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Utilization of FIA-UV/ED for detection of adenine derivates

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Abstract

A purine derivative adenine poses many biological functions. Besides the fact that this molecule is one of the building blocks for RNA and DNA, there are many derivates with their specifics attributes. 2-aminopurine is well known as mutagen. 2,6-diaminopurine is able to replace purine basis in nucleic acids. Benzylaminopurine belongs to phytohormones.

Keywords: Purine, adenine, oxidation, DNA, electrochemical determination

Introduction

Biological harmfulness of reactive oxygen species is given by the subsequent oxidation of essential cellular structures. They can therefore peroxide lipids to form hydrocarbon radicals and thus alter the structure and function of biomembranes. In the case of proteins, the amino acid oxidation, cleavage peptide linkages and other changes in the structure, function and protein-protein interactions occur. Nucleic bases (purines and pyrimidines) can be also oxidized by reactive oxygen species. The oxidized bases are than removed from the DNA chain. Unfortunately, all these changes may give rise to single-strand and double-strand breaks in DNA leading to irreversible cell damage. It has been demonstrated that molecule, which shows very well on DNA oxidation, is the creation of 8-oxo-2-deoxyguanosine (8-oxo-DG). Besides oxidation of guanine the oxidation changes in the molecules adenine (8-oxo-2-deoxyadenosine) were also observed (Quinlivan and Gregory 2008; Singh et al. 2009; Zhou et al. 2008). For the detection of this marker of oxidative damage number of methods (liquid chromatography, capillary electrophoresis) was developed. Electrochemical determination of these biological markers appears to be advantageous for its sensitivity and selectivity.

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Materials and methods

In this study we aimed at study of electrochemical behaviour of derivates of adenine (adenosine-monophosphate, cyclic adenosine monophosphate, adenosine-triphosphate, 2-aminopurine, adenine, nicotinamide adenine dinucleotide, 2,6-diaminopurine, adenosine, 6-benzyl-aminopurine, S-adenosyl-L-Methionine). For this purpose the technique of flow injection analysis with electrochemical detection (FIA-ED) was employed. The FIA-ED system was consisting of one solvent-delivery pump, injection valve. Two serially connected detectors in tandem were presented. First was UV detector which was set on 260nm wavelength and second one was Analytical electrochemical cell (5040, ESA, USA) which is consisted of glassy carbon working electrode, hydrogen-palladium electrode as reference electrode and auxiliary electrode, and Coulochem III as a control module. All adenine derivates were diluted in the Mili Q water. 10 µl of sample was injected in the system. Samples of matrixes were centrifuged at 14 000 G by time of 20 minutes. Supernatant was then directly analyzed.

Results and discussion

First of all, we optimized detection method, where hydrodynamic voltammograms within the range from 100 to 1,300 mV for all above mentioned analytes were measured. Based on the obtained results, we chose 1.000 mV as suitable for sensitive detection of all derivates of nucleic acids bases. Influences of pH and flow rate on signal height were also tested. pH optimum was 5 and suitable flow rate was determined as 0.75 $ml.min^{-1}$ (Fig 2). Dose-response curves were measured within the range from 1 to 100 µM for adenine and 2-aminopurine; for other analytes, concentration range from 1 to

1,000 μM was chosen. Moreover, we investigated behaviour of adenine and its derivatives in the presence of two types of matrices.

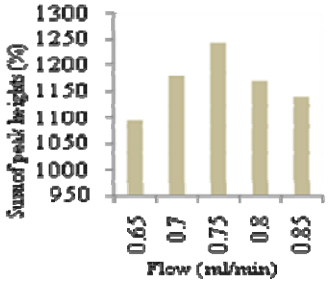

Figure 1: Dependence of peak height of adenines on flow rate.

Human urine and extract of BY-2 tobacco cells were chosen as suitable biological matrices. Obtained recoveries for each analytes demonstrate various interactions with these two matrices. Samples of matrixes were first injected to obtain one electrochemical signal under 1000mV which indirectly indicates the concentration of adenine derivates. After spiking of matrix by each adenine derivate we obtained information about approximate ability of matrix to scavenge of particular derivative. While the peak of spike+sample is higher in comparison to only sample peak the ability of matrix to scavenge particular derivate is theoretically low (Fig 2).

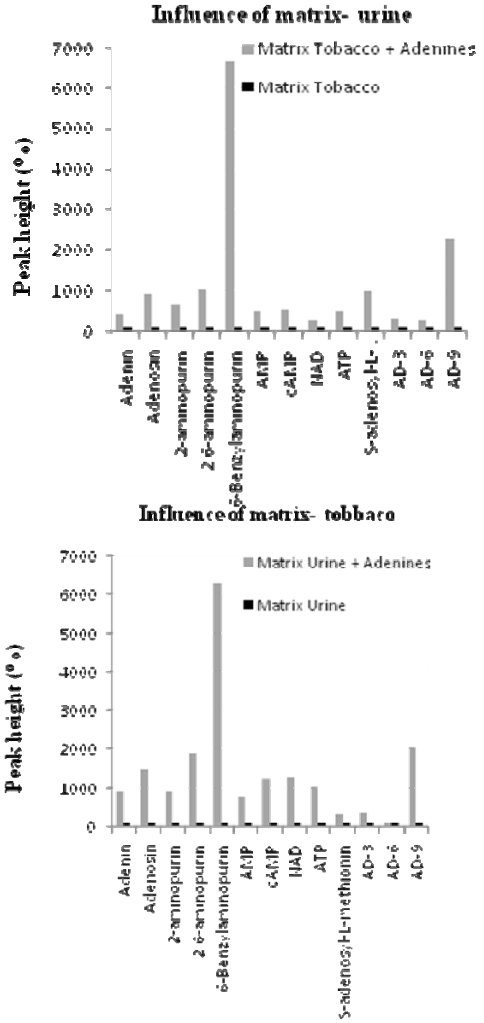

Figure 2: Influence of matrix urine & tobbaco

Conclusion

Liquid chromatography represents so called “golden standard” for analysis of complex mixtures and matrices. Its connection with electrochemical detector brings higher sensitivity and selectivity.

Acknowledgements

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