

Modelling of the interaction between receptor mimetics and their ligands at solid / liquid interface



Ádám Juhász

PhD Theses

Supervisors:

Dr. Imre Dékány, Professor Emeritus

Dr. Gábor Tóth, Full Professor

Doctoral School of Chemistry

Faculty of Science and Informatics

Department of Physical Chemistry and Materials Science

University of Szeged

Szeged

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1. Introduction and main goals

Analytical methods based on the surface plasmon resonance (SPR) phenomena have become widely used in the last decades as a result of the rapid development of the electronics and information technology background. In the early stages of the development of this technique, the method was primarily characterized by the study of compounds bound to the surface of the sensor, but nowadays it is also possible to study complex systems such as the interaction of lipid bilayers that model cell membranes or liposomes formed from phospholipids and proteins. Among the quasi two-dimensional sensor techniques, the unlabelled, quantitative, real-time and temperature-dependent characterizations of receptor-ligand type interactions can be carried out by using SPR technique at the interface between the receptor-coated sensor surface and the ligand solution. Internationally, the SPR is an extremely widely used sensor technology, but according to the publications at Hungarian Institutes there are only a few papers which interpret the results of the detailed characterization of molecular interactions. During my research work, one of the main goal was to determine the individual cross sectional area of several proteins, amino acid, and di- and tripeptides. In order to confirm the applicability of this technique the experimentally determined cross sectional areas have been compared with analogous and structural data provided by quartz crystal microbalance (QCM) method and small angle X-ray scattering investigation, respectively.

Beyond the determination of the quantitative relationship between the surface-immobilized macromolecules and their ligands, the definition of the rate and equilibrium constants of the binding process by developing an appropriate data processing and evaluation process is also an integral part of my dissertation. Among others, according to the above-mentioned evaluation process, the study of the pH-dependent binding process of ibuprofen (IBU) onto the bovine serum albumin (BSA)-functionalized sensor surface has been carried out. Based on the fitting of the registered sensorgrams with kinetic models a spreadsheet-based software solution has been developed which resulted in the determination of the association and dissociation kinetic constants. In order to verify the binding parameters isotherm titration calorimetry (ITC) studies have also been performed.

The thermodynamic state functions determined by studying the temperature-dependence of the equilibrium constant can reinforce or refute the validity of a supposed reaction mechanism. During the preparation of my dissertation, the implementation and evaluation of temperature-dependent SPR measurements formed the backbone of my research work and these results have been presented in detail. The aim of the temperature-dependent measurements was the determination of the thermodynamic parameters of the interaction between AMPA receptor

model peptides synthesized by solid-phase Fmoc synthesis (University of Szeged, Department of Medical Chemistry) and kynurenic acid (KYNA) and their synthetic derivatives. Moreover, comparative studies have also been carried out where the model receptor polypeptides were substituted by serum proteins and lysozyme (LYZ). In view of the thermodynamic parameters the validity of the assumed binding mechanism was confirmed by independent ITC measurements, where the compounds were available in enough quantities.

2. Experimental methods

A two-channel, wavelength modulated, *in-situ* and temperature-controlled SPR apparatus developed at the Institute of Photonics and Electronics (Prague, Czech Republic) was used for my research work which is located at the University of Szeged, Department of Physical Chemistry and Materials Science. The spectrometer communicates with a PC via USB connection and data registration is carried out by SPR UP software.

A quartz crystal microbalance model QCM200 (Stanford Research Systems, SRS, USA) with QCM25 (5 MHz) chrome/gold electrode and a flow cell adapter was used to determine the cross-sectional area of *L*-cysteine (Cys) and *L*-glutathione (GSH) on gold surface.

Thermometric titration experiments were performed at 298.15 K with a computer-controlled VP-ITC power-compensation microcalorimeter (MicroCal). The enthalpograms (calorimeter power signal vs time) were evaluated by means of Origin Microcal 7.1. software.

SAXS curve of the LYZ was recorded with a slit-collimated Kratky compact small-angle system (KCEC/3 Anton-Paar KG, Graz, Austria) equipped with a position-sensitive detector (PSD 50M from M.Braun AG, Munich, Germany) in the range of $2\Theta = 0,05-8^\circ$. Cu $K\alpha$ radiation was generated by a Philips PW1830 X-ray generator operating at 40 kV and 30 mA.

3. New scientific results

T.1 Determination of the surface concentration (adsorbed amount) of covalently bound proteins, di- and tripeptides and amino acid on the surface of gold-coated sensor via SPR spectroscopy studies at solid/liquid interface. Based on the results of independent experimental techniques, the proof of the suitability of the SPR technique on the determination of the cross-sectional area and surface orientation of small biomolecules. For proteins the above-mentioned parameters can be estimated by SPR.

