentian University, Bharti School of Engineering, Sudbury, ON, Canada

ARTICLE INFO

Keywords:

NGF
Drug binding
Pain
Therapeutic
Surface plasmon resonance
Pharmacokinetics

ABSTRACT

The study of the interactions between a drug and plasma serum proteins are necessary in determining pharmacological and toxicological properties for therapeutic development. Small molecule nerve growth factor (NGF) inhibitors have been investigated for their abilities to inhibit NGF binding to TrkA as a potential therapeutic option for the treatment of neuropathic and inflammatory pain. In this study, surface plasmon resonance (SPR) spectroscopy and ¹²⁵I-NGF radioisotope binding assays were carried out to better understand the role of serum albumin (SA) in small molecule binding to NGF. SA has been characterized as a universal drug carrier with up to seven binding domains on its surface to transport drug molecules to target tissues. Here, we use SPR kinetic analysis to analyze the change in specificity of small molecules to immobilized NGF in the presence and absence of SA. In the presence of SA an overall increase in small molecule binding affinity for NGF was observed compared to binding in the absence of SA. Our results suggest a crucial role for SA in the pharmacokinetics of small molecule binding to NGF. This effect will require consideration when developing therapeutic agents.

1. Introduction

Nerve growth factor (NGF) functions not only in prenatal nerve growth, but also has a significant role in pain and immune function in adults [1,2]. During tissue damage, NGF, interleukins and tumour necrosis factor- α are secreted by immune cells resulting in painful pathologies [3,4]. NGF in particular leads to hyperalgesia and allodynia by triggering peripheral and central neuronal sensitization by activating the receptor TrkA [5,6]. In such conditions, having an analgesic with the ability to block NGF binding to TrkA and the resulting painful signal would hold great therapeutic advantage.

It is estimated that 20–30% of adults suffer from chronic pain, many of whom are not adequately relieved from the painful symptoms with current therapeutic options [7,8]. Targeting NGF-TrkA signalling for the development of novel therapeutic agents has been the focus of several studies. Currently the humanized monoclonal anti-NGF antibody Tanezumab is in Phase III clinical trials for sequestering free NGF for the treatment of arthritic joint pain [9]. Despite being highly selective for NGF, there are many challenges to antibodies as a therapeutic agent including autoimmune responses, delivery challenges and production capacity [10].

Small molecule-based inhibitors have emerged as a possible therapeutic option for inhibiting NGF. Small molecule NGF-inhibitors such as PD 90780 [11], ALE 0540 [12], Ro 08-2750 [13] and PQC-083 [14] have demonstrated their efficacy in inhibiting NGF *in vitro* and in some cases *in vivo*. Flexible docking experiments suggest that these small molecule inhibitors bind to NGF at the loop I/IV cleft to alter the molecular topology in a manner which alters the binding capacity for the receptor TrkA [15]. More recently, a series of bivalent naphthalimide analogues were screened using surface plasmon resonance (SPR) spectroscopy to identify lead small molecule NGF-inhibitors with nanomolar inhibitory efficiency binding to immobilized TrkA (in press).

Serum albumin (SA) is an abundant plasma protein in the circulatory system that functions as the main transport vehicle for fatty acids [16,17]. SA is also capable of transporting a wide variety of drug molecules, which affects the pharmacokinetics and pharmacodynamics of these agents [18,19]. Binding to SA alters the plasma half-life of a drug, significantly lowers the rate of clearance and changes the volume and the pattern of drug distribution [20]. In addition, SA has a complex binding capacity with small molecules, which makes it difficult to study. There are at least seven hydrophobic binding pockets on the surface of human serum albumin (HSA) that act as universal receptors

* Corresponding author at: Northern Ontario School of Medicine, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, Canada.
E-mail addresses: allkennedy@nosm.ca (A.E. Kennedy), greg.ross@nosm.ca (G.M. Ross).

for drug molecules and modulate cell delivery [21,22]. Due to the high ligand binding capacity of SA, it has become an interest to pharmaceutical companies for drug development although yet to be investigated in respect to small molecule NGF-inhibitor binding.

