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Highly Sensitive Novel Biosensor Based on an Immobilized *lac* Repressor**

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Sequence-specific interactions of proteins with DNA are central to all aspects of the utilization of genetic information in any organism. The lactose repressor of *E. coli* served as a paradigm for such interactions even before the chemical structure of the interacting partners was elucidated.^[1] The *lac* repressor protein recognizes the *lac* operator, a particular region of base pairs in the chromosome of *E. coli* and binds to it tightly with a dissociation constant of 10^{–11}–10^{–13} M.^[2] Site-specific recognition of DNA by the *lac* repressor is interrupted by an inducer, such as lactose, to allow the production of the enzymes necessary for the utilization of this carbon source.^[3] A major conformational change in the *lac* repressor structure takes place as the result of inducer binding.^[4, 5] The majority of inducers that bind to the *lac* repressor are galactose derivatives, such as isopropyl-*D*-thiogalactoside (IPTG), *o*-nitrophenyl-*D*-galactoside (ONPG), and 1,6-allo-lactose.^[6] Other sugars like *o*-nitrophenylfucoside (ONPF) also bind strongly to the *lac* repressor.^[7]

Capacitance measurements have been successfully used as a basis for the construction of biosensors for sensitive detection of the human chorionic gonadotropin (HCG) hormone, by immobilization of antibodies on the electrode surface.^[8] Heavy metals can also be detected, with heavy metal binding proteins as recognition elements.^[9, 10] The capacitive transduction principle has now been used for the development of a biosensor to monitor inducer molecules or DNA, through the use of a repressor protein as the biological recognition element.

Biosensors prepared by immobilizing the *lac* repressor protein on a gold surface modified with thioctic acid have been used in the experimental set-up presented schematically, along with the detection principle in Figure 1. The specificity of *lac* repressor based biosensors for operator DNA was tested by injections of plasmid p310 DNA, two linearized plasmid DNAs, and genomic DNA. Plasmid p310 DNA (2455 base pairs (bp) in length) was constructed by cloning a 24 bp fragment that contains the *lac* ideal operator into the *Nhe*I site of plasmid pEE4. One linearized plasmid DNA was obtained by digestion of the plasmid p310 DNA with *Eco*RI, and the target *lac* operator (the second linearized plasmid DNA) was excised as an 84 bp fragment by cutting with *Eco*RI and *Hind*III.

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[**] This work was supported with grants given by the Swedish Engineering Research Council (TFR). Prof. Muller Hill and Dr. Andrew Barker are acknowledged for providing plasmids and the *Escherichia coli* BMH8117 strain.

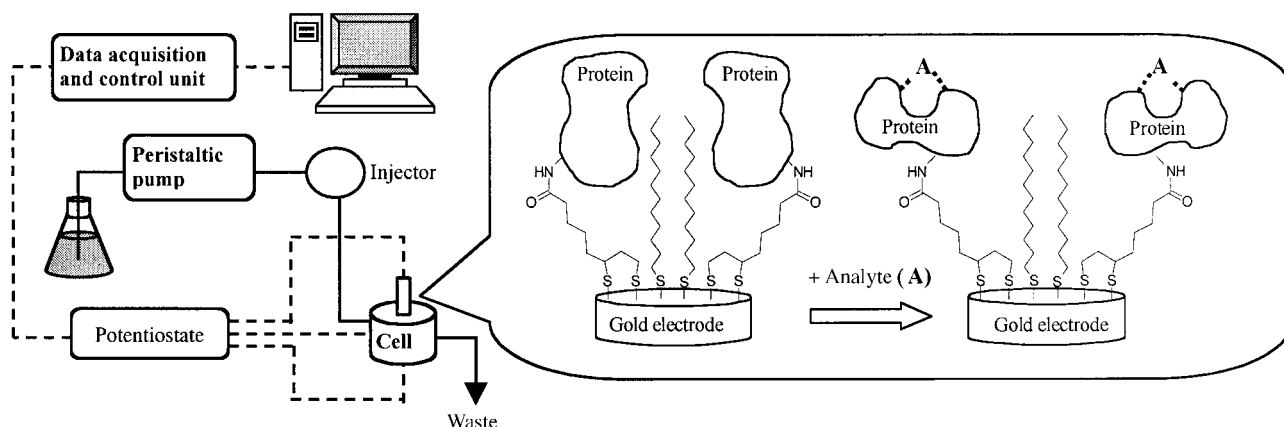


Figure 1. Experimental set-up and detection principle.

