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Short communication

Gold surface patterned with cyclodextrin-based molecular nanopores for electrochemical assay of transglutaminase activity

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ABSTRACT

A novel electrochemical method for measuring transglutaminase activity was reported