SANGER SEQUENCING AS A TOOL FOR INVESTIGATION OF INTERACTIONS BETWEEN PLATINUM-BASED CYTOSTATIC DRUGS AND DNA

Kristýna Šmerková[a], Simona Dostálová[b], Markéta Ryvolová[a,c], Helena Škutková[b], Vojtěch Adam[a,c], Ivo Provazník[b], René Kizek[a,c] *

[a] *Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, European Union-Czech Republic*
[b] *Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Kolejni 4, CZ-612 00 Brno, European Union-Czech Republic*
[c] *Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, European Union-Czech Republic*
kizek@sci.muni.cz

Abstract

Platinum-based cytostatic drugs such as cisplatin, carboplatin and oxaliplatin play an important role in the battle with cancer. The mechanism of their activity is widely studied and the quantification of the drug incorporated in the DNA structure is in the center of attention. In this study we investigated the behavior of the platinum-based cytostatic drug and DNA adducts during the well-established Sanger sequencing method involving capillary electrophoretic (CE) separation. Three selected platinum-based cytostatic drugs (cisplatin, carboplatin and oxaliplatin) were incubated with the DNA fragment to create adducts and subsequently sequenced.

INTRODUCTION

Cancer is the main reason of health problems in developed countries where every fourth death is caused by tumor disease [1]. For tumor therapy the chemotherapeutics including platinum based cytostatic drugs (cisplatin, carboplatin, oxaliplatin) are one of the most commonly used [2]. The biological activity of the first platinum-based cytostatic drug (cispaltin, *cis*-Diamminedichloroplatinum(II)) was discovered by Rosenberg in 1965 [3]. „The second generation" of platinum cytostatics represented by carboplatin (*cis*diammine-1,1-cyclobutane dicarboxylate) was developed in 80s as a less toxic alternative of cisplatin causing less serious side effects [4]. Cisplatin and carboplatin are effective for therapy of brain, neck, and/or lung cancer; however they have severe toxic and mutagenic effect on both cell and animal model samples [5,6]. Both of these cytostatics form same Pt-DNA complexes *in vivo* and it is known that cisplatin is ineffective to call lines resistant to carboplatin and vice versa. Therefore "third generation" – oxaliplatin ((*trans-R,R*)1,2-Diaminocyclohexaneoxalatoplatinum(II)) - was developed in 90s overcoming the resistance [7] and moreover providing lower mutagenity [8]. The analysis of Pt-DNA adducts is routinely performed by electrochemical methods [9,10], however the application of sequencing reaction combined with capillary electrophoresis represents an alternative method for quantification of platinum-based cytostatic drug intercalated in the DNA structure.

EXPERIMENTAL

Experimental procerure is shown in Fig. 1.

