Colloids and Surfaces B: Biointerfaces 123 (2014) 924–929

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Determination of binding capacity and adsorption enthalpy between Human Glutamate Receptor (GluR1) peptide fragments and kynurenic acid by surface plasmon resonance experiments

E. Csapó[a], Z. Majláth[b], Á. Juhász[a], B. Roósz[c], A. Hetényi[d], G.K. Tóth[d], J. Tajti[b], L. Vécsei[b,e], I. Dékány[a,d,*]

[a] MTA-SZTE Supramolecular and Nanostructured Materials Research Group, University of Szeged, H-6720 Dóm tér 8, Szeged, Hungary
[b] Department of Neurology, University of Szeged, H-6725 Semmelweis u. 6, Szeged, Hungary
[c] Department of Chemistry and Chemical Informatics, University of Szeged, Juhász Gyula Faculty of Education, H-6725 Boldogasszony sgt. 6, Szeged, Hungary
[d] Department of Medical Chemistry, Faculty of Medicine, University of Szeged, H-6720 Dóm tér 8, Szeged, Hungary
[e] MTA-SZTE Neuroscience Research Group, University of Szeged, H-6725 Semmelweis u. 6, Szeged, Hungary

ARTICLE INFO

Article history:
Received 9 August 2014
Received in revised form 17 October 2014
Accepted 22 October 2014
Available online 30 October 2014

Keywords:
AMPA receptor
GluR1
Kynurenic acid
Adsorption
Enthalpy

ABSTRACT

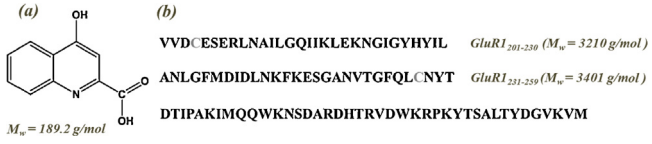
The interaction between kynurenic acid (KYNA) and two peptide fragments (ca. 30 residues) of Human Glutamate Receptor 201–300 (GluR1) using surface plasmon resonance (SPR) spectroscopy was investigated. Because of the medical interest in the neuroscience, GluR1 is one of the important subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR). AMPARs are ionotoropic glutamate receptors, which are mediating fast synaptic transmission and are crucial for plasticity in the brain. On the other hand, KYNA has been suggested to have neuroprotective activity and it has been considered for apply in therapy in certain neurobiological disorders. In this article the adsorption of the $GluR1_{201-230}$ and $GluR1_{231-259}$ peptides were studied on gold biosensor chip. The peptides were chemically bonded onto the gold surface via thiol group of L-cysteine resulted in the formation of peptide monolayer on the SPR chip surface. Because the $GluR1_{231-259}$ peptide does not contain L-cysteine the Val256 was replaced by Cys256. The cross sectional area and the surface orientation of the studied peptides were determined by SPR and theoretical calculations (LOMETS) as well. The binding capability of KYNA on the peptide monolayer was studied in the concentration range of 0.1–5.0 mM using 150 mM NaCl ionic strength at pH 7.4 (±0.02) in phosphate buffer solutions. In order to determine the binding enthalpy the experiments were carried out between +10 °C and +40 °C. The heat of adsorption was calculated by using adsorption isotherms at different surface loading of KYNA on the SPR chip.

1. Introduction

The kynurenine pathway (KP) is the main route of tryptophan metabolism, which produces several neuroactive metabolites. While KYNA is the only known endogenous glutamate-receptor inhibitor, another metabolite produced in the KP, quinolinic acid (QUIN) is an N-methyl-D-aspartate receptor (NMDAR) agonist. QUIN exerts a neurotoxic effect through the stimulation of NMDAR and it is also capable of inducing lipid peroxidation. Excessive activation of glutamate receptors results in excitotoxic neuronal damage, and this process has been implicated in the pathophysiology of several neurological disorders such as stroke, epilepsy or traumatic brain injury [1,2]. On the contrary, an increasing evidence suggests that KYNA is an endogenous neuroprotective metabolite, which is capable of preventing excitotoxic neuronal damage [3,4]. Alterations in the delicate balance of KP metabolites have been demonstrated in a number of neurological disorders including Parkinson's disease, Huntington's disease, stroke and dementia [5,6]. KYNA exerts its effects mainly by antagonizing NMDA receptors, but it is also inhibitor of α7-nicotinic acetylcholine receptors, thereby modulating presynaptic glutamate release [7,8]. It has therefore been suggested to have a neuromodulatory role in the central nervous system, as it can influence both dopamine and glutamate release [9,10]. AMPARs are also able to bind KYNA, but here

