Sensing and Bio-Sensing Research 15 (2017) 1–4

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

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 ^{125}I-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).

https://doi.org/10.1016/j.sbsr.2017.06.001
Received 10 March 2017; Received in revised form 24 May 2017; Accepted 12 June 2017

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 µL/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 µL/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/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).

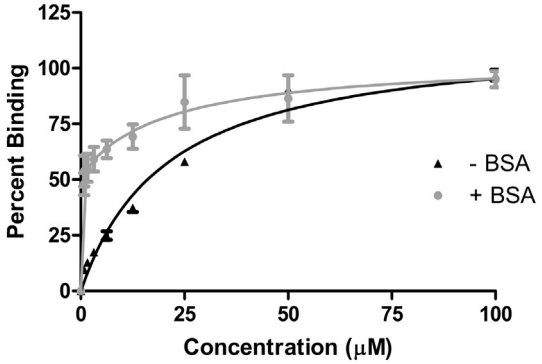

Fig. 1. Binding curve of BVNP-0197 to immobilized NGF. Two-site steady-state binding curve of small molecule NGF-inhibitor BVNP-0197 binding to immobilized NGF. X-axis: concentration (µM); Y-axis: percent binding subtracted response (response measured from active flow cell minus response measured from 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

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

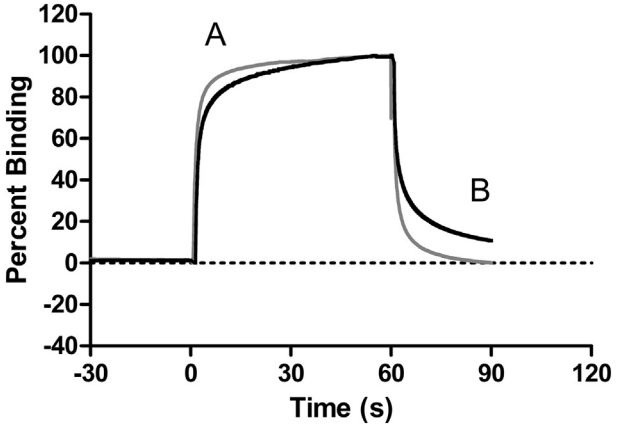

Fig. 2. Kinetic rate analysis of BVNP-0197 binding to immobilized NGF. Subtracted sensogram of BVNP-0197 (200 μM) outlining the changes in kinetic rate of small molecule binding to immobilized NGF. Time 0–60 s represents the association of BVNP-0197 to NGF; at 60 s the analyte flow stops. A dissociation period from 60 to 90 s is monitored before regeneration of the chip occurs before the next sample is run. Black: BVNP-0197 binding to NGF in the absence of BSA. Grey: BVNP-0197 binding to NGF in the presence of BSA. (A) BVNP-0197 shows a decreased rate of association with the presence of BSA to immobilized NGF. (B) BVNP-0197 shows an increased rate of dissociation with the presence of BSA from immobilized NGF.

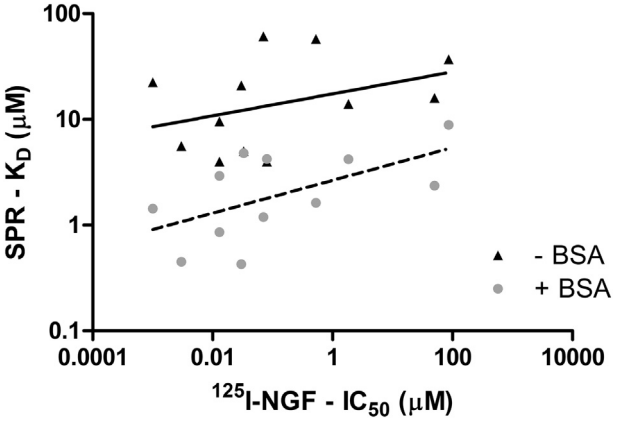

Fig. 3. Relationship between binding data from cell-free and cell-based assays. X-axis: IC_{50} measured from cell-based ^{125}I-NGF binding (μM); Y-axis: High affinity K_D measured using cell-free SPR binding to immobilized NGF (μM). SPR analysis was completed in the presence (black triangle) and absence (grey circles) of 1% BSA during binding to immobilized NGF. SPR binding in the presence of BSA was correlated to the cell-based binding measurements ($F(1,12) = 5.070$; $p = 0.0480$; $R^2 = 0.3364$) (dotted black line). This relationship was not statistically significant when SPR binding was completed in the absence of BSA (solid black line).