Herein, we report the change in binding affinity for NGF during small molecule interactions with bovine serum albumin (BSA). In this study, BSA was used as a model protein because of its structural homology with HAS [23], and to enable comparison to receptor binding assays used for NGF studies. In the presence of BSA, small molecule-based inhibitors showed an overall increase in binding affinity for immobilized NGF. This increase in binding affinity accounts in part for the discrepancy observed when comparing binding data of small molecule-based inhibitors using cell-free systems, such as SPR, and traditional *in vitro* methods. In addition, these findings may provide valuable information necessary for drug delivery and drug design of NGF-inhibitor pain therapeutics.

2. Materials and methods

2.1. Surface plasmon resonance spectroscopy

A BIAcore T200 spectrometer was obtained from GE Healthcare Life Science (Piscataway, NJ, USA). Full length (1–118) mouse NGF purified by HPLC from 2.5S NGF (purity > 95%) was obtained from Cedarlane Laboratories (Toronto, ON) and coupled to a CM5 sensor chip using standard amine coupling methods. Data was collected at a temperature of 25 °C. Individual compound samples were tested from lowest to highest concentrations, separated by a 15 second stabilization period after each sample in each compound series. During each sample cycle, analyte was injected for 60 s at a flow rate of 30 $\mu\text{L}/\text{min}$. A dissociation period was monitored for 30 s after analyte injection before regeneration occurred with a 1.0 M NaCl for 120 s at a flow rate of 30 $\mu\text{L}/\text{min}$ to wash any remaining analyte from the sensor chip before running the next sample. Synthetic organic compounds were synthesized by Sussex Research (Ottawa, ON, Canada) and diluted to concentrations ranging from 0.78 μM to 200 μM using HBS-EP buffer with 0.5% DMSO (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA and 0.05% v/v Tween 20; pH 7.5). To determine binding affinity, the response obtained from each sample run in a series was plotted against concentration using the BIA evaluation software version 2.0 and was evaluated using a two steady-state site binding model as previously described [24]. Data was also analyzed using a heterogeneous ligand model to determine kinetic rate constants.

2.2. Binding of ^{125}I -NGF to PC12 cells

The iodination of NGF was performed as previously described [25]. The ^{125}I -NGF obtained (typically 80–120 c.p.m./pg) was purified by size exclusion chromatography on a PD10 column (Pharmacia) pre-equilibrated with HKR buffer (10 mM HEPES [pH 7.35] containing 125 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 1 g/L glucose, 1 g/L BSA). PC12 cells were grown in RPMI with 10% fetal calf serum. Each data point set up in a single tube containing ^{125}I -NGF (at the required concentration), 400,000 cells (for a final concentration of $10^6/\text{mL}$) and NGF (at 10 nM for nonspecific binding) as required. The tubes were incubated for 2 h at 4 °C, and 100 μL aliquots (providing triplicate data points for each sample) were transferred to 400 μL microcentrifuge tubes containing 200 μL of 10% glycerol in HKR. Tubes were centrifuged for 2 min at 5000 rpm, the tip containing the cell pellet was cut off, and radioactivity bound to the cells was determined.

3. Results

A series of small molecule NGF inhibitors were screened using SPR spectroscopy for their binding affinity to immobilized NGF. A two-site binding model was applied using BIAevaluation software (Version 2.0).