* Corresponding author. Tel.: +36 62 544 210; fax: +36 62 544 042.
E-mail address: i.dekany@chem.u-szeged.hu (I. Dékány).

http://dx.doi.org/10.1016/j.colsurfb.2014.10.046
0927-7765/



Scheme 1. Structural formula of the KYNA (a) and the peptide sequence of Human GluR1 201–300 (b) highlighted the two studied fragments (GluR1$_{201-230}$ and GluR1$_{231-259}$).

the effect is concentration-dependent: at a higher concentration level KYNA inhibits them, but in the lower, nanomolar concentration range it may facilitate these glutamate receptors [11,12]. KYNA inhibits glutamate receptors by binding to the ligand-binding domain, but the facilitatory effect described on AMPARs is most possibly a consequence of allosteric modulation via an extracellular domain [11]. Allosteric modulation of AMPARs stands in the focus of research interest, as it might be a promising therapeutic tool in the management of various neuropsychiatric diseases [13,14]. However, excessive stimulation of glutamate receptors might cause neuronal damage, therefore the understanding of the delicate mechanisms of receptor potentiation is crucial. KYNA has been demonstrated to be able to modulate AMPARs, but the exact molecular mechanism is not yet clarified.

The objective of this work was to describe a novel method for examination of receptor–ligand binding mechanisms, and to investigate the interactions of KYNA with a subunit of AMPAR. The extracellular domain of GluR1 was used to examine if there is a positive allosteric modulation site for KYNA. Scheme 1 shows the structural formula of the studied KYNA (a) and the sequence of the Human GluR1 201–300 peptide (b). In the course of our studies two shorter peptide fragments of GluR1 (Scheme 1b) were synthesized in our laboratory. However the GluR1 201–300 is commercially available but take into account the prize/amount of this product we synthesized different fragments of this peptide in our laboratory. Anyway, the aim of our work was to investigate that which part of the GluR1 peptide shows higher affinity toward KYNA drug molecules.

Surface plasmon resonance (SPR) spectroscopy is one of the most applied technique to measure biomolecule interactions in real-time in a label free environment [15]. In most cases interactions between DNA–DNA, DNA–protein, lipid–protein and protein/peptides–drug molecules can be investigated [16]. While one of the interactants is immobilized to the surface of sensor, the other are free in solution and passed over the surface. Association and dissociation is measured in arbitrary units and displayed in a graph called the "*sensorgram*". However, numerous research groups have published their results in the field of characterization of different biomolecule interactions [15–17], but only a few articles provide deeper information about the temperature-dependent interactions between peptides and polymers or potential drug molecules [18] by using SPR. Due to this *quasi* two dimension technique the thermodynamic data of the adsorption can also be determined [18]. Besides the SPR the isothermal titration calorimetry (ITC) is the commonly used 3D technique to determine the thermodynamic parameters of interactions in solution.

In this article the binding capability of KYNA on gold-coated surface were studied at different temperatures after immobilized the surface with GluR1$_{201-230}$ and GluR1$_{231-259}$ peptides. These peptides were covalently bonded on the gold surface via —SH group of L-cysteine (Cys204 and Cys256). The cross sectional area of the prepared peptides were determined by SPR and theoretical calculations as well. In all cases the adsorption isotherms at given temperatures derived from the measured plasmonic curves (sensorgrams). Analysis of these isotherms provided ΔH^{o}_{ad} data for a peptide/KYNA interaction at different surface coverage (Θ). For calculation of ΔH^{o}_{ad} enthalpy data the following equation was used:

$$\Delta H^{o}_{ad} = -R\left\{\frac{[d(\ln c)]}{[d(1/T)]}\right\}_{\Theta} \quad (1)$$

where ΔH^{o}_{ad} is the isosteric heat of adsorption, R is the ideal gas constant (8.3144621 J/mol·K), $\boldsymbol{c}$ is the equilibrium concentration of the KYNA solution, at T is the absolute temperature.

2. Materials and methods

2.1. Materials

The following chemicals were used in the experiments: kynurenic acid (≥98%, Sigma), NaCl (≥98%, Molar), NaH_2PO_4 ((≥99%, Sigma–Aldrich), Na_2HPO_4 (≥98.5%, Sigma). The studied GluR1$_{201-230}$ and GluR1$_{231-259}$ peptides were synthesized in our laboratory. All the starting materials were used without further purification. The KYNA and peptide stock solutions were freshly prepared in all cases using Milli-Q ultrapure water (18.2 MΩ cm at 25 °C).