The signals obtained (Figure 2) with linearized plasmid DNAs were higher than those obtained for circular plasmid DNA, probably due to the steric constraints that diminish the ability of the *lac* repressor to bind to the operator when the operator is included in the circular plasmid. Nonspecific genomic DNA gave low signals; these results show the ability of the sensor modified with the *lac* repressor to distinguish the operator DNA from the nonoperator genomic DNA or from nonlinearized circular plasmid DNA.

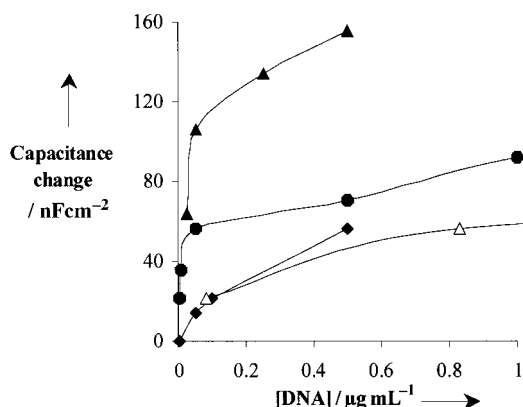


Figure 2. Specificity of the biosensor for different DNA sequences: Plasmid DNA linearized with *EcoRI* (▲); plasmid DNA linearized with *EcoRI* and *HindIII* (●); plasmid DNA (◆); genomic DNA (△). Measurements were carried out in 10 mM potassium phosphate buffer (pH 7.2) containing 1 mM dithiothreitol (DTT) with a flow rate of 0.25 mL min⁻¹ and a sample injection volume of 250 μL.

The linearized DNA sequences of different size that contained the *lac* operator produced different capacitance signals (Figure 2). When the linearized plasmid with the complete base sequence (2455 bp) was used, the observed capacitance signal was about threefold higher than the one obtained with the target *lac* operator (84 bp fragment with the mixture of nontarget larger fragment). In the latter case, lower capacitance changes were obtained due to the smaller size of the specific operator fragment. The different sensitivities observed indicate clearly that the electrode modified with the *lac* repressor protein specifically recognizes the operator DNA fragment.

The sensitivity of the modified electrodes towards the inducer molecules decreased in the order: $S_{\text{ONPG}} > S_{\text{IPTG}} > S_{\alpha\text{-D-lactose}}$ (Figure 3). The sugar derivative with the highest

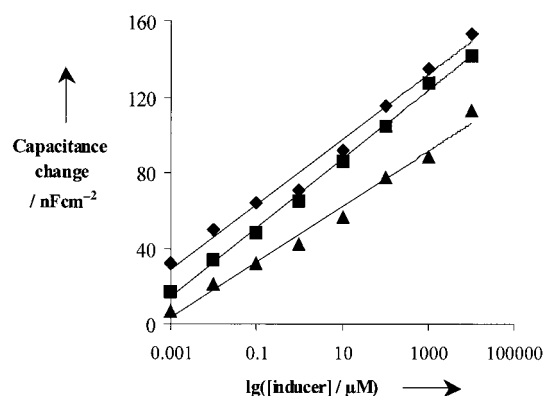


Figure 3. Calibration curves for different inducer molecules: ONPG (◆); IPTG (■); lactose (▲). Experimental conditions were as given in Figure 2.

molecular weight ($M_w = 310$), α -D-lactose, gave the lowest signal, while IPTG ($M_w = 230$) and ONPG ($M_w = 301$) yielded similar higher responses. All three inducers were detected in the 1 nM–10 mM concentration range. When saturating amounts of IPTG were injected in the system, the sequential injection of the plasmid DNA linearized with *EcoRI* did not cause any further change in capacitance, which suggests that the operator binding did not occur. The binding of the inducer to the immobilized *lac* repressor protein prevents the repressor binding to the operator.

The obtained results clearly demonstrate the possibility of utilizing capacitance biosensors based on the *lac* repressor protein for the assay of specific DNA sequences and inducers in a highly sensitive manner (nanomolar range). The use of repressor proteins in biosensors represents a new general approach in bioanalysis, which is comparable in selectivity and affinity to methods involving monoclonal antibodies (mAbs). However, mAbs are normally not so specific for small molecules like the inducers, and mAbs against specific DNA sequences may be rather difficult to obtain. There are many repressor proteins both in the human proteome and in various bacteria, hence, one could expect numerous repressor pro-

teins to exist which exhibit high selectivity and affinity towards both specific DNA sequences and different inducers.