TI.1 Assuming monomolecular coverage, for Cys, *L*-cysteinyl-tryptophan (Cys-Trp) and GSH, in view of the adsorbed amount on gold surface the cross-sectional areas are the followings: 0,32; 0,47 and 0,62 nm², respectively. For Cys and GSH these data are in good agreement with the results of independent QCM technique (0,30 and 0,52 nm²).

TI.2 Assuming monomolecular coverage for proteins (BSA, HSA and LYZ) in view of the adsorbed amount on gold surface the cross-sectional areas are the followings: 171,5; 173,0 and 21,9 nm². Based on the results of SAXS studies the determined D_{\max} values (8,4; 8,7 and 4,8 nm, respectively) clearly confirm the existence of a SPR-based acceptable adsorption model.

T.2 Contributing to the design of the BSA/IBU colloidal drug delivery system, the quantitative characterization of the interaction between the carrier protein and the drug molecules at solid/liquid interface. Developing a spreadsheet-based evaluation method which allows the fitting of the registered sensorgrams by pseudo-first-order kinetic model. Successful application of the developed evaluation procedure in order to the determine the association as well as the dissociation rate constants of the binding complex.

The quotient of the determined $k_a = 56.4 \pm 4.4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ and $k_d = 0.022 \pm 0.019 \text{ s}^{-1}$ rate constant provided the equilibrium constant, which is $K_A = 2.51 \times 10^3 \pm 2.00 \times 10^2 \text{ dm}^3 \text{ mol}^{-1}$ at the given measuring temperature. The result of the SPR-based evaluation at solid/liquid interface and the evaluation using the kinetic approach shows a surprisingly good agreement with the analogous result of the solution phase and equilibrium ITC study ($K_A = 2.47 \times 10^3 \pm 5.33 \times 10^1 \text{ dm}^3 \text{ mol}^{-1}$).

T.3 Modelling of the interaction between the GluR1₂₇₀₋₃₀₀ polypeptide fragment of AMPA receptor and KYNA at SPR sensor surface.

T3.1 Assuming monomolecular coverage, the vertical surface orientation of the polypeptide in the adsorption layer on gold sensor surface has been verified. This assumption is confirmed by independent molecule dynamic calculations and AFM studies.

T3.2 The sorption isotherms of KYNA on GluR1₂₇₀₋₃₀₀ layer have been determined at four temperatures. Using the temperature-dependence of the functions determined by fitting of the isotherms, I calculated the change in the isosteric adsorption heat as a function of surface coverage of KYNA. Analyzing the dependence of the isosteric enthalpy change on the surface coverage, it can be concluded that the formation of the 1: 1 stoichiometric binding complex on the sensor surface is beneficial.

T.4 Determination of the enthalpy-, entropy- and heat capacity changes of the interaction between the KYNA and the GluR1₂₇₀₋₃₀₀ polypeptide via the van't Hoff analysis of the temperature-dependence of the equilibrium constant.

T4.1 The apparent rate constants of the binding complex have been determined via the discrete and global fitting of the registered sensorgrams of the interaction between KYNA and the immobilized GluR1₂₇₀₋₃₀₀ fragment model at neutral medium (pH = 7.4) by using pseudo-first-order kinetic model. Based on the concentration-dependence of the apparent rate constants the real rate constants of the formation and decomposition of the binding complex have been calculated.

T4.2 The enthalpy-, entropy- and heat capacity changes of the reversible interaction of KYNA with GluR1₂₇₀₋₃₀₀ polypeptide-functionalized SPR sensor surface have been determined via the van't Hoff analysis of the temperature-dependence of the equilibrium constant by using nonlinear fitting. The calculated parameters are $\Delta H^\circ = -27.91 \pm 5.27 \text{ kJ mol}^{-1}$, $\Delta S^\circ = -60.33 \pm 17.95 \text{ J mol}^{-1}\text{K}^{-1}$ and $\Delta C_p = -1.28 \pm 0.54 \text{ kJ mol}^{-1}\text{K}^{-1}$, respectively.

T4.3 Based on the sign as well as the values of the determined thermodynamic parameters it can be concluded that the enthalpy-controlled binding of the KYNA can be assumed via electrostatic and hydrogen bonds. Salt bridge is formed between the positively charged side chains of arginine of the solvated GluR1₂₇₀₋₃₀₀ polypeptide and the negatively charged KYNA, which is confirmed by molecule dynamic calculations as well.

T.5 Modelling of the interaction between the GluR1₂₃₁₋₂₅₉ polypeptide fragment of AMPA receptor and KYNA at SPR sensor surface

T5.1 The apparent rate constants of the binding complex have been determined at six different temperatures via the discrete fitting of the registered sensorgrams of the interaction between KYNA and the immobilized GluR1₂₃₁₋₂₅₉ fragment model at neutral medium (pH = 7.4) by using pseudo-first-order kinetic model. Based on the concentration-dependence of the apparent rate constants the real rate constants of the formation and decomposition of the binding complex have been calculated.