2.2. Syntheses of GluR1$_{201-230}$ and GluR1$_{231-259}$

The two peptide fragments of the AMPA receptor subunit 1 (201–230, VVDCESERLNAILGQIIKLEKNGIGYHYIL, peptide GluR1$_{201-230}$ and 231–259, V256C, ANLGFMDIDLNKFKESGANVTGFQLCNYT, peptide GluR1$_{231-259}$) were synthesized by solid-phase technique utilizing Fmoc chemistry. The peptide chains were elongated on a Rink MBHA resin (0.76 mmol/g) and the syntheses were carried out on a CEM Liberty synthesizer. The applied protecting groups were the followings: Fmoc-Asp(OBut), Fmoc-Cys(Trt), Fmoc-Glu(OBut), Fmoc-Ser(But), Fmoc-Arg(Pbf), Fmoc-Lys(Boc), Fmoc-Tyr(But) Fmoc-His(Trt), Fmoc-Thr(But). Couplings were performed twice with HBTU. After the final Fmoc removing the completed peptide resins were treated with TFA/DTT/water (90:5:5), on RT for 2.5 h. Than the TFA was evaporated, the residues were triturated with ether and the resulted crude peptides were solubilized in 10% aqueous acetic acid, filtered and lyophilized. The crude peptides were purified by semipreparative RP-HPLC on a Phenomenex Jupiter Proteo (Torrance, CA) C18 10 μm column (15 mm × 250 mm). The applied flow rate was 3 ml/min, the gradient was 20–45% in 50 min. The homogeneity of the resulted peptides were evaluated by analytical RP-HPLC on a 4.6 mm × 250 mm Phenomenex Jupiter 5 C18 300 column at 1.2 ml/min flow rate and a gradient of 35–50% in 15 min. R_t = 9.14 (GluR1$_{201-230}$) or a Phenomenex Luna 10 C18 100 column at 1 ml/min flow rate and a gradient of 40–55% in 15 min R_t = 10.49 (GluR1$_{231-259}$). The peptides were further characterized by mass spectrometry using a Finnigan TSQ 7000 tandem quadrupol mass spectrometer equipped with electrospray ion source. The results are the followings: Mw_{calc} = 3210.61, measured = 3209.98 for GluR1$_{201-230}$, and Mw_{calc} = 3400.97, measured = 3400.56 for GluR1$_{231-259}$.

2.3. Surface plasmon resonance spectroscopy

SPR measurements were carried out in order to determine the adsorbed amounts and the monomolecular coverage of GluR1$_{201-230}$ and GluR1$_{231-259}$ peptides on the bare gold surface, as well as the sorption of KYNA on the gold surface functionalized with the above-mentioned peptides. The adsorbed amount of KYNA on bare gold surface was also studied. A two-channel SPR sensor platform developed at the Institute of Photonics and Electronics (Prague) was applied. The SPR chip is a thin gold layer

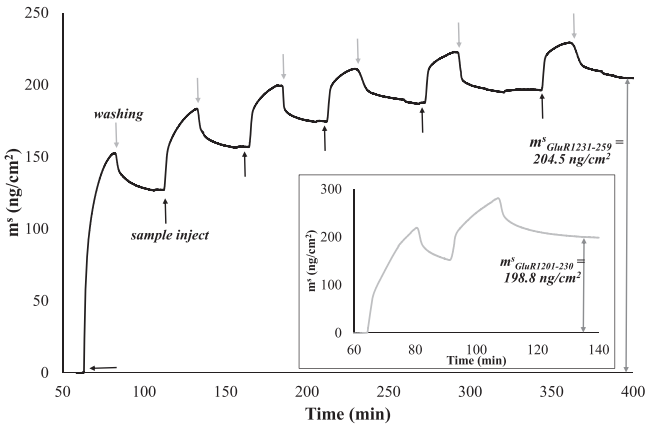


Fig. 1. Adsorption of $GluR1_{231–259}$ and $GluR1_{201–230}$ peptides (inset) on bare gold surface (c = 0.03 mM, I = 150 mM (KCl), pH = 7.40, T = 20 °C, flow rate: 25 μl/min).

(50 nm thickness), deposited on a glass substrate. During measurements the flow rate of 25 and 50 μL min^{-1} was used at constant temperature, and the adsorption isotherms were determined between +10 °C and +40 °C. The interaction of KYNA with the subunits of AMPA receptor was studied in the concentration range of 0.1–5.0 mM in physiological condition (using 150 mM NaCl in phosphate buffer solution at pH = 7.40 (±0.02)). The SPR spectra were analyzed in real-time by a special software package that allows determination of the resonant wavelength in both sensing channels.

