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RATE OF BINDING OF HOST MOLECULES TO ARTIFICIAL RECEPTORS FORMED BY SELF-ORGANISATION OF LIPIDATED OLIGOPEPTIDES

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Abstract: The arrays of artificial receptors were synthesized and used in the studies of binding of triphenylmethyl dyes. Rate of binding of Brilliant Black was determined by the measurements of decrease of optical density of dye solution. Considering the fast process of removing excess of dye and uniform coloration of receptors, Brilliant Black was evaluated as the most versatile reporter dye for Indicator Displacement Assay (IDA).

Keywords: docking, chemosensors, IDA, cellulose

Conventionally, chemosensors are designed based on the use of an indicator–spacer–receptor approach (ISR). In this solution, an indicator (chromophore or fluorophore) is covalently bind with a receptor through a spacer (1). Thus, construction of complex indicator–spacer–receptor molecule enabled measurement of changes in fluorescence or absorbance after introduction of an analyte that binds to the receptor. The major defect of this approach is that attachment of the indicator to the receptor may require difficult syntheses. An alternate approach that circumvents this problem is the indicator–displacement assay (IDA) (2). In an IDA approach, an indicator at the first binds reversibly to a receptor and then, an analyte is introduced into the system causing the displacement of the indicator from the host and binding of an analyte to the host (3).

The most significant demand for an IDA is an affinity between the indicator and the receptor, and between the analyte and the receptor. The mechanism of these interactions involves H-bonding (4), electrostatic interactions (5), and complexing with metal centers (6), therefore, they depend on the geometry, charge, hydrophobicity, and the solvent system (7). Signal modulation in an IDA is possible based on several mechanisms: photoinduced electron transfer (PET) (8), fluorescence resonance energy transfer (FRET) (9), electronic energy transfer (EET) (10), or simple changes in local ionic strength or pH (11).

The IDA methodology has many advantages over traditional sensing assays. One of them is that IDA does not require the indicator to be covalently attached to the receptor. Second, because there are no covalent bonds between the receptor and the indicator, the same receptor can work with several different indicators. Third, the assay works well in both organic and aqueous media. The most important, the methodology can be easily adapted to different receptors and platforms for quick analysis. The noncovalent attachment of the signaling unit makes IDAs very flexible, because the nature of the indicator as well as the indicator/receptor ratio (12) can be varied according to the sensing problem.

IDA methodology can be divided into three types. The colorimetric IDA (C-IDA) which utilizes colorimetric indicators. The second class is the fluorescent IDA (F-IDA) which uses fluorescent indicators, and the third class is the metal complexing IDA (M-IDA) that utilizes a metal center with either a colorimetric or fluorescent indicator, that means that M-IDA is a subset of C-IDA and F-IDA. Naked-eye detection of binding of a ligand to the receptors has been the inspiration for the development of C-IDAs.

Herein we attempted to use C-IDA methodology for selecting suitable colorimetric indicators for studies of binding colorless ligand to artificial receptors formed by self-organisation of N-lipidated oligopeptides attached to surface cellulose support.

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EXPERIMENTAL

Immobilization of 2,4-dichloro-6-methoxy-1,3,5-triazine (DCMT) on cellulose

147 plates (10 × 10 cm) of filter paper Whatman-7 were immersed in 1 M aq. NaOH (1600 mL) and gently shaken for 15 min. An excess of solution was removed by effective impression. Then, the wet, alkalized plates of paper were soaked in suspension of finely grounded sodium bicarbonate (20 g) in 1 M solution of 2,4-dichloro-6-methoxy-1,3,5-triazine in THF (1600 mL) and gently shaken for 45 min at room temperature. The plates were removed and thoroughly washed by successive treatment with solution of THF, (3 × 500 mL), and acetone (3 × 500 mL), spreaded out to remove the remaining solvent and dried in vacuum desiccator over P_2O_5 and KOH to the constant mass.

Elemental analysis: for pure cellulose: %N 0.00-0.05, %Cl 0.01-0.05; for modified cellulose found: %N 3.64, %Cl 2.66.

Loading of the cellulose plate

Loading based on nitrogen content = 2.60 mmola (N) /L g; what is equivalent to 0.87 mmol (triazine) /L g.