T5.2 The enthalpy-, entropy- and heat capacity changes of the reversible interaction of KYNA with GluR1₂₃₁₋₂₅₉ polypeptide-functionalized SPR sensor surface have been determined via the van't Hoff analysis of the temperature-dependence of the equilibrium constant by using nonlinear fitting. The calculated parameters are $\Delta H^\circ = 42.79 \pm 5.73 \text{ kJ mol}^{-1}$; $\Delta S^\circ = -11.61 \pm 0.0197 \text{ J mol}^{-1} \text{ K}^{-1}$ and $\Delta C_p = 6.42 \pm 0.65 \text{ kJ mol}^{-1} \text{ K}^{-1}$. It was found that, the reversible binding of KYNA is the result of an enthalpy-controlled process. Similar to the previously studied GluR1₂₇₀₋₃₀₀, the negative signs of the ΔH° and ΔS° strongly refer the presence of hydrogen bonds and electrostatic interactions, which can be interpreted by the salt bridge formed between the deprotonated carboxyl group of KYNA and the protonated amino group at position 242 (and/or position of 244) of lysine.

T.6 Comparative studies of the binding of KYNA onto the BSA- and HSA-functionalized SPR sensor surface. In contrast to the AMPA receptor model polypeptide fragments, the confirmation of the less specific binding of KYNA to the serum proteins.

T6.1 The apparent rate constants of the binding complex have been determined at four different temperatures via the discrete fitting of the registered sensorgrams of the interaction between KYNA and the immobilized serum proteins (BSA, HSA) at neutral medium (pH = 7.4) by using pseudo-first-order kinetic model. Based on the concentration-dependence of the apparent rate constants the real rate constants of the formation and decomposition of the binding complex have been calculated.

T6.2 The enthalpy-, entropy- and heat capacity changes of the reversible interaction of KYNA with serum protein-functionalized SPR sensor surface have been determined via the van't Hoff analysis of the temperature-dependence of the equilibrium constant by using nonlinear fitting. In case of BSA the calculated parameters are $\Delta H^\circ = -1.94 \pm 0.25 \text{ kJ mol}^{-1}$; $\Delta S^\circ = 0.025 \pm 0.0008 \text{ J mol}^{-1} \text{ K}^{-1}$ and $\Delta C_p = -2.17 \pm 0.18 \text{ kJ mol}^{-1} \text{ K}^{-1}$, while for HSA the following data were obtained: $\Delta H^\circ = -1.87 \pm 0.22 \text{ kJ mol}^{-1}$; $\Delta S^\circ = 0.0255 \pm 0.0008 \text{ J mol}^{-1} \text{ K}^{-1}$

and $\Delta C_p = -2.95 \pm 0.09 \text{ kJ mol}^{-1} \text{ K}^{-1}$. It was found that, the reversible binding of KYNA is the result of an enthalpy- and entropy-controlled process. Comparing the thermodynamic parameters of the interaction between BSA/HSA and KYNA and the corresponding parameters of the AMPA receptor model fragments (GluR1₂₃₁₋₂₅₉ és GluR1₂₇₀₋₃₀₀) it can be concluded that, for polypeptide fragments, the results of the SPR-based measurements confirm the presence of a more specific receptor-ligand type binding.

4. List of publications

Publications related to the scientific topic of the dissertation:

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Impact factor: **13.036**

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Magyar Kémiai Folyóirat, 125. évfolyam, 1. szám (2019)
2. L. Janovák, Á. Turcsányi, É. Bozó, Á. Deák, L. Mérai, D. Sebők, **Á. Juhász**, E. Csapó, M. M. Abdelghafour, E. Farkas, I. Dékány, F. Bari, Preparation of novel tissue acidosis- responsive chitosan drug nanoparticles: characterization and *in vitro* release properties of Ca²⁺ channel blocker nimodipine drug molecules
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- 1. Juhász Ádám**, A kinurénsav kötődésének felületi plazmon rezonancia spektroszkópiás vizsgálata receptor modelleken
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- 2. Juhász Ádám**: Makromolekulák és ligandumaik önszerveződésének felületi plazmon rezonancia spektroszkópiás kinetikai és termodinamikai jellemzése
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- 4. Dékány, N. Varga, E. Csapó, V. Hornok, D. Ungor, Á. Juhász, D. Sebők**, Self-assembled core-shell nanoparticles for drug delivery: structural properties and kinetic of the release process
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- 4. D. Ungor, E. Csapó, Á. Juhász, I. Dékány**, Interaction of cysteine and cysteine-containing peptides with gold in two- and three-dimensional systems
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- 5. Á. Juhász**, E. Csapó, H. Szokolai, D. Ungor, I. Dékány, Kinetics and thermodynamics characterization of the interactions between kynurenic acid and human glutamate receptor fragments by surface plasmon resonance studies
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