Abstract

The aim of my thesis was the synthesis of the modified 2'-deoxyribonucleoside triphosphates (dNTPs) bearing electrochemically oxidizable labels and their incorporation into DNA for the application in bioanalysis.

In the first part of my thesis, I developed the synthesis of modified dNTPs bearing 2,3-dihydrobenzofuran (DHB) or 2-methoxyphenol (MOP) labels at 5-position of 2'-deoxycytidine 5′-*O*-triphosphate and at the 7-position of 7-deaza-2'-deoxyadenosine 5′-*O*-triphosphate by Suzuki-Miyaura cross-coupling reactions. Then modified dNTPs were used as substrates for DNA polymerases in enzymatic synthesis of modified DNA by PCR and primer extension. Electrochemical properties of the DHB and MOP-labeled nucleosides, dNTPs and DNA were studied by using of a square-wave voltammetry (SWV) at the pyrolytic graphite electrode (PGE) giving signals of MOP oxidation around 0.5 V and DHB oxidation around 0.85 V. The use of DHB group in combination with other electrochemical active labels was limited by close position of its oxidation peak to the signals of oxidation of natural nucleobases, whereas MOP moiety was successfully used for redox coding of nucleobases in combination with aminophenyl or benzofurazane label giving two independently readable redox signals in each case.

In the second part of this thesis, phenothiazine (PT) was tested as a new redox label. Synthesis of PT-modified nucleosides and dNTPs were performed by Suzuki-Miyaura and Sonogashira cross-coupling reactions. Modified DNA bearing PT labels were enzymatically synthesized by primer extension, PCR and nicking enzyme amplification reactions. Modified nucleosides and dNTPs containing PT-group through the acetylene linker also displayed fluorescence properties. Electrochemical properties of the PT-modified nucleosides, nucleotides and DNA were studied by cyclic (CV) and square-wave voltammetry at the pyrolytic graphite electrode showing anodic peaks in the region 0.66 V and 0.86 V. PT moiety was also studied as a label for multipotential coding of DNA bases in combination with benzofurazane or nitrophenyl moiety.

Finally, new substituted ferrocene (Fc) derivatives with electron donating and electron withdrawing groups were studied as new redox labels with tunable redox potential. I designed and prepared 1-(N,N-dimethylaminocarbonyl)-1'-ethynylferrocene and 1-fluoro-1'-ethynylferrocene as building blocks for the synthesis of modified nucleosides and nucleoside triphosphates. These new derivatives, as well as known 1-ethynyl-1',2,2',3,3',4,4',5-octamethylferrocene and propargylamidoferrocene, were used for Sonogashira cross-coupling

reactions with halogenated dNTPs. The obtained nucleotides bearing modified Fc labels were used as substrates for enzymatic synthesis of modified DNA. So far, electrochemical properties of modified nucleosides were studied by SWV, whereas further electrochemical studies and applications of Fc-modified dNTPs and DNA will be examined in near future in collaboration with the Fojta group.