

Study of properties of nucleic acid dependent photosensitizer In(III)(pyropheophorbide-a)chloride by using a LB 940 Mithras plate reader coupled to a red diode array light source

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- Photochemical process for detection of nucleic acids in vitro
- Using the Mithras LB 940 multimode reader for monitoring the photochemical reactions by absorbance

Abstract

Photochemical reactions controlled by nucleic acids are applied for the detection of nucleic acids both in vitro and in live cells. They can be controlled both temporally and spatially, since light can be switched on and off at the desired time and it can be easily focused on the desired area of the sample (e.g. on an organelle in the cell). Optimization of such processes includes variations of conditions like the intensity of the light, the concentration of a fluorogenic dye, probes, a nucleic acid template and buffer additives. Such studies can be quickly and precisely conducted using a LB 940 Mithras plate reader combined with a diode array light source. Herein we demonstrated an application of this instrument for the study of an exemplary photochemical process: a reported before, red-light induced, nucleic acid dependent and singlet oxygen mediated reaction of bleaching of a yellow dye 2,5-(4-carboxyphenyl)isobenzofurane.

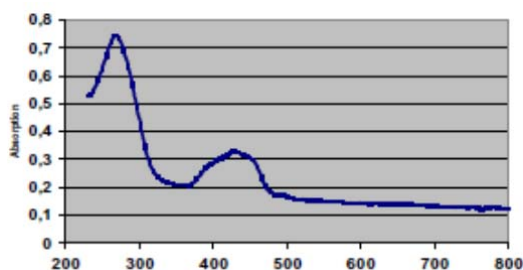


Figure 1: Absorption spectra of 2,5-(4-carboxyphenyl)isobenzofurane

For example, we confirmed that this reaction can be used for monitoring nucleic acids in vitro.

Introduction

Nucleic acid controlled, chemical reactions occur with either formation (ligation) or cleavage of chemical bonds [1]. These reactions can be applied both for detection of nucleic acids in vitro and in live cells and for the cell specific generation of drugs. These drugs can e.g. selectively kill cancer cells, which are genetically different from normal cells. For example, Saito et al. and Tanabe and Nishimoto et al. have described a stoichiometric, UV-light induced generation of model drugs [2]. The addition of the specific nucleic acid increases yields of the products in these reactions 2- to 7-fold. Gothelf et al. have reported a reaction generating toxic ¹O₂ (drug) from non-toxic ³O₂ (pro-drug) [3]. In particular, they have prepared a duplex from the (pyropheophorbide-a)-oligodeoxyribonucleotide-conjugate (PS~ODN1, Figure 1) and the complementary Q~ODN2 (Q= quencher). In this associate, the PS is fully quenched by the Q, whereas in the presence of an analyte nucleic acid the PS~ODN1 conjugate is related and the PS is reactivated. In the active form the PS induces the generation of ¹O₂ when exposed to light. This effect could be observed only at rather high concentrations of the duplex (5 µM) and in D2O buffer: conditions, which are not compatible with live cells. This reaction is not catalytic since the PS is quickly decomposed in the presence of ¹O₂. In 2010 we have optimized the system of Gothelf [4]. In particular, we have found that the substitution of pyropheophorbide-a in the PS~ODN1 conjugate for its In(III) complex improves the system substantially. The In(III)-complex-based PS is highly active both in vitro and in live cells. Moreover, it exhibits high photo-stability and is able to generate over 6000 eq singlet oxygen. Nucleic acid dependent reactions that are compatible with live cells are especially interesting, because they can be applied

not only for monitoring endogenous RNAs in situ but also the RNA-dependent release of cytotoxic drugs. In this note we report on the application of this catalyst for the detection of nucleic acids in vitro by using a LB 940 Mithras plate reader coupled to a red diode array light source ($\lambda_{\text{max}} = 650 \text{ nm}$, Figure 2). We make use of the ability of this instrument to monitor 96 photochemical reactions simultaneously by using absorbance measurements. It allows conducting optimizations within short times. Potentially, the throughput can be further increased by using microtiter plates with the larger number of samples. Moreover, other types of optical responses (fluorescence, chemiluminescence) can be measured using the same instrument.

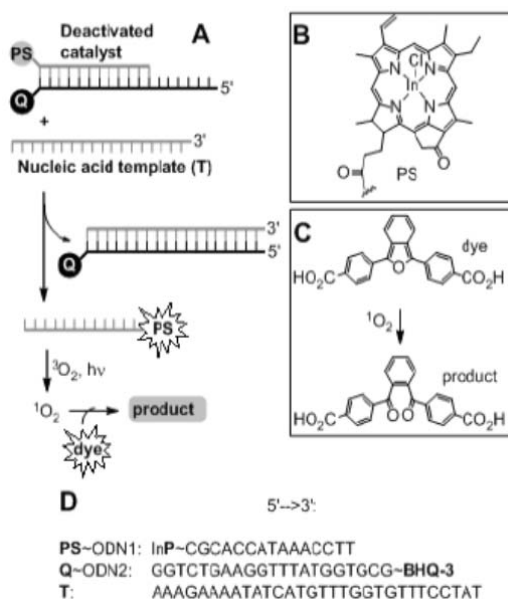


Figure 2: A: An outline of a concept of nucleic acid dependent photosensitizers (PS) and the mechanism of their activation; B: a structure of the photosensitizer (In(III)(pyropheophorbide-a)chloride) used in this work; C: a structure of the dye used for monitoring formation of singlet oxygen and the mechanism of its bleaching in the presence of singlet oxygen; D: sequences of probes and the nucleic acid template T