2.4. Theoretical calculations

Molecular structures of the $GluR1_{201–230}$ and $GluR1_{231–259}$ peptides were generated with LOMETS. LOMETS a Local Meta-Threading-Server, for quick and automated predictions of protein tertiary structures and spatial constraints was developed by Wu and Zhang. It generates 3D models by collecting high-scoring target-to-template alignments from eight (8) locally installed threading programs (FUGUE, HHsearch, MUSTER, PPA, PROSPECT2, SAM-T02, SPARKS, and SP3). For a given target, 160 models are generated by the eight (8) component servers where each server generates 20 models, as sorted by their Z-scores in each algorithm. The best 10 models are selected from the 160 models based on a scoring function. The LOMETS server is freely available to the academic community at http://zhang.bioinformatics.ku.edu/LOMETS. (Wu and Zhang, 2007) [19].

3. Results and discussion

3.1. Adsorption on bare gold surface

In order to study the interaction between KYNA and GluR1 peptide fragments by SPR spectroscopy firstly the synthesized peptides were immobilized on the surface of gold-coated SPR chip from aqueous solutions (c = 0.03 mM). The registered SPR sensorgrams are presented in Fig. 1. As it can be seen the adsorption of both peptides are not fully reversible, substantial part of the adsorbed amount remains irreversibly bound at gold surface. Most probably the peptides were covalently bounded via Cys204 and Cys256, respectively [20]. The immobilization process was repeated at several times while measurable adsorption was detected. Most probable monomolecular peptide layer was formed on the gold surface. It was found that the monolayer amount of $GluR1_{201–230}$ and $GluR1_{231–259}$ are m^s = 198.8 ng/cm^2 and 204.5 ng/cm^2, respectively. The adsorption capacity at the maximal concentration (Γ_m/nmol cm^{-2}) and the cross sectional area (a_m/nm^2) of the studied peptides are summarized in Table 1. In Table 1. the monolayer capacity for adsorption of KYNA on bare gold surface is also presented. For calculation of a_m the Eq. (2) was used [20]:

$$a_m\ (\mathrm{nm}^2) = 0.166 \times \frac{1}{\Gamma_m} \quad (2)$$

However the SPR sensorgrams of KYNA adsorption on bare gold chip are not presented here, but we can conclude that the kynurenic acids were reversible bonded onto the gold surface. In this case if we plotting the adsorbed amounts (before washing process) as a function of KYNA concentration (Γ vs. c_{kyna}) the saturation region of Langmuir-type isotherm provides the monolayer adsorption capacity (m_s = 130.0 ng/cm^2) using the reciprocal Langmuir presentations (see data in Table 1.)

The cross sectional area of the peptides are also determined by theoretical calculations. Table 1. summarizes the calculated cross sectional area ($a_{m,calc}$) for peptides and also for KYNA using MarvinSketch [21] and LOMETS [19] softwares. Using MarvinSketch only the theoretical minimal and maximal projection area for molecules can be determined at the lowest energy conformer. The determined data for simple KYNA molecule by SPR (a_m = 0.242 nm^2) is in good agreement with the calculated area ($a_{m,calc}$ = 0.277 nm^2) obtained by MarvinSketch. For peptides the calculated MarvinSketch data are somewhat larger than the measured values because the above-mentioned software takes no count of the binding of peptide onto the gold surface via Cys residues. In contrast, the calculated surface area by using LOMETS software provided quite correct surface area values for KYNA and peptides as well. Fig. 2 shows the representative final minimum energy molecular structure for $GluR1_{201–230}$ peptide [19]. As it can be seen in Table 1, the $a_{m,calc}$ values are roughly similar to the tentatively determined area by using SPR.

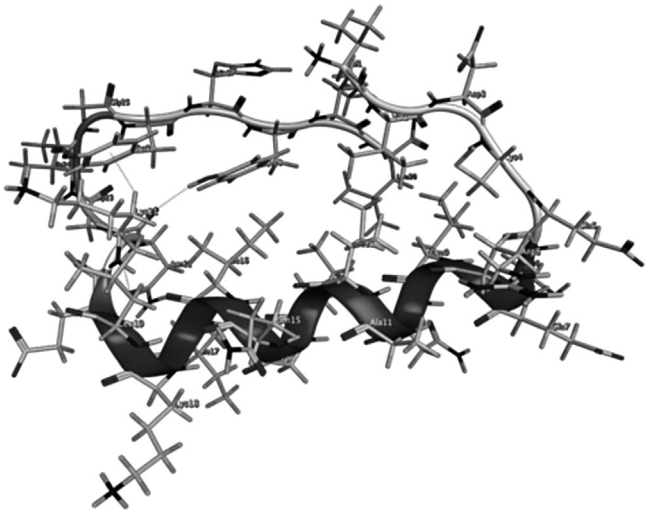


Fig. 2. Final minimum energy molecular structure for $GluR1_{201–230}$ peptide, obtained by using LOMETS [19].