respective IC_{50} measured using *in vitro* ^{125}I-NGF binding was found to be statistically significant when SPR binding was completed in the presence of BSA ($F(1,12) = 5.070$; $p = 0.0480$; $R^2 = 0.3364$) (Fig. 3). This relationship was not statistically significant when SPR binding was completed in the absence of BSA ($F(1,12) = 1.643$; $p = 0.2289$; $R^2 = 0.1411$).

4. Discussion

Early phase drug discovery is focused on identifying compounds with optimal affinity and selectivity of drug targets. Methods for determining target receptor affinity is being shifted towards cell-free systems which can accurately measure direct binding of a compound to its target receptor without the influence of other identities in the system [26,27]. SPR is one technology available to measure drug specificity to a target protein using a label-free method with high enough sensitivity to look at small molecule compounds (< 500 Da) binding to larger target proteins (> 26 kDa) [28,29].

Although drug interactions are often described as a IC_{50} or K_D value, emphasis on drug kinetics is also ideal for identifying lead compounds during drug development [27,30,31]. A drug with a shorter association phase translates into higher target receptor specificity and likely reduced side effects if used as a therapeutic. In addition, a longer target receptor dissociation phase is generally ideal for reducing therapeutic doses and extending the drug effects. Alternatively, if side effects related to drug toxicity are of concern, a shorter target receptor dissociation phase can be targeted.

Small molecule NGF-inhibitors have been an area of focus for nearly two decades as a potential therapeutic option for pain. NGF plays a large role in pain signalling through the TrkA receptor in conditions where an inflammatory pain responses is persistent, such as in autoimmune diseases or arthritic pain [32–34]. Recently, screening for lead small molecule NGF-inhibitors has seen success with cell-free SPR techniques, with identifying novel small molecule compounds with higher specificity for NGF and increased inhibitory potential in altering NGF-TrkA binding than any previously reported compound [24,35,36]. However, with this transition of measuring binding in cell-free systems, a discrepancy exists when comparing values to traditional cell-based techniques. These discrepancies may be a result of a carrier protein influencing *in vitro* small molecule-NGF binding, which is not present during SPR cell-free assays.

SA has been identified as a universal drug carrier to aid drug delivery to targeted tissues [18,37,38]. Cell culture protocols, such as ^{125}I-NGF binding experiments, require the addition of SA to media to deliver important nutrients to cells, as well as to bind toxins and free radicals reducing cell damage [39]. SA may also play a critical role during small molecule binding to NGF *in vitro*. The presence of 1% BSA during cell-free analysis of small molecule compounds binding to immobilized NGF measured an overall increase in affinity when compared to binding in the absence of BSA. Kinetic rate analysis determined that in the presence of 1% BSA, small molecule compounds had a decreased rate of association for immobilized NGF in addition to an increased rate of dissociation (Fig. 2). These changes in kinetic rate account for the increase in measured binding affinity and could possibly translate into an increased specificity for NGF with less possible toxic effects if used as therapeutic agents.

SPR analysis of small molecule binding to immobilized NGF in the presence of 1% BSA was found to be statistically correlated to values determined through ^{125}I-NGF binding (Fig. 3). This relationship is not statistically significant in the absence of BSA. Thus, it is possible that BSA acts as a carrier protein for the small molecules for increased affinity for NGF. It is hypothesized that BSA binds small molecule compounds, enabling a small axial rotation of the compound's structure, placing the small molecule in a conformation ideal for high affinity binding to available NGF.