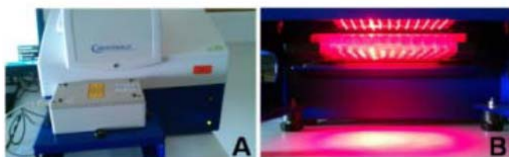


Figure 3: A: A photo of a LB 940 Mithras plate reader coupled to a diode array red light source (650 nm); view from the top with the light source switched off; B: view from the front with the light source switched on

Experimental Procedures

Conjugates of oligodeoxyribonucleotides (PS~ODN1 and Q~ODN2, Figure 1D), singlet oxygen - sensitive dye 2,5-(4-carboxyphenyl)isobenzofuran (dye, Figure 1C) and nucleic acid template (T, Figure 1D) were synthesized as described elsewhere [4]. Each conjugate mixture was equilibrated / annealed by applying the following temperature gradient: heating from 22 to 90 °C in 20 min, 10 min at 90 °C and slow (2 °C / min) cooling to 22 °C. Each mixture contained the dye. Its bleaching was monitored at 430 nm.

Material

- Mithras LB 940 multimode microplate reader (Berthold Technologies)
- Diode array red light source, $\lambda_{\text{max}} = 650 \text{ nm}$
- Absorption filter: 430 nm (Berthold Technologies, order no.: 39500)
- 96-well culture plate, sterile, F-bottom (Greiner Bio-one, order no.: 655180)
- Conjugates prepared according to reference 4
- Buffer: phosphate 10 mM, pH 7 containing NaCl 150 mM

Instrument Settings & Data Analysis

Data were collected using the MikroWin2000 software. Absorbance at 430 nm was measured as a function of time to monitor bleaching of the dye. Absorbance values were corrected for the background, which was determined by measuring absorbance at 430 of a well containing the buffer only. All experiments were conducted at 22 °C. Data were processed by using Microsoft Excel software.

Results

The goal of this study was to evaluate the applicability of the nucleic dependent photochemical amplification system reported elsewhere [4] (Figure 1) for the detection of nucleic acids in vitro. Upon irradiation of the photosensitizer PS~ODN1 with red light (650 nm) at aerobic conditions singlet oxygen is generated. For detection we added 2,5-(4-carboxyphenyl)-isobenzofuran (dye, Figure 3A), which is bleached in the presence of $^1\text{O}_2$ according to the mechanism outlined in Figure 1C. The absorbance at 430 nm in relation to the irradiation time enabled us to calculate initial reaction rates: $(dA/dt)_0$. To optimize the concentration of the dye we plotted $(dA/dt)_0$ as a function of the dye

concentration (Figure 3B). We observed a saturation effect above 200 μM dye concentration. In all further experiments the concentration of the dye equal to 300 μM was selected. Next, mixtures of PS~ODN1 (200 nM), Q~ODN2 (200 nM), the dye (300 μM) and variable template T concentrations in the phosphate buffer were irradiated with red light and initial reaction rates $((dA/dt)_0)$ were determined. We observed a linear relationship $((dA/dt)_0)$ to the template concentration in the range 0 to 200 nM, which indicates that this amplification system can be applied for the detection of nucleic acids in vitro.

References

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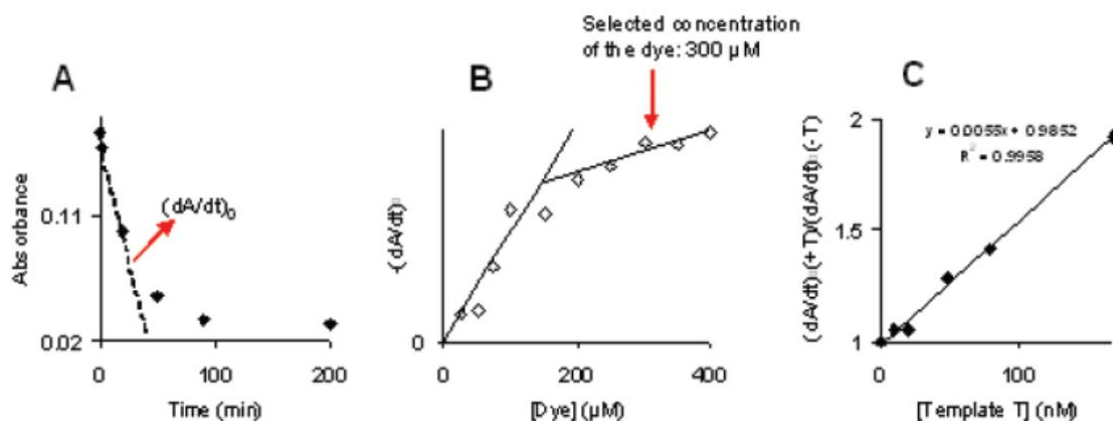


Figure 4: A: Representative kinetics of bleaching of the $^1\text{O}_2$ -sensitive dye (300 μM) in the presence of PS~ODN1 (200 nM) and upon irradiation with red light (650 nm); other conditions: absorbance at 430 nm was plotted as a function of irradiation time; buffer - phosphate 10 mM, pH 7, NaCl 150 mM, 22 °C; B: Optimization of the concentration of the $^1\text{O}_2$ -sensitive dye; the first derivative of dependence of the absorbance at 430 nm from time, which was determined at the initial reaction time as it is shown in figure 1A $((dA/dt)_0)$, was plotted as a function of concentration of the dye; other conditions: [PS~ODN1]=[Q~ODN2]= 200 nM, phosphate 10 mM, pH 7, NaCl 150 mM, 22 °C; 300 μM was selected for all other experiments; C: Calibration curve for determination of the concentration of the template nucleic acid T: conditions are as indicated for B