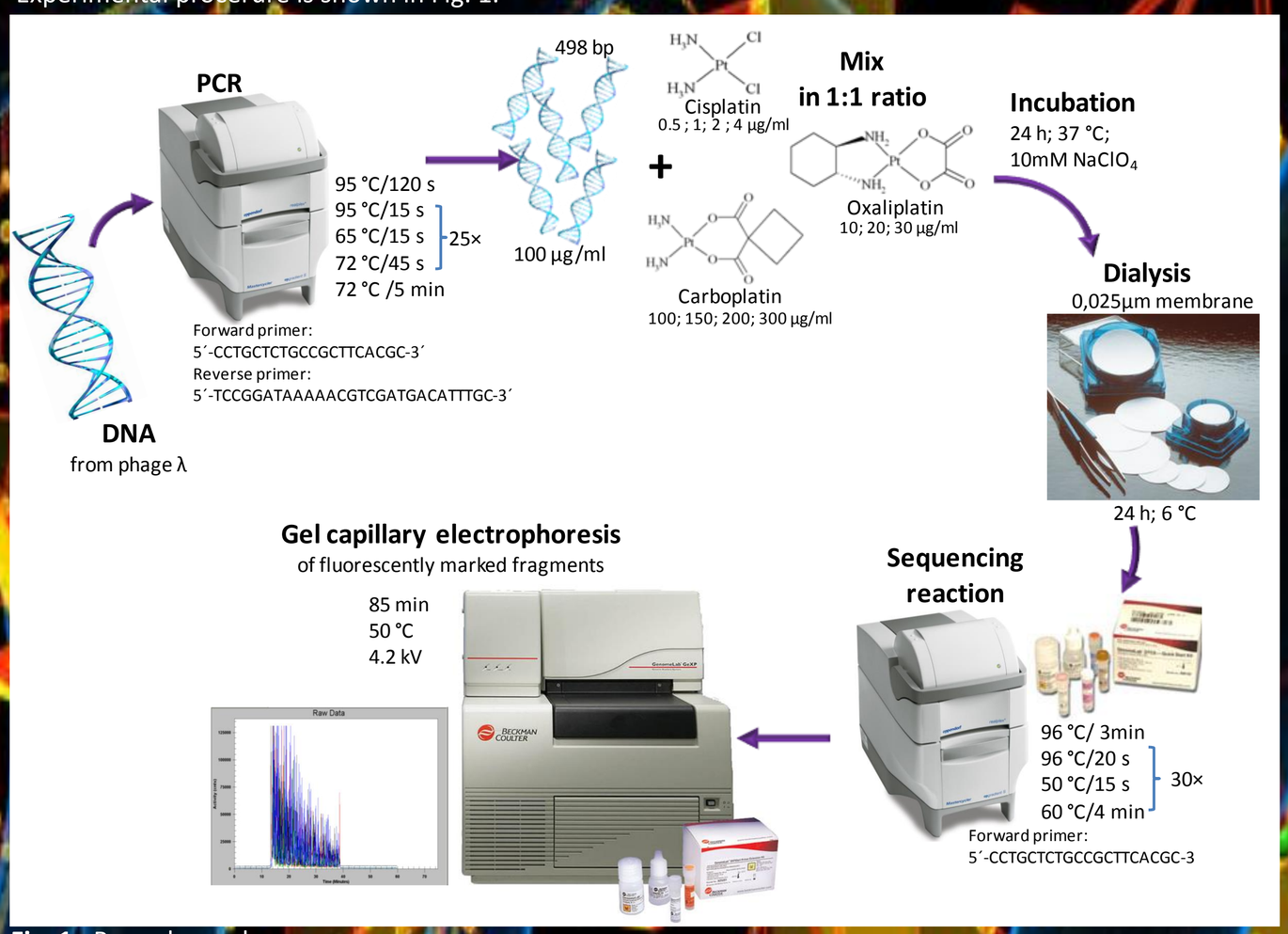

Fig. 1: Procedure scheme

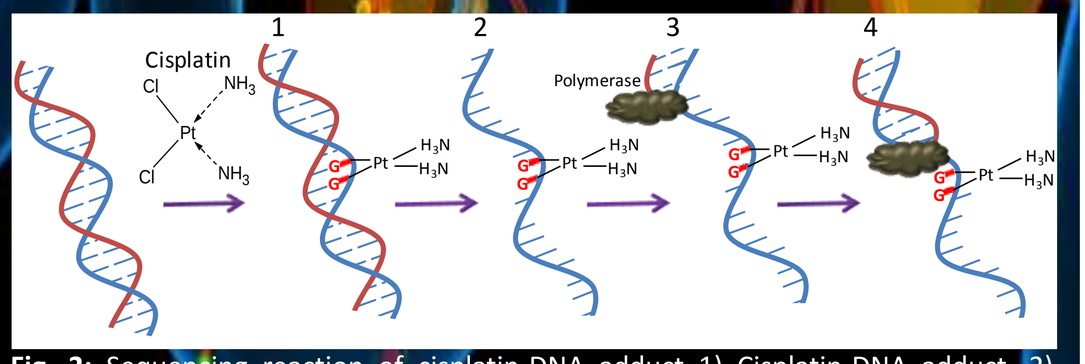

Fig. 2: Sequencing reaction of cisplatin-DNA adduct 1) Cisplatin-DNA adduct, 2) Denaturation, 3) Polymeration, 4) Termination of polymeration at cisplatin binding position

CONCLUSION

Using Sanger sequencing method, the influence of platinum-based cytostatics on DNA was investigated. The intensity of the signal decreased linearly with the amount of the cytostatics in the sample in case of all four DNA bases. Moreover it was found that different types of cytostatics have different behavior and influence on the sequencing reaction. These results may also suggest that each generation of the therapeutics has different mechanism of action and efficiency for cancer therapy.

ACKNOWLEDGEMENT

The financial support by NanoBioTECell GA ČR P102/11/1068 , NanoBioMetalNet CZ.1.07/2.4.00/31.0023 and IGA IP19/2012 is highly acknowledged.

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RESULTS

It was found that the signal intensity decreased with increasing concentration of the cytostatic drug. In Fig. 3, the analysis of carboplatinated DNA is shown. The signal of non-platinated DNA (control) is taken as 100%. In Fig. 3A, the signal of thymine was evaluated showing 50% decrease for 50 µg/ml of applied carboplatin. The signal decreased linearly with the concentration and it is shown that when 150 µg/ml of carboplatin is applied, the measured signal is only 6.3% of the control signal. Signal decrease was observed in case of all four bases; however the decrease was 36.5% (Fig. 3B), 31.4% (Fig. 3C) and 13.2% (Fig. 3D) in case of guanine, cytosine and adenine, respectively (when 50 µg/ml of carboplatin is applied).

From the results can be concluded that cytostatics bound to the DNA influence the LIF signal for all four bases the same way which suggests that it is not affecting the CE-LIF analysis but the labeling reaction and the fragment synthesis by polymerase. We believe that when the drug is bound to the DNA the polymerase stops the synthesis of the complementary fragment and therefore the signal decreases.

