Detection of Ureasa Enzyme Analysis Using Electrode Modified with Vertically Aligned Ni Nanopillars

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Urease, enzyme catalyzing the hydrolysis of urea into carbon dioxide and ammonia, was firstly isolated from *Cannavalia enzyformis* (*Fabacae*) in 1926 [1]. Afterward it has been shown that urease (EC 3.5.1.5, amidohydrolases) is abundant enzyme in plants and, moreover, it can be found at numerous of eukaryotic microorganisms and bacteria [2-5]. The existence of this enzyme at higher organisms has not been shown yet. The highest activity of urease was determined in embryonic plant tissues, first of all, in seeds of *Fabaceae* and *Curcubitaceae* species [6-12]. In addition, a highly active isoenzym of urease was found at developing embryos. The activity of this enzyme is very dependent on nickel presence in its active centre [13]. This enzyme is substrate-specific, which means that the enzyme catalyzes the hydrolysis of urea only [14]. This feature is a basic diagnostic criterion used in the determination of many bacteria species, which produce highly active urease. *Helicobacter pylori* belong to such bacteria species. Many cases of peptic ulcers, gastritis, and duodenitis are caused by *H. pylori* infection. The presence of urease is used in the diagnosis of Helicobacter species [15-20]. The aim of this work is to use nickel nanoelectrode for selective and sensitive detection of urease

Nanoelectrodes preparation

The formation of nanostructures (nanopillars) is based on using an alumina template (Whatman Anodisc with pore diameter 100 nm) with hexagonally arrayed nanopores. One of the template sides is sputtered by metal (Au) which forms a conductive layer on the surface of the template to a cathode in an electroplating cell. During the electroplating process under galvanostatic conditions the selected metal fills the nanopores of the template. As an electrolyte, Watts Bath (250 g/l $NiSO_4$, 50 g/l $NiCl_2$, 34 g/l H_3BO_3) was used. The temperature of the solution was 55 °C, the pH was usually ranging between 3 and 3.5. The current density was 15 mA/cm^2.The circular electrode was prepared on the bottom of alumina template using fotolitography and etching of gold layer. Cu wire was fixed to the gold layer for electrode interconnection.) and the system has been sealed using epoxy resin (Cyborbond) and curred in UV light. After dissolving the template the required vertically aligned and ordered nanopillars (nanoelectrodes) on the gold layer are obtained.

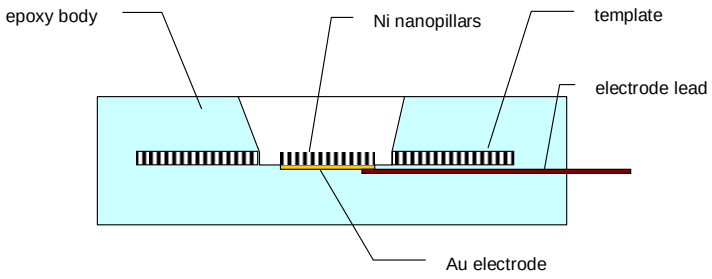

Figure 1. Coross-sectional view of the gold electrode construction with Ni nanoelectrodes for urease detection.

The Ni nanoelectrodes were utilized for a detection of urease. Measurements were carried out in acetate buffer (pH 4.6). Urease (10 µl) was accumulated at the surface of Ni nanoelectrode for various times (from 30 s to 10 min). The optimal time of accumulation was 240 s. Under these experimental conditions urease gave oxidative signal at 0.8 V. Previously we investigated the influence of various denaturing conditions (physical and chemical) on signals of various proteins (lactoferrin, protein p53) [21, 22]. However, we have not utilized the stationary electrochemical instrument to measure denaturation of protein yet. Therefore we were interested in the issue whether we were able to observe a difference between signal of native and heat denatured urease. The protein was denatured for 30 min at 99 °C. Subsequently the urease was measured at the Ni nanoelectrode. The voltammograms obtained are shown in Fig. 8 (red curve – native protein, blue curve – denatured protein). Based on the results obtained the native urease gave approximately six times lower signal compared to the denatured protein.

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