5. Conclusions

SPR cell-free techniques provide an advantage in drug development by eliminating other potentially influencing proteins traditionally found in cell-based binding assays. By introducing proteins independently, SPR allows for a more clear understanding of the binding events which occur *in vitro* affecting drug activity. By identifying carrier proteins which participate in small molecule binding to NGF the development of future pain therapeutics will evolve to account for such protein interactions *in vivo*. BSA has shown to alter small molecule binding to immobilized NGF in a cell-free system. This shift in binding affinity for NGF in the presence of BSA eliminates the previously observed variance in binding measurements when compared to cell-based assays. These results suggest that BSA plays a critical role in small

molecule binding efficacy to NGF *in vitro*. In addition, these results will allow tailoring future development strategies to account for the influence of carrier proteins to identify therapeutic lead agents.

Conflict of interest

The authors don't have any conflict of interest.

Funding

There are no funding sources associated with this research program.

References

[1] L. Aloe, M.L. Rocco, P. Bianchi, L. Manni, Nerve growth factor: from the early discoveries to the potential clinical use, J. Transl. Med. 10 (2012) 239, http://dx.doi.org/10.1186/1479-5876-10-239.
[2] R.K.H. Gerber, H. Nie, L. Arendt-Nielsen, M. Curatolo, T. Graven-Nielsen, Local pain and spreading hyperalgesia induced by intramuscular injection of nerve growth factor are not reduced by local anesthesia of the muscle, Clin. J. Pain 27 (2011) 240–247, http://dx.doi.org/10.1097/AJP.0b013e3182048481.
[3] M.V. Chao, R. Rajagopal, F.S. Lee, Neurotrophin signalling in health and disease, Clin. Sci. (Lond.) 110 (2006) 167–173, http://dx.doi.org/10.1042/CS20050163.
[4] S.D. Skaper, Nerve growth factor: a neurokine orchestrating neuroimmune-endocrine functions, Mol. Neurobiol. 24 (2001) 183–199, http://dx.doi.org/10.1385/MN:24:1-3:183.
[5] L. McKelvey, G.D. Shorten, G.W. O'Keeffe, Nerve growth factor-mediated regulation of pain signalling and proposed new intervention strategies in clinical pain management, J. Neurochem. 124 (2013) 276–289, http://dx.doi.org/10.1111/jnc.12093.
[6] M.B. Max, W.F. Stewart, The molecular epidemiology of pain: a new discipline for drug discovery, Nat. Rev. Drug Discov. 7 (2008) 647–658, http://dx.doi.org/10.1038/nrd2595.
[7] M. Hirose, Y. Kuroda, E. Murata, NGF/TrkA signaling as a therapeutic target for pain, Pain Pract. 16 (2016) 175–182, http://dx.doi.org/10.1111/papr.12342.
[8] D. Schopflocher, P. Taenzer, R. Jovey, The prevalence of chronic pain in Canada, Pain Res. Manag. 16 (2011) 445–450.
[9] D.S. Chang, E. Hsu, D.G. Hottinger, S.P. Cohen, Anti-nerve growth factor in pain management: current evidence, J. Pain Res. 9 (2016) 373–383, http://dx.doi.org/10.2147/JPR.S89061.
[10] H. Samaranayake, T. Wirth, D. Schenkwein, J.K. Räty, S. Ylä-Herttuala, Challenges in monoclonal antibody-based therapies, Ann. Med. 41 (2009) 322–331, http://dx.doi.org/10.1080/07853890802698842.