To verify the hypothesis, DNA adducts with cisplatin and oxalipaltin were prepared and analyzed by the same means. We observed that the concentration of the drug causing the same level of signal decrease differed for each type of cytostatic drug. To compare the results we calculated the concentration of the drug causing the decrease for 75% of the for control signal. It was found that for carboplatin 75 µg/ml is required. In case of oxalipaltin only 7 µg/ml is needed and finally only 0.3 µg/ml of cisplatin is causing such decrease.

From these results it can be concluded that different cytostatics have different abilities to form adducts with DNA.

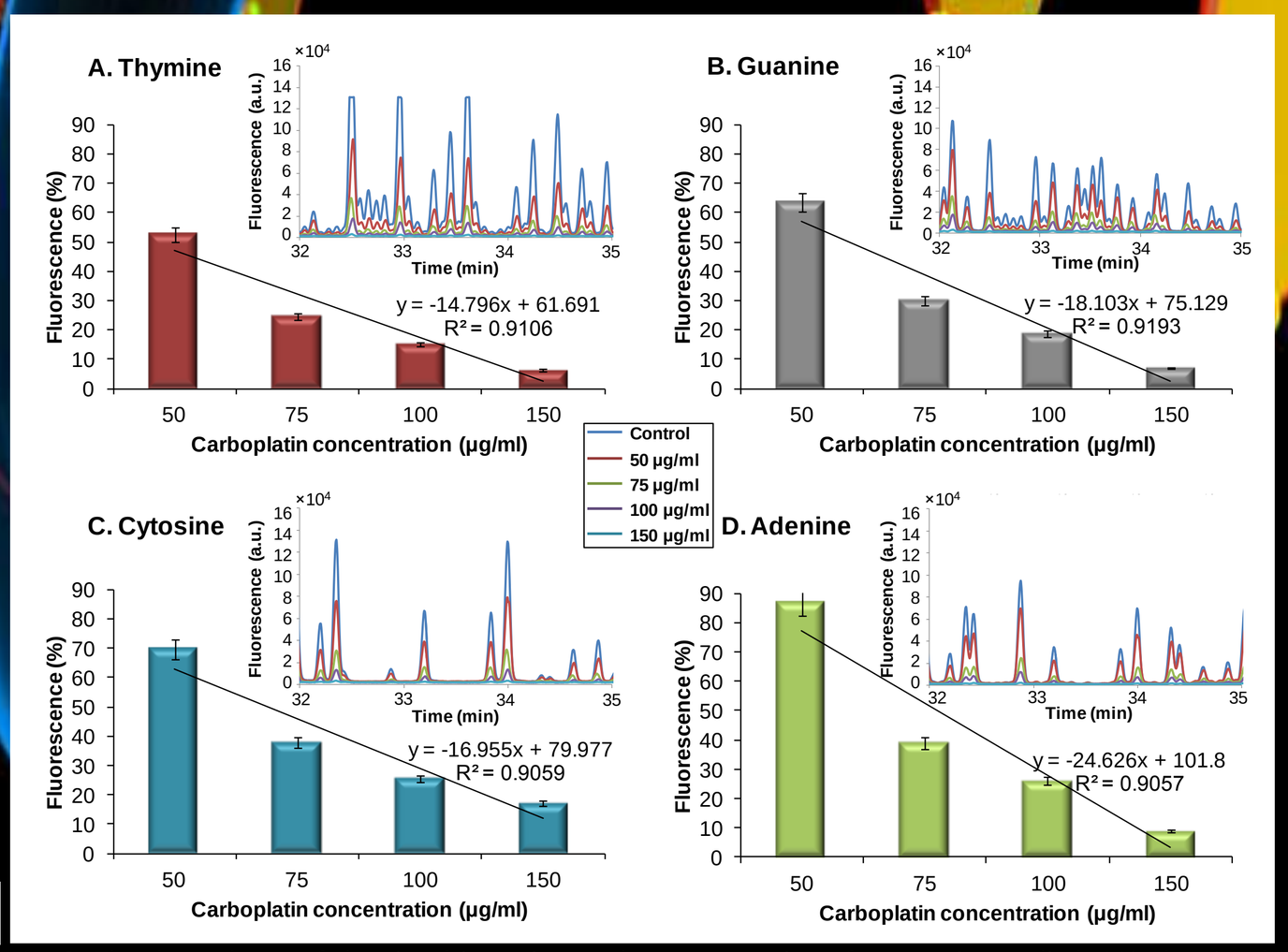

Fig. 3: The dependence of the signal intensity on the concentration of cytostatisc applied. 1A) The signal intensity dependence of thymine (inset: raw electrophoretic data used), 1B) The signal intensity dependence of guanine (inset: raw electrophoretic data used), 1C) The signal intensity dependence of cytosine (inset: raw electrophoretic data used), 1D) The signal intensity dependence of adenine (inset: raw electrophoretic data used).