Experimental Section

Biosensors were prepared by immobilizing the *lac* repressor protein through carbodiimide covalent coupling onto a gold surface modified with thioctic acid.^[8] The *lac* repressor was purified as described previously from *Escherichia coli* BMH8117 (genotype: F⁻, $\Delta(lac-proAB)$ *thi*, *gyrA* (Nal^R), *supE*, λ).^[11] The Ap^R plasmid pWB1000, which constitutively over-expresses wild-type *lac* repressor, and plasmid p310, which contains the *lac* ideal operator, were cloned into the *NheI* site of plasmid pEE4.^[12] Competent *E. coli* BMH8117 cells were transformed with the plasmids according to standard procedures.^[13] The cells were grown in double YT medium in 1 L culture flasks at 37 °C with shaking, harvested after 16–20 h of incubation, washed twice with buffer (0.2 M tris(hydroxymethyl)amino-methane–HCl (Tris-HCl; pH 7.2) containing 0.2 M KCl, 10 mM MgCl₂, 5 % (v/v) glycerol, 1 mM NaN₃, 0.3 mM DTT, and 1 mM phenylmethanesulfonyl fluoride (PMSF)), and stored frozen.

The plasmid DNA containing the *lac* operator was isolated from the cells harboring plasmid p310 with the miniprep Qiagen kit method according to the manufacturer's instructions. When performing measurements with linearized plasmid DNA, the plasmid DNA was digested with *EcoRI* (for the complete 2455 bp sequence) or *EcoRI* and *HindIII* (for the 84 bp sequence). The plasmids were linearized by digestion with the respective enzymes at 37 °C for 1 h.

Gold electrodes were polished, treated with ultrasound, plasma cleaned, and then pretreated with thioctic acid, as described earlier.^[8] Next, the thioctic acid self-assembled electrodes were thoroughly washed with pure ethanol, dried, and activated in a 1 % solution of 1-(3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride in dried acetonitrile for 5 h. After washing with 100 mM potassium phosphate buffer (pH 8), the electrodes were dipped into a protein solution (approximately 0.05 mg mL⁻¹) at 4 °C for 24 h. The electrodes were washed again with phosphate buffer and immersed for 20 minutes in a 10 mM solution of 1-dodecanethiol in ethanol. A final washing of the protein-modified electrode with phosphate buffer completed the electrode preparation.

The electrode modified with the *lac* repressor was inserted as the working electrode in a three-electrode flow cell with a dead volume of approximately 10 μ L. A platinum foil and a platinum wire served as the auxiliary and reference electrodes, respectively. An extra Ag/AgCl reference electrode was placed in the outlet stream to compare the potential with the platinum reference electrode just before measurements were made. In order to apply 50 mV on the working electrode, a computer was used to compare the potential of the Pt with the potential of the Ag/AgCl before applying the pulse on the working electrode. The carrier buffer (10 mM potassium phosphate buffer, pH 7.2) containing 1 mM DTT was degassed before use and pumped at a flow rate of 0.25 mL min⁻¹. Samples of 250 μ L volume were injected in the carrier flow.

Measurements were made as described earlier by applying a 50 mV potential pulse and recording the current transient.^[8] The current values were collected with a frequency of 50 kHz, and the first 10 values were used for the evaluation of capacitance.

Received: February 9, 2001 [Z16590]

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Noncovalent Assembly of [2]Rotaxane Architectures**

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Self-organization is an attractive approach to the construction of complex molecular architectures such as grids, cages, and topological objects.^[1–3] The synthesis of catenanes and rotaxanes employs noncovalent binding interactions to template the formation of the covalently interlocked structures.^[4] The key noncovalent intermediate is the [2]pseudorotaxane, where a guest molecule is threaded through the plane of a macrocycle.^[5, 6] The latent topological properties of this intermediate are kinetically trapped by macrocyclization to give [2]catenanes or by the introduction of bulky stopper groups to give [2]rotaxanes. Recently, there have been reports of catenane structures composed of interpenetrating self-assembled macrocycles: metal–ligand interactions have been used to construct [2]catenanes from as many as eight separate molecular components (Figure 1 a).^[7, 8] To date, only covalent [2]rotaxanes have been constructed using two interlocked molecules.^[4, 9] Here we describe a three-molecule approach to noncovalent [2]rotaxane architectures, where the macrocycle self-assembles around a complementary guest (Figure 1 b).

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[**] We thank Dr. C. J. Craven and Dr. J. P. Waltho for the 600 MHz NMR spectra. We also thank the Royal Society/NATO & FCO Chevening (M.O.V.), the Lister Institute (C.A.H.), the EPSRC (M.J.P.), and the James Black Foundation (C.Z.) for funding.