Enantioselective Artificial Receptors Formed by the Spreader-Bar Technique

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Received: September 18, 2002
Final version: November 27, 2002

Abstract

Chiroselective binding sites have been created on thin gold films by application of the spreader-bar approach. Impedometric techniques and surface plasmon resonance were applied to detect binding. (R)-(±)-1,1′-Binaphthyl-2,2′-diod (R-BNOH) and (S)-(−)-1,1′-binaphthyl-2,2′-diod (S-BNOH) were used as model analytes. The artificial receptors were prepared by co-adsorption of 16-mercaptophexadecane (matrix) with a thiol-modified chiral selector (template). The conjugates of d,l-thiotic acid and (R)-(−) or (S)-(−)-1,1′-binaphthyl-2,2′-diamine were used as templates. Different concentration ratios of the matrix and template were tested. No chiral selectivity of surfaces formed by either the matrix or the template alone was observed. The use of alkylthiols shorter than 16-mercaptophexadecane led to the formation of surfaces with no chiral selectivity. The gold electrodes coated by the spreader-bar technique displayed an enantioselectivity of up to 4.76 or up to 2.55 as measured by the capacitive and SPR methods, respectively.

Keywords: Enantioselective artificial receptors, Spreader-bar technique, Capacitive chemosensor, Surface plasmon resonance

Interest in the development of chiroselective analytical methods increased essentially during the last years, mainly due to needs of pharmaceutical industry. This resulted from the increasing pressure of regulatory authorities against the marketing of racemic mixtures [1, 2] since different enantiomers frequently result in quite different biological effects. Nowadays, enantiomerically pure pharmaceuticals represent approximately a third of all drug sales worldwide [3].

Chiral recognition is currently a subject of high research interest. Surfaces modified by chiral molecules have been applied successfully to asymmetric synthesis [4], to development of heterogeneous enantioselective catalysts [5] and enantioselective molecular recognition. The latter direction includes attempts to develop artificial receptors for applications in gas [6] or liquid phases [7, 8]. The enantioselective binding of phenylalanine and mandelic acid to surfaces modified by chiral molecules, were detected by quartz crystal microbalance [7] and atomic force microscopy [8]. A highly enantioselective crystallization of l-leucine on a monolayer formed by the corresponding isomer immobilized on gold surface precoated with alkylthiol was observed [9]. Optical sensors for chiral recognition of enantiomers of propranolol and phenethylamines were described [10, 11].

Here we report a new method to form chirally sensitive artificial chemoreceptors on a solid support. The approach is based on the spreader-bar technique previously used for formation of selective binding sites for DNA bases and others purines and pyrimidines [12, 13]. The binding can be detected both optically and electrically.

Table 1 presents changes in the capacitance of gold electrodes coated by various self-assembled monolayers on addition of racemic mixtures of phenylalanine and model target compound BNOH. It is notable that the electrodes coated by the conjugates of racemic thiotic acid with different enantiomers of 1,1′-binaphthyl-2,2′-diamine (R- and S-conjugates correspondingly), being used alone or in a mixture with matrix molecules, displayed no capacitive increase on addition of phenylalanine, even in millimolar concentrations. However, these electrodes were very sensitive to the addition of even micromolar concentrations of racemic R/S-BNOH solution. Deviations of the recognition properties of the receptors formed by the R- and S-conjugates from ideal symmetric behavior are most probably caused by favorable binding of definite optical isomers of thiotic acid during the conjugation, thus leading to the formation of molecules with two chiral centers and loss of the mirror symmetry. The binding of the model analyte to the conjugate may be explained by π-electron interactions. The ability of such surfaces to discriminate enantiomers was studied by measuring of the changes in the electrode capacitance on addition of R- or S-enantiomers of BNOH, the concentration varied from 6.25 to 50 µM. Capacitance changes for a number of electrodes coated by different organic monolayers are presented in the Table 2. The ratio of the apparent capacitance changes, assumed to be proportional to the adsorbed amount [14] and therefore to the enantioselectivity, was taken as a criterion for the chiral recognition properties of each of the tested coatings. The
results showed that the concentration ratio between matrix and template molecules in the coating solution is the key parameter determining the chiral recognition properties of the surfaces obtained.