[11] A. Colquhoun, G.M. Lawrance, I.L. Shamovsky, R.J. Riopelle, G.M. Ross, Differential activity of the nerve growth factor (NGF) antagonist PD90780 [7-(benzolylamino)-4,9-dihydro-4-methyl-9-oxo-pyrazolo[5,1-b]quinazoline-2-carboxylic acid] suggests altered NGF-p75NTR interactions in the presence of TrkA, J. Pharmacol. Exp. Ther. 310 (2004) 505–511, http://dx.doi.org/10.1124/jpet.104.066225.
[12] J.B. Owolabi, G. Rizkalla, A. Tehim, G.M. Ross, R.J. Riopelle, R. Kamboj, M. Ossipov, D. Bian, S. Wegert, F. Porreca, D.K. Lee, Characterization of anti-allodynic actions of ALE-0540, a novel nerve growth factor receptor antagonist, in the rat, J. Pharmacol. Exp. Ther. 289 (1999) 1271–1276.
[13] O. Niederhauser, M. Mangold, R. Schubenel, E.A. Kusznir, D. Schmidt, C. Hertel, NGF ligand alters NGF signaling via p75(NTR) and trkA, J. Neurosci. Res. 61 (2000) 263–272, http://dx.doi.org/10.1002/1097-4547(20000801)61:3<263::AID-JNR4>3.0.CO;2-M.
[14] J.K. Eibl, B.C. Strasser, G.M. Ross, Identification of novel pyrazoloquinazolinecarboxilate analogues to inhibit nerve growth factor in vitro, Eur. J. Pharmacol. 708 (2013) 30–37, http://dx.doi.org/10.1016/j.ejphar.2013.03.029.
[15] J.K. Eibl, B.C. Strasser, G.M. Ross, Structural, biological, and pharmacological strategies for the inhibition of nerve growth factor, Neurochem. Int. 61 (2012) 1266–1275, http://dx.doi.org/10.1016/j.neuint.2012.10.008.
[16] S. Curry, H. Mandelkow, P. Brick, N. Franks, Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites, Nat. Struct. Biol. 5 (1998) 827–835, http://dx.doi.org/10.1038/1869.
[17] J.R. Simard, P.A. Zunszain, J.A. Hamilton, S. Curry, Location of High and Low Affinity Fatty Acid Binding Sites on Human Serum Albumin Revealed by NMR Drug-competition Analysis, (2006), http://dx.doi.org/10.1016/j.jmb.2006.06.028.
[18] L. Buchholz, C.-H. Cai, L. Andress, A. Cleton, J. Brodfuehrer, L. Cohen, Evaluation of the human serum albumin column as a discovery screening tool for plasma protein binding, Eur. J. Pharm. Sci. 15 (2002) 209–215, http://dx.doi.org/10.1016/S0928-0987(01)00219-6.
[19] S. Esmaeilzadeh, H. Valizadeh, P. Zakeri-Milani, The effects of pH, temperature and protein concentration on the in vitro binding of flutamide to human serum albumin, Pharm. Dev. Technol. (2016) 1–10 http://dx.doi.org/10.3109/10837450.2016.1163392.
[20] L.Z. Benet, B.A. Hoener, Changes in plasma protein binding have little clinical relevance, Clin. Pharmacol. Ther. 71 (2002) 115–121, http://dx.doi.org/10.1067/mcp.2002.121829.
[21] J. Reichenwallner, D. Hinderberger, Using bound fatty acids to disclose the functional structure of serum albumin. - PubMed - NCBI, Biochim. Biophys. Acta 1830 (2013) 5382–5393, http://dx.doi.org/10.1016/j.bbagen.2013.04.031.
[22] U. Pal, S. Pramanik, B. Bhattacharya, B. Banerji, N.C. Maiti, Binding interaction of a gamma-aminobutyric acid derivative with serum albumin: an insight by fluorescence and molecular modeling analysis, Spring 5 (2016) 1121, http://dx.doi.org/10.1186/s40064-016-2752-x.
[23] B.X. Huang, H.-Y. Kim, C. Dass, Probing three-dimensional structure of bovine serum albumin by chemical cross-linking and mass spectrometry, J. Am. Soc. Mass Spectrom. 15 (2004) 1237–1247, http://dx.doi.org/10.1016/j.jasms.2004.05.004.
[24] A.E. Kennedy, K.S. Sheffield, J.K. Eibl, M.B. Murphy, R. Vohra, J.A. Scott, G.M. Ross, A surface plasmon resonance spectroscopy method for characterizing small-molecule binding to nerve growth factor, J. Biomol. Screen. 21 (2016) 96–100, http://dx.doi.org/10.1177/1087057115607814.