Loading based on nitrogen content = 31.9×10^{-6} mol (N) /cm^2; what is equivalent to 10.6×10^{-6} mol (triazine)/cm^2.

Loading based on chlorine content = 0.75 mmol (Cl) /L g; what is equivalent to 9.2×10^{-6} mol (Cl)/cm^2.

Immobilization of *m*-phenylenediamine

The plates were separated and immersed in 1 M solution of *m*-phenylenediamine in THF (1600 mL) and gently shaked for 24 h at room temperature. Then, the plates were removed from *m*-phenylenediamine solution, soaked with dry filter paper and washed successively with THF (2 × 1600 mL), DMF (3 × 1000 mL), and again with THF (3 × 1600 mL) and then dried in vacuum desiccator.

Loading of the first amino acid

The Fmoc-protected amino acid (15 mmol), N-methyl-N-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholinium *p*-toluenosulfonate (DMT/NMM/ p-TsO$^-$) (6.19 g, 15 mmol) and HOBt (2.02 g, 15 mmol) were dissolved in DMF (30 mL) and then NMM (3.3 mL, 30 mmol) was added. 147 functionalized cellulose plates were immersed in the mixture and gently shaked for 24 h. An excess of acylating reagent was removed, then plates were successively washed by gentle shaking in DMF (3 × 100 mL) and CH_2Cl_2 (2 × 100 mL) then dried in vacuum desiccator.

This general procedure was used for preparation of *sub*-libraries using N-Fmoc protected amino acids: Fmoc-Ala-OH (4.93 g; 15 mmol); Fmoc-Trp-OH (6.40 g; 15 mmol); Fmoc-Pro-OH (5.06 g; 15 mmol); Fmoc-Glu-(OtBu)-OH (6.38 g; 15 mmol); Fmoc-His(Trt)-OH (9.29 g; 15 mmol); Fmoc-Lys(Boc)-OH (7.03 g; 15 mmol); Fmoc-Ser(tBu)-OH (5.75 g; 15 mmol).

Removal of Fmoc protecting group

147 plates were immersed in 25% piperidine in DMF (150 mL) and gently shaked for 20 min, then thoroughly washed with DMF (3 × 150 mL), CH_2Cl_2 (2 × 150 mL) and immediately used in the next synthetic stage.

Introduction of the second amino acid

The Fmoc-protected amino acid (7 mmol), DMT/NMM/ p-TsO$^-$ (2.89 g, 7 mmol), HOBt (0.94 g, 15 mmol) was dissolved in DMF (15 mL) and then NMM (1.65 mL, 15 mmol) was added. 147 functionalized cellulose plates were immersed in the mixture and gently shaked for 24 h. An excess of acylating reagent was removed, then the plates were successively washed by gentle shaking in DMF (3 × 100 mL) and CH_2Cl_2 (2 × 100 mL), then dried in vacuum desiccator.

This general procedure was used for preparation of *sub*-libraries using N-Fmoc protected amino acids: Fmoc-Ala-OH (2.30 g; 7 mmol); Fmoc-Arg(Pbf)-OH (4.54 g; 7 mmol); Fmoc-Pro-OH (2.36 g; 7 mmol); Fmoc-Glu-(OtBu)-OH (2.98 g; 7 mmol); Fmoc-His(Trt)-OH (4.34 g; 7 mmol); Fmoc-Lys(Boc)-OH (3.28 g; 7 mmol); Fmoc-Ser(tBu)-OH (2.68 g; 7 mmol).

Removal of Fmoc protecting group

147 plates were immersed in 25% piperidine in DMF (150 mL) and gently shaked for 20 min, then thoroughly washed with DMF (3 × 150 mL), CH_2Cl_2 (2 × 150 mL) and immediately used in the next synthetic stage.

Introducing of lipid fragment

The vigorously stirred solution of DMT/NMM// p-TsO$^-$ (6.19 g, 15 mmol) in CH_2Cl_2 (50 mL) was cooled to 0-5^{o}C, treated with carboxylic acid (15 mmol) and NMM (0.8275 mL, 7.5 mmol). Stirring at 0-5^{o}C was continued for 4 h and activated carboxylic acids were immediately used for lipidation of the plates.