As revealed from the results of Table 2, the sensitivity of the sensors to the analyte increases on increasing the template concentration in the mixture with the matrix molecule. This is additional evidence for the important role of the template to create cavities of a specific size that act as a mould for the target analyte. The highest enantioselectivities were obtained for the gold electrodes coated by template/matrix mixtures with molar ratios of 1:3.5 and 1:2. The efficiency of artificial receptors was compared for two types of matrices, namely the long chain alkylthiols (1,16-mercaptohexadecanoic acid, 16-mercaptohexadecane) and the shorter one (12-mercaptododecane). A decrease of the matrix thickness, realized by the substitution of 16-mercaptohexadecane by 12-mercaptododecane, led to the essential decrease of enantioselectivity.

The kinetics of the capacitance change on additions of analytes is shown in Figure 1. An addition of the S-isomer results in up to 30% decrease of the electrode capacitance while the effects on addition of R-isomer were several times lower. The effects magnitude is similar to that observed for surfactants adsorption on hydrophobic self-assembled monolayer [14] but much less than for antigen/antibody interaction [15]. The reason of the capacitive effect is mainly a
replacement of highly polarizable water molecules and ions by the much less polarizable analyte molecules. The fact that the dielectric constant of the analyte is low and therefore more similar to hydrophobic chains of surfactants than to proteins, explains high capacitive effects. A rough estimation of the binding constant from the concentration, corresponding to 50% of the maximal effect, gives the value of about 3 × 10^4 M⁻¹, which is similar to that estimation from the data [12].

The impedance spectra of gold electrodes modified by the same template with different matrices are presented in Figure 2. The electrodes were coated with a mixture of 16-mercaptOHexadecane (0.1 mmol L⁻¹) and S-conjugate (0.03 mmol L⁻¹) in ethanol/dioxane (9/1) (●) or by a mixture of 12-mercaptododecane (0.1 mmol L⁻¹) and S-conjugate (0.03 mmol L⁻¹) in ethanol/dioxane (9/1) (●). Measurement conditions are described in Experimental.

![Impedance spectra of gold electrodes coated by a mixture of 16-mercaptOHexadecane (0.1 mmol L⁻¹) and S-conjugate (0.03 mmol L⁻¹) in ethanol/dioxane (9/1) (●) or by a mixture of 12-mercaptododecane (0.1 mmol L⁻¹) and S-conjugate (0.03 mmol L⁻¹) in ethanol/dioxane (9/1) (●). Measurement conditions are described in Experimental.](image1)

The chiral selectivity of the spreader-bar structures with S-conjugate as the template was further examined by means of surface plasmon resonance measurements. We applied the ratio of the template and matrix concentrations that had provided the highest enantioselectivity in the capacitive study (Table 2). As can be seen in Figure 3, the signal changes upon addition of the S- and R-BNOH is higher in the case of S-BNOH. The enantioselectivity was calculated as a ratio of stationary SPR shifts on addition of corresponding enantiomers; a value of 2.55 was obtained. The unusual kinetics of the SPR signal indicates on a contribution of some additional process; it could be conformational changes in the receptor layer or a crystallization of the adsorbed analyte (as in [9]).

The proposed methodology is widely applicable and can be used to form chirally selective receptors for a large variety of species. The measured values of enantioselectivity (4.76 and 2.55 for capacitive and SPR technique correspondingly) are similar to value of 2.0 obtained for separation of the same compounds by capillary electrophoresis [16]. One can expect further increase of the chiral sensitivity by conjugating chiral spreader bar to a non-chiral thiolinker. Such sensors may be used for analysis of chiral compounds in complex mixtures, for quality control of chiral drugs and food additives, and in related applications.