[25] G.M. Ross, I.L. Shamovsky, G. Lawrance, M. Solc, S.M. Dostaler, S.L. Jimmo, D.F. Weaver, R.J. Riopelle, Zinc alters conformation and inhibits biological activities of nerve growth factor and related neurotrophins, Nat. Med. 3 (1997) 872–878.
[26] S. Núñez, J. Venhorst, C.G. Kruse, Target-drug interactions: first principles and their application to drug discovery, Drug Discov. Today 17 (2012) 10–22, http://dx.doi.org/10.1016/j.drudis.2011.06.013.
[27] R.A. Copeland, D.L. Pompliano, T.D. Meek, Drug-target residence time and its implications for lead optimization, Nat. Rev. Drug Discov. 5 (2006) 730–739, http://dx.doi.org/10.1038/nrd2082.
[28] N.J. Wittenberg, B. Wootla, L.R. Jordan, A. Denic, A.E. Warrington, S.-H. Oh, M. Rodriguez, Applications of SPR for the characterization of molecules important in the pathogenesis and treatment of neurodegenerative diseases, Expert. Rev. Neurother. 14 (2014) 449–463, http://dx.doi.org/10.1586/14737175.2014.896199.
[29] S.C.B. Gopinath, P.K.R. Kumar, Biomolecular discrimination analyses by surface plasmon resonance, Analyst 139 (2014) 2678–2682, http://dx.doi.org/10.1039/c3an02052e.
[30] D.G. Myszka, Analysis of small-molecule interactions using Biacore S51 technology, Anal. Biochem. 329 (2004) 316–323, http://dx.doi.org/10.1016/j.ab.2004.03.028.
[31] S. Geschwindner, J.F. Carlsson, W. Knecht, Application of optical biosensors in small-molecule screening activities, Sensors (Basel) 12 (2012) 4311–4323, http://dx.doi.org/10.3390/s120404311.
[32] D.L. Bennett, Neurotrophic factors: important regulators of nociceptive function, Neuroscientist 7 (2001) 13–17.
[33] S. Capsoni, S. Covaceuszach, S. Marinelli, M. Ceci, A. Bernardo, L. Minghetti, G. Ugolini, F. Pavone, A. Cattaneo, Taking pain out of NGF: a “painless” NGF mutant, linked to hereditary sensory autonomic neuropathy type V, with full neurotrophic activity, PLoS One 6 (2011) e17321, , http://dx.doi.org/10.1371/journal.pone.0017321.
[34] P.A. Barker, High affinity not in the vicinity? Neuron 53 (2007) 1–4, http://dx.doi.org/10.1016/j.neuron.2006.12.018.
[35] K.S.A. Sheffield, R. Vohra, J.A. Scott, G.M. Ross, Using surface plasmon resonance spectroscopy to characterize the inhibition of NGF-p75(NTR) and proNGF-p75(NTR) interactions by small molecule inhibitors, Pharmacol. Res. 103 (2016) 292–299, http://dx.doi.org/10.1016/j.phrs.2015.12.005.
[36] K.S.A. Sheffield, A.E. Kennedy, J.A. Scott, G.M. Ross, Characterizing nerve growth factor-p75(NTR) interactions and small molecule inhibition using surface plasmon resonance spectroscopy, Anal. Biochem. 493 (2016) 21–26, http://dx.doi.org/10.1016/j.ab.2015.09.019.
[37] A. Belatik, S. Hotchandani, J. Bariyanga, H. Tajmir-Riahi, Binding sites of retinol and retinoic acid with serum albumins. - PubMed - NCBI, Eur. J. Med. Chem. 48 (2012) 114–123, http://dx.doi.org/10.1016/j.ejmech.2011.12.002.
[38] X. Ma, J. Yan, Q. Wang, D. Wu, H. Li, Spectroscopy study and co-administration effect on the interaction of mycophenolic acid and human serum albumin, Int. J. Biol. Macromol. 77 (2015) 280–286, http://dx.doi.org/10.1016/j.ijbiomac.2015.03.052.
[39] T. Peters, All About Albumin, Academic Press, San Diego, 1995, http://dx.doi.org/10.1016/B978-012552110-9/50009-X.