3.2. Interaction of KYNA with $GluR1_{201–230}$ and $GluR1_{231–259}$ peptides on gold surface

The binding capability of kynurenic acid on $GluR1_{201–230}$ and $GluR1_{231–259}$ functionalized gold surfaces was studied in the concentration range 0.1–5.0 mM at different temperatures (from +10 °C to 40 °C). As it can be seen on Fig. 3a and b increase in concentration of the applied KYNA solutions results in higher wavelength shift from ~0.5 nm to almost 2 nm at 10 °C and 20 °C. Similar trend was observed at the other studied temperatures as well. Fig. 3 clearly shows that washing procedure was applied after each

Table 1
Monolayer amount (m_s), adsorption capacity (Γ_m), the measured (a_m) and the calculated ($a_{m,calc}$) cross sectional area of the KYNA and the peptides.

Molecules on gold surface		Monolayer amount, m^s (ng/cm^2)	Adsorption capacity at measured max. concentration, Γ_m (nmol/cm^2)	Cross sectional area, a_m (nm^2) MarvinSketch	Calculated cross sectional area, $a_{m,calc}$ (nm^2)*1 LOMETS
KYNA (20 °C)	130.0	0.687	0.242	0.277	0.346
GluR1$_{201-230}$ (20 °C)	198.8	0.059	2.814	3.630	2.948
GluR1$_{231-259}$ (20 °C)	204.5	0.064	2.594	3.770	3.063

KYNA on peptide-covered gold surface at different temperatures		Monolayer amount, m^s (ng/cm^2)	Adsorption capacity, Γ_m (nmol/cm^2)*2
GluR1$_{201-230}$	(10 °C)	18.9	0.100
	(20 °C)	16.8	0.089
	(30 °C)	15.1	0.080
	(40 °C)	14.4	0.076
GluR1$_{231-259}$	(10 °C)	19.9	0.105
	(25 °C)	18.2	0.096
	(40 °C)	17.6	0.093

*1 $a_{m,calc}$ are calculated by the MarvinSketch [21] and LOMETS [19] programs.
*2 Calculated from Eq. (3).

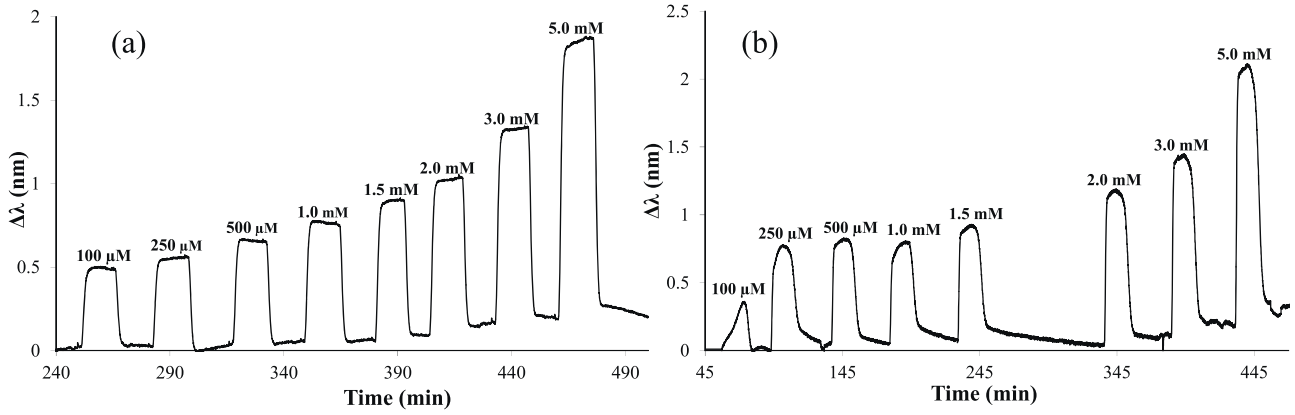


Fig. 3. Representative SPR curves of adsorption of KYNA on GluR1$_{201-230}$ (a) and GluR1$_{231-259}$ (b) functionalized gold surface at different KYNA concentrations (I = 150 mM (KCl), pH = 7.40, flow rate: 25 µl/min, T = 20 °C (a) and 10 °C (b)).

immobilization steps. These washing steps reduced the adsorbed mass of KYNA to nearly zero so that one may conclude that the interaction of KYNA with the subunits of AMPA receptor is fully reversible. The adsorption isotherms of KYNA derived from the sensorgrams are presented in Fig. 4. The measured isotherms were fitted with Brunauer–Emmet–Teller (BET), Langmuir and Freundlich isotherm equations as well [22,23]. Taking into account the R^2 values which were calculated by least-squares curve fitting method[1] we found that the BET isotherm function (Eq. (3)) follows supremely the trend of the measured data.

$$\Gamma = \frac{c_e K_B \Gamma_m}{(c_e - c_s)\left\{1 + (K_B - 1)\left(\frac{c_e}{c_s}\right)\right\}} \quad (3)$$

where Γ is the amount of adsorbed kynurenic acid molecules per unit surface of solid at equilibrium, Γ_m monolayer adsorption capacity, c_e equilibrium concentration of KYNA remaining in solution when adsorbed amount equals Γ, c_s saturation (solubility limit) concentration of the KYNA and K_B parameter related to the binding intensity for all layers.