**Experimental**

(R,+)−1,1′-binaphthyl-2,2′-diol (R-BNOH), (S,−)−1,1′-binaphthyl-2,2′-diol (S-BNOH), (R,+)−1,1′-binaphthyl-2,2′-diamine, (S,−)−1,1′-binaphthyl-2,2′-diamine, 1-phenylalanine, d-phenylalanine and d,L-thiopic acid were purchased from Sigma. 16-MercaptOHexadecane, 12-mercaptododecane, N,N′-dicyclohexyl-carbodiimide and N-hydroxysuccinimide were obtained from Aldrich. ‘Milli-Q’ (Millipore) water was used throughout.

Conjugates were prepared by mixing of 0.4 mmol L⁻¹ d,L-thiopic acid, 30 mmol L⁻¹ of N,N′-dicyclohexyl carbodiimide, 30 mmol L⁻¹ of N-hydroxysuccinimide and 0.2 mmol L⁻¹ S- or R-BNH in dioxane (all the concentrations are referred to the final solution) and stirred for 5 days at room temperature. Then the mixture was centrifuged to remove the precipitate. A formation of the conjugates was indicated by thin layer chromatography (chloroform/methanol/water...
concentrated H$_2$SO$_4$), then rinsed with water, and dried.

Artificial binding sites for R-BNOH and S-BNOH were created on a gold surface. Gold electrodes consisted of a 700 µm diameter circular active area (0.38 mm$^2$) and a contact pad (1 x 2 mm). Electrical connection was provided by a 7 mm length and 10 µm thick gold wire. A gold layer (150 nm) was sputtered on a glass support with Ti/Pd (50/50 nm) adhesive layers. Prior to use, gold electrodes were self-cleaned by piranha solution (a 1:3 mixture of 30% H$_2$O$_2$/50% H$_2$SO$_4$), then rinsed with water, and dried under nitrogen. Caution: piranha solution reacts violently with most organic materials and must be handled with extreme care. For the preparation of the artificial receptors by the spreader-bar technique, the electrodes were immersed into a solution of 16-mercaptohexadecane and R- or S-conjugate in ethanol/dioxane (9/1) for 72 h at 22 ºC, then rinsed with ethanol, and dried under nitrogen. A statistical deviation of the initial capacitance of coated electrodes prepared in the same conditions was typically within 10%.

Capacitive measurements were conducted with a homemade 8-channel lock-in amplifier with 8 independent current inputs which is based on the modules of Femto Messtechnik (Germany) by using a two-electrode configuration under mild stirring. Gold electrodes were used as the working electrodes and a Ag/AgCl wire (area more than 50 mm$^2$) was used as the reference electrode. All potentials are referred to this electrode. The electrode capacitance was measured by registration of the 90º component of the AC current at 80 Hz, under a DC potential of +0.3 V. The amplitude of the sine voltage was 10 mV. Impedance spectroscopy (IS) experiments were performed with the Frequency Response Analyzer 2 (PGSTAT12/FRA2, Eco Chemie, The Netherlands) in a three-electrode cell under quiescent conditions in the presence of 10 mmol L$^{-1}$ ferricyamide in 50 mmol L$^{-1}$ KCl, pH 7.2 as supporting electrolyte. A gold electrode was used as a working electrode, a saturated calomel electrode as a reference electrode, and a platinum wire as an auxiliary electrode. The impedance spectra were recorded in the frequency range from 1 Hz to 100 kHz by using a sinusoidal excitation signal. The DC potential was 0.4 V. An excitation amplitude of 10 mV was used. Surface plasmon resonance (SPR) experiments were carried-out with the BIOSUPLAR-2 SPR-spectrometer (Analytical µ-Systems, Germany) onto gold coated glass slides that had been coated via the above procedure. All capacitive and SPR measurements were performed in 15 mmol L$^{-1}$ phosphate, 50 mmol L$^{-1}$ KCl, pH 7.3 at ca. 22 ºC.

Acknowledgement

M. P. is grateful to DAAD for a short term fellowship supporting his visit to the University of Regensburg.

References