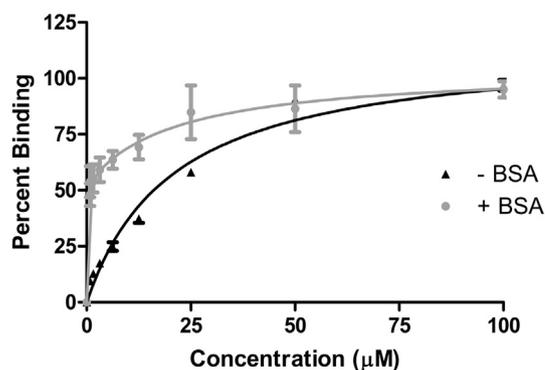


Fig. 1. Binding curve of BVNP-0197 to immobilized NGF. Two-site steady-state binding curve of small molecule NGF-inhibitor BVNP-0197 binding to immobilized NGF. X-axis: concentration (μM); Y-axis: percent binding subtracted response (response measured from active flow cell minus response measured from reference flow cell). Samples run in the absence of BSA are represented in black triangles; samples containing 1% BSA are represented in grey circles. Standard error of the mean is shown with error bars ($n = 3$).

The response obtained due to binding from each analogue concentration was plotted against concentration to determine binding affinity (K_D). To determine the effect of BSA on small molecule binding to immobilized NGF, samples were suspended in HBS-EP buffer with 0.5% DMSO in the presence and absence of 1% w/v BSA. Fig. 1 represents the two-site steady-state affinity plot for the binding of BVNP-0197 (500–0.78 μM) to immobilized NGF. The binding affinity of BVNP-0197 for NGF in the absence of BSA was measured at 21.0 μM . The addition of 1% BSA increased the binding affinity of BVNP-0197 for NGF to 0.428 μM . Table 1 describes the changes in SPR measured binding affinity for all small molecule compounds to immobilized NGF in the presence and absence of BSA.

Kinetic rates were determined using a heterogeneous ligand model in the BIAevaluation software (Version 2.0). The marked increase in binding affinity of BVNP-0197 was measured by a decrease in rate of association as well as an increase in rate of dissociation (Fig. 2). The subtracted sensogram of BVNP-0197 (200 μM) was chosen to represent the changes in kinetic rates (Fig. 2) however each analyte injection showed similar changes in their respective phases. All small molecule compounds were subjected to kinetics rate analysis which demonstrated the same trend in results (data not shown).

Binding saturation studies with ^{125}I -NGF *in vitro* were completed to measure inhibitory potential for each compound. A half maximal inhibitory concentration (IC_{50}) was determined for each small molecule to determine the concentration by which ^{125}I -NGF binding was inhibited by 50% (Table 1). The relationship between SPR high affinity binding of small molecule compounds to immobilized NGF and the respective IC_{50} measured using *in vitro* ^{125}I -NGF binding was found to be statistically significant when SPR binding was completed in the

Table 1
Binding measurements to NGF in cell-free and cell-based assays.

| Compound | K_D to NGF-SPR binding (μM) | | IC_{50} ^{125}I -NGF Binding (μM) |
|------------|--|--------|--|
| | 0% BSA | 1% BSA | |
| BVNP-0193 | 22.4 | 1.43 | 0.001 |
| BVNP-0198 | 5.61 | 0.45 | 0.003 |
| BVNP-0183 | 4.03 | 0.86 | 0.013 |
| BVNP-0195 | 9.61 | 2.92 | 0.013 |
| BVNP-0197 | 21.0 | 0.43 | 0.030 |
| BVNP-0191 | 5.13 | 4.81 | 0.033 |
| BVNP-0196 | 61.0 | 1.19 | 0.070 |
| BVNP-0190 | 4.34 | 4.23 | 0.080 |
| BVNP-0182 | 57.7 | 1.62 | 0.530 |
| BVNP-0188 | 14.0 | 4.21 | 1.830 |
| BVNP-0194 | 16.0 | 2.37 | 50.00 |
| Ro 08-2750 | 36.9 | 8.88 | 86.00 |

Download English Version:

<https://daneshyari.com/en/article/5019688>

Download Persian Version:

<https://daneshyari.com/article/5019688>

[Daneshyari.com](https://daneshyari.com)