Fig. 4a and b shows that increase in temperature from 10 °C to 40 °C results the decrease in the adsorbed amount of KYNA at given temperatures. This experimental result shows a reversible adsorption process, that means certain thermodynamic state functions of the interaction could be determined from the adsorption isotherms [18,20]. The adsorption capacity values of KYNA on peptide functionalized gold surface was also summarized in Table 1 using Eq. (3) BET-equation. As it can be seen substantially less amount of KYNA (m_s = 19–14 ng/cm^2, ca. 15–10 m/m (%)) bounded on the peptide-covered surface compared to the bare gold surface (m_s = 130 ng/cm^2). According to Eq. (1) the adsorption enthalpy (isosteric heat of adsorption) could be calculated from the temperature dependency of the equilibrium concentration of the adsorbed molecules in the bulk solution at constant surface concentration (surface coverage, Θ).[2] The values of adsorption heats (enthalpies) were calculated by Eq. (1), at chosen surface concentration, assuming linearity of ln$c$–1/T function (Supplementary 1). The Suppl. 1 represents the fitting linear at five different $n_{peptide}$:n_{KYNA} molar ratios (1:1; 1:1.3; 1:2; 1:3 and 1:4) and according to the Eq. (1) the slope of each linear provides only one adsorption enthalpy value[3] at chosen surface concentration. If we plotting the *lnc* data as a function of 1/T at numerous surface concentration, we obtain important information about the change of the adsorption enthalpy as function of the surface coverage/loading of KYNA molecules (Θ).

[1] R^2 values are the follows: R^2 > 0.86 (BET), R^2 > 0.80 (Langmuir) and R^2 > 0.79 (Freundlich).

[2] $\Theta = \Gamma_{(KYNA)}/\Gamma_{m\,(KYNA)}$.

[3] In this case this adsorption enthalpy value is actually the binding enthalpy of KYNA to the peptide immobilized on gold surface.

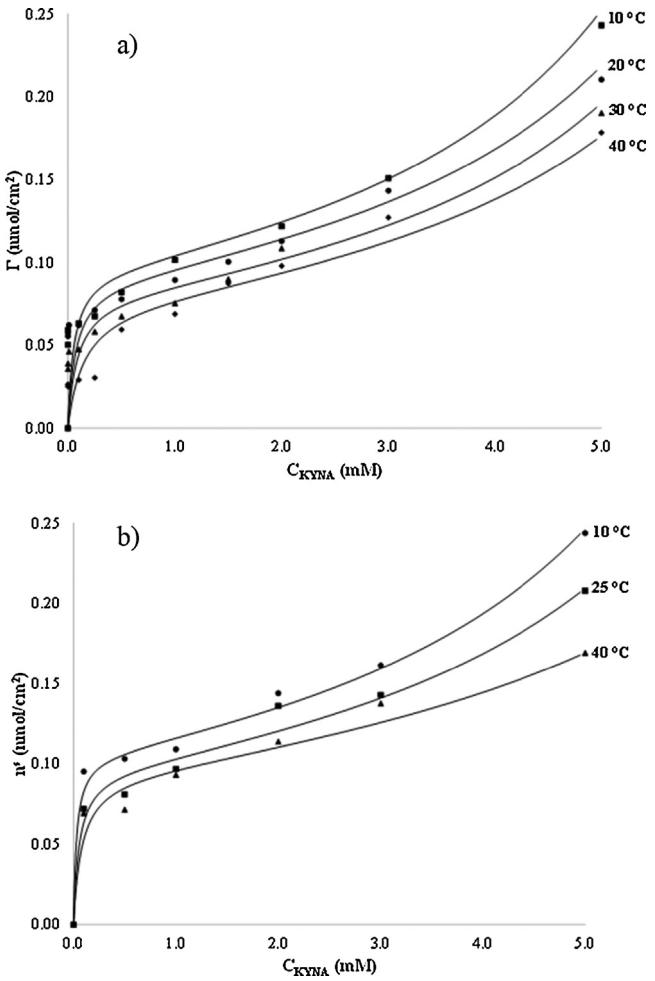


Fig. 4. Adsorption isotherms of KYNA on gold surface after immobilized the chip with GluR1$_{201-230}$ (a) and GluR1$_{231-259}$ (b) peptides at different temperatures (the experimental error is ±4.8%).