Functionalized cellulose plates were immersed in the mixture obtained after activation of carboxylic acids and gently shaked at room tem-

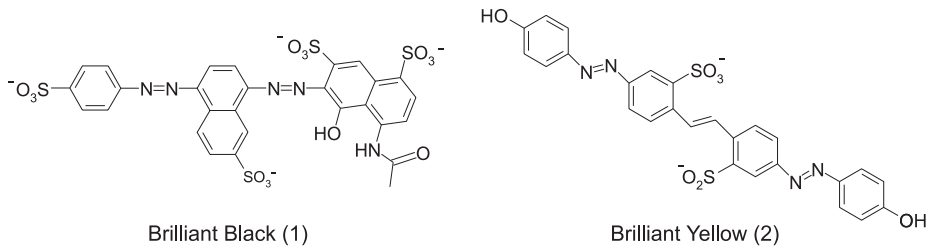

Figure 1. Structure of effective reporter dyes.

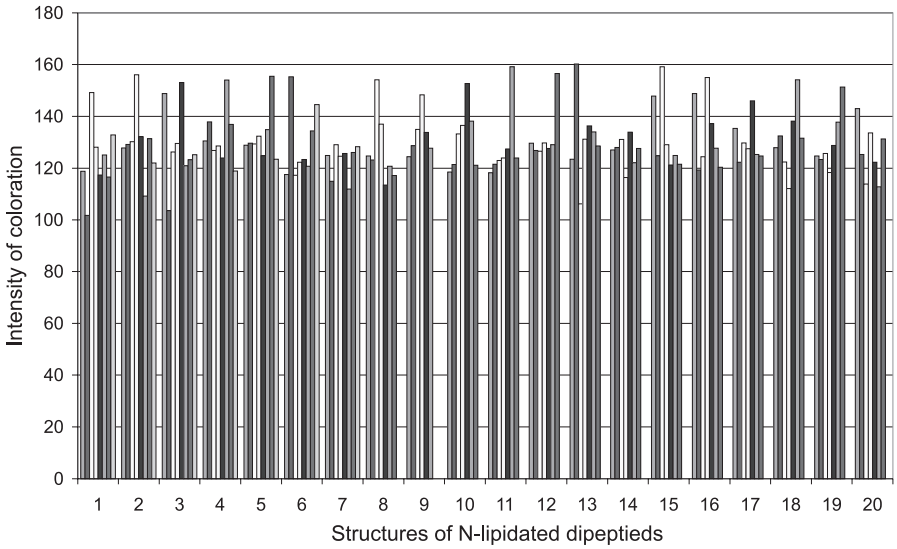

Figure 2. Intensity of binding of Brilliant Black to the library of receptors with deprotected functional groups at side chains of amino acid residues inside the binding pocket.

perature for 24 h. The plates were soaked and thoroughly washed with DCM (3 × 50 mL), the mixture of THF (20 mL) and water (20 mL), DMF (3 × 50 mL) and DCM (50 mL) and dried in vacuum desiccator.

This general procedure was used for acylation with following carboxylic acids:
hexadecanoic acid (3.85 g; 15 mmol), (R)-12-hydroxyoctadec–9-enoic acid (4.48 g; 15 mmol) and octanoic acid (2.58 g; 15 mmol).

Removal of side chain protecting groups

Functionalized cellulose plates were treated with 100 mL solution of TFA in DCM (50% v/v) with triisopropylsilane (TIS) (2% v/v) and water (3% v/v) for 4 h. Then the plates were washed with DCM (5 × 100 mL), EtOH (2 × 100 mL), DCM (2 × 100 mL) and dried in the desiccator.

RESULTS AND DISCUSSION

The arrays of artificial receptors formed by N-lipidated oligopeptides immobilized, in the highly ordered fashion, on the cellulose support were synthesized and used in the studies on molecular recognition. Thus, even in the case, when the single receptor in a differential array does not necessarily have selectivity for a particular analyte the combined fingerprint response can be extracted as a diagnostic pattern visually, or using any chemometric tools.

The previous studies confirmed that alternation of the binding pattern could detect even tiny structural changes of guest molecules and therefore offer a new tool for medical diagnostics. For example, an assay of physiological fluids and tissue homogenates has been found useful for diagnosis of thyroid tumors (13).