Fig. 5 shows the calculated adsorption enthalpy as a function of surface coverage/loading) for KYNA-GluR1$_{201-230}$ (gray) and for KYNA-GluR1$_{231-259}$ (black) interactions. It can be seen in Fig. 5 that no significant difference between the two studied system is observed. Increase in the surface coverage to $\Theta \sim 1$ results the increase in the isosteric heat of adsorption from ~−25 kJ/mol to ~−40 kJ/mol. Further increase in the value of Θ to ~2.5 the ΔH is decreased to ~−5 kJ/mol, because of the lower binding energy of interaction between the peptide coated surface and KYNA at higher surface loading. In Fig. 5, the dashed vertical lines correspond to different $n_{peptide}$:n_{KYNA} = 1:1; 1:2; 1:3 and 1:4 molar ratios. On the whole, we can summarize that the maximal isosteric heat of adsorption (~40 kJ/mol) is observed at $\Theta \sim 1$ surface coverage where the $n_{peptide}$:n_{KYNA} molar ratio is 1:1.3. Taking into account the structure of the peptide and KYNA molecules different coordination/binding modes are possible. Based on the findings of SPR the sorption of KYNA on peptide-functionalized gold surface is reversible, so we can conclude that formation of H-bonds between COO^- groups or the evolvement of π–π stacking interaction between KYNA aromatic rings and Tyr or His residues of peptide is also play an important role in the binding of drug molecules on the surface of subunit of AMPAR. Scheme 2 shows schematic representation of one possible binding of kynurenic acid molecules on the peptide-coated gold surface. At higher surface coverage (at ~5 mM maximum KYNA concentration) weaker electrostatic interaction (~5 kJ/mol) between the KYNA drug molecules and peptide fragments of AMPA receptor exists. (e.g. weak electrostatic interaction between COO^- and NH_3^+ groups).

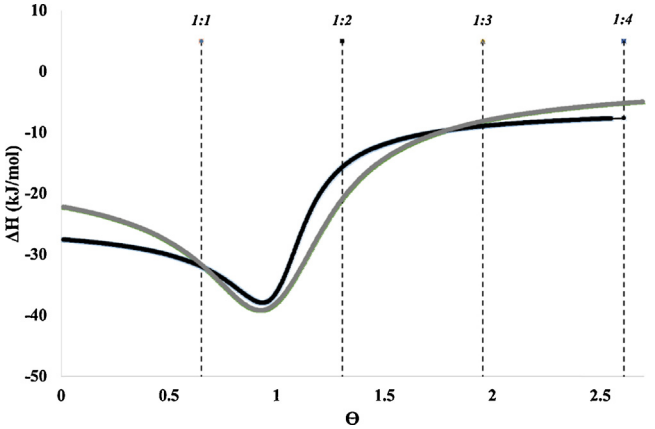
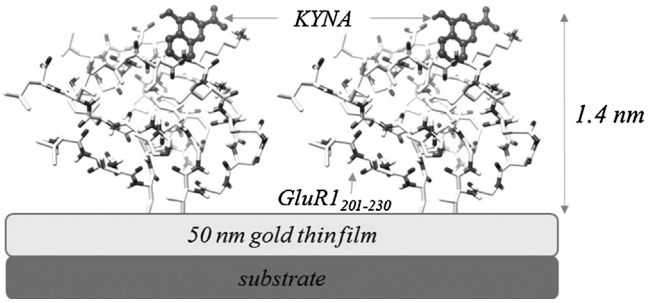


Fig. 5. Isosteric heat of adsorption for KYNA-GluR1$_{201-230}$ (gray) and for KYNA-GluR1$_{231-259}$ (black) interaction as a function of surface coverage.



Scheme 2. Schematic representation of one possible binding of KYNA molecules on peptide-coated gold surface.

4. Conclusion

KYNA has a wide range of different targets, interacting with the NMDA and AMPA subtypes of glutamate receptors, alpha7 nicotinic acetylcholine receptors and GPR35 receptors as well. As it may influence glutamatergic, dopaminergic, GABAergic and holinergic neurotransmission, it has been suggested to play an important neuromodulatory role in the CNS. Of importance is to note its concentration-dependent dual effect on AMPA receptors, which might form the basis of its regulatory effect. Understanding the exact molecular mechanisms of KYNA at the level of receptor domains might elucidate its exact role in different physiological and pathophysiological processes, and thereby promote the development of novel therapeutic tools. This work described the novel methodology for determining the thermodynamic parameters of the interaction between certain AMPA receptor subunits and kynurenic acid drug molecules using quasi 2D adsorption experiments like surface plasmon resonance spectroscopy. The different peptide fragments of GluR1 synthesized in our laboratory were covalently (irreversible) bounded onto the gold surface via Cys residues resulted in the formation of uniform monolayer peptide surface on gold biosensor chip. In contrast, we found that the bonding of kynurenic acid on bare gold surface is reversible. We measured the reversible adsorbed amount of KYNA molecules on the subunits of AMPA receptor-modified gold surface at different temperatures under physiological conditions. Depending on the temperature from +10 °C to +40 °C ca. *19–14 nanogramm* drug molecules are bounded on given (1 cm^2) peptide-covered

gold surface which amount is only ca. 15–10% compared to the adsorbed amount of KYNA on bare gold chip. The interaction of KYNA and subunits of AMPA receptor is reversible, the calculated adsorption enthalpy function ΔH^{o}_{ad} vs.Θ shows maximum value ($\sim$−40 kJ/mol) at $n_{peptide}$:n_{KYNA} = 1:1.3 (at $\Theta \sim 1$) but at higher $n_{peptide}$:n_{KYNA} ratio measurable less interaction was observed (from $\Theta \sim 1.5$).

Acknowledgements

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.2.A-11/1/KONV-2012-0047.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.10.046.

References

[1] A.M. Palmer, D.W. Marion, M.L. Botscheller, P.E. Swedlow, S.D. Styren, S.T. DeKosky, J. Neurochem. 61 (1993) 2015.
[2] D. Zádori, P. Klivényi, L. Szalardy, F. Fülöp, J. Toldi, L. Vécsei, J. Neurol. Sci. 322 (2012) 187.
[3] D. Zádori, P. Klivényi, E. Vámos, F. Fülöp, J. Toldi, L. Vécsei, J. Neural Transm. 116 (2009) 1403.
[4] V. Hornok, T. Bujdosó, J. Toldi, K. Nagy, I. Demeter, C. Fazekas, I. Krizbai, L. Vécsei, I. Dékány, J. Neural Transm. 119 (2012) 115.
[5] L. Vécsei, L. Szalárdy, F. Fülöp, J. Toldi, Nat. Rev. Drug Discov. 12 (2013) 64.
[6] Z. Majláth, J. Tajti, L. Vécsei, Ther. Adv. Neurol. Disord. 6 (2013) 386.
[7] R. Carpenedo, A. Pittaluga, A. Cozzi, S. Attucci, A. Galli, M. Raiteri, F. Moroni, Eur. J. Neurosci. 13 (2001) 2141.
[8] C. Hilmas, E.F. Pereira, M. Alkondon, A. Rassoulpour, R. Schwarcz, E.X. Albuquerque, J. Neurosci. 21 (2001) 7463.
[9] H.Q. Wu, A. Rassoulpour, R. Schwarcz, J. Neural Transm. 114 (2007) 33.
[10] H.Q. Wu, E.F. Pereira, J.P. Bruno, R. Pellicciari, E.X. Albuquerque, R. Schwarcz, J. Mol. Neurosci. 40 (2010) 204.
[11] C. Prescott, A.M. Weeks, K.J. Staley, K.M. Partin, Neurosci. Lett. 402 (2006) 108.
[12] E. Rózsa, H. Robotka, L. Vécsei, J. Toldi, J. Neural Transm. 115 (2008) 1087.
[13] S.E. Ward, M. Harries, Curr. Med. Chem. 30 (2010) 3503.
[14] G.J. Lees, Drugs 59 (2000) 33.
[15] J. Homola, S.S. Yee, G. Gauglitz, Sens. Actuators B 54 (1999) 3.
[16] H. Sípová, J. Homola, Anal. Chim. Acta 773 (2013) 9.
[17] K. Gaus, E.A.H. Hall, Anal. Chim. Acta 470 (2002) 3.
[18] N. Singh, S.M. Husson, Biomacromolecules 6 (2005) 9.
[19] S. Wu, Y. Zhang, Nucleic Acids Res. 35 (2007) 3375.
[20] D. Sebők, E. Csapó, T. Preocanin, G. Bohus, N. Kallay, I. Dékány, Croat. Chem. Acta 86 (2013) 287.
[21] MarvinSketch. http://www.chemaxon.com/products/marvin/marvinsketch
[22] X. Li, S.M. Husson, Biosens. Bioelectron. 22 (2006) 336.
[23] S. Yamaguchi, T. Mannen, T. Zako, N. Kamiya, T. Nagamune, Biotechnol. Prog. 19 (2003) 1348.