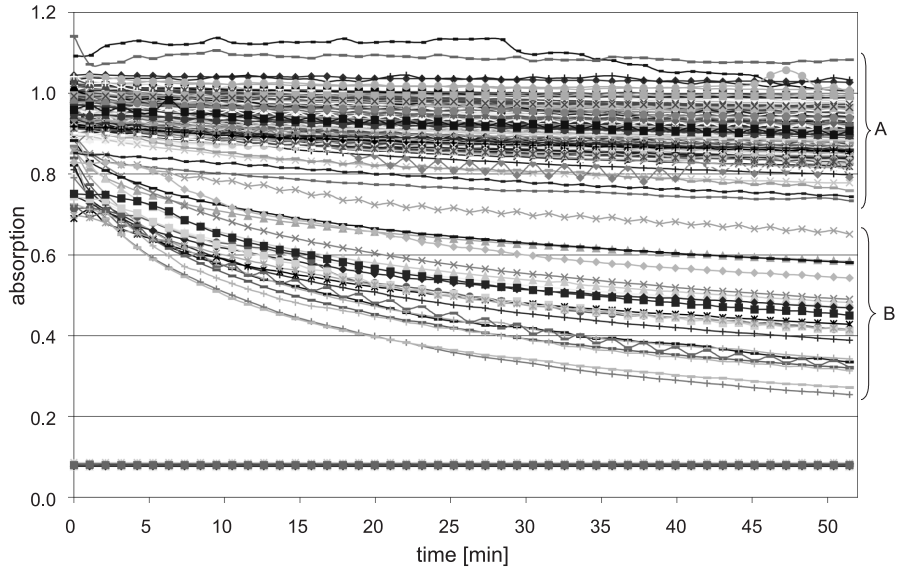

Figure 3a. Rate of binding of Brilliant Black by 147 library members (determined by Sunrise Microplate reader with Magellan software).

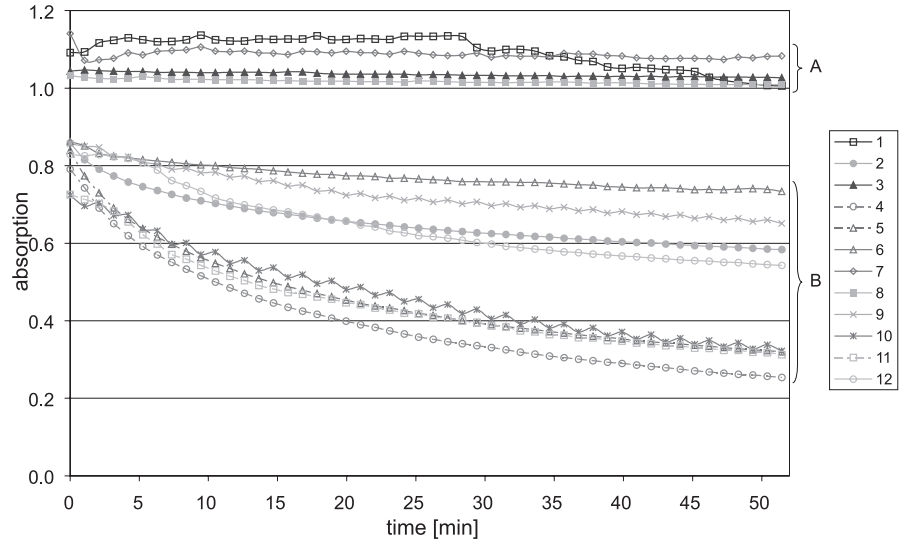

Figure 3b. Rate of binding of Brilliant Black by 12 randomly selected examples from 147 library members (determined by Sunrise Microplate reader with Magellan software); 1) hexadecanoyl-AlaGlu~; 2) hexadecanoyl-HisTrp~; 3) hexadecanoyl-LysSer~; 4) hexadecanoyl-ArgAla~; 5) (R)-12-hydroxyoctadec-9-enoyl-AlaHis~; 6) (R)-12-hydroxyoctadec-9-enoyl-GluLys~; 7) (R)-12-hydroxyoctadec-9-enoyl-LysTrp~; 8) (R)-12-hydroxyoctadec-9-enoyl-LysPro~; 9) octanoyl-AlaHis~; 10) octanoyl-HisHis~; 11) octanoyl-LysHis~; 12) octanoyl-ArgHis~.

Further successful application of arrays of artificial receptors for profiling of metabolome depends on efficiency of competitive binding of guest molecules and colored indicator dye. The most versatile indicator should bind fast and unselectively to all binding pockets with the equal or almost equal strengths. It has been found that in the presence of strongly polar functional groups of the peptide fragment inside the binding pocket, binding of lipophilic molecules of typical reporter dyes is strongly disfavored. Moreover, an increase of length of peptide chain and removing protecting groups from the side chains of aminoacids enlarged the binding pocket (14). The screening has shown that most of typical

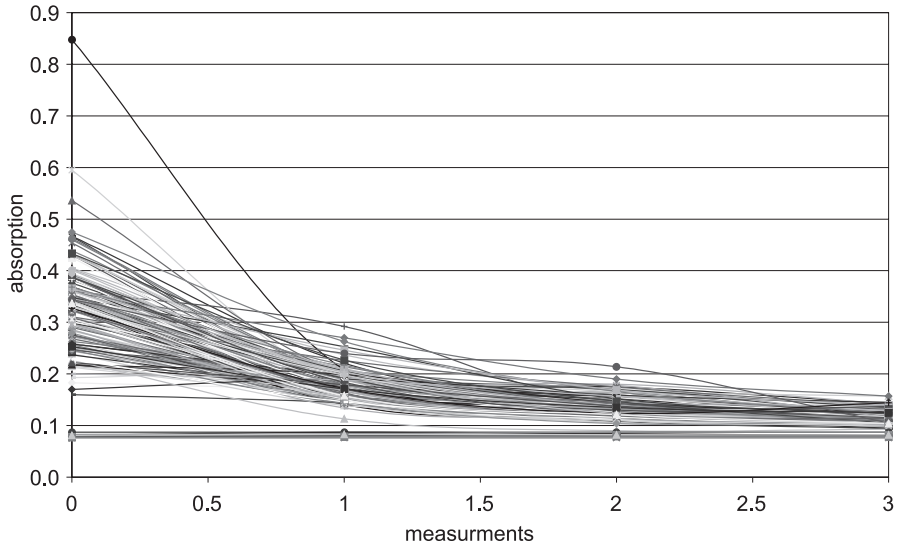

Figure 4. Profile of washout of excess of Brilliant Black.

triphenylmethyl dyes (Thymol Blue, Bromothymol Blue, Bromocresol Purple, Bromocresol Green, Bromochlorophenol Blue, Phenol Red, Bromophenol Blue, Chromeazurol S and Sulfasalazine) bind in very irregular fashion with most of receptors remained uncolored. As the most promising for this purpose two dyes were selected with structures depicted below on Figure 1. Considering the yellow coloration as less suitable for analysis of natural products, only the Brilliant Black was used in further experiments. The rate and intensity of binding of Brilliant Black to the artificial receptors was measured (see Figure 2).

The decrease of optical density of Brilliant Black solution from the absorbance 1.200 of starting solution to the plateau proceeded in most of the cases faster (less than 1 min) than measurable under experiment condition (see Figure 3a, marked as A). The slow decrease of absorbance observed for several binding profiles (see Figure 3a, marked as B), reflects probably slow, unspecific diffusion of the dye into interior of the cellulose support, probably *via* fragments with irregular structure. However, the rate of this unspecific process is so slow, when compared to the fast specific binding, that it should not to be recognized as concomitant, disturbing process, and therefore in all dying experiments proceeding in less than 5 min period, could be neglected.

The rate of binding of the reporter dye, Brilliant Black from its solution by randomly selected library members with information concerning the structure of binding pocked has been presented on Figure 3b.

It has been found that Brilliant Black profile of washout of excess of dye was very advantageous for all receptors studied. Probably, due to the presence of strongly polar sulfonic groups most of nonspecifically bound reporter dye has been successfully removed after second washing procedure. Considering this fast process of removing excess of dye and fast process of fairly uniform coloration of receptors, Brilliant Black could be evaluated as a versatile reporter dye for Indicator Displacement Assay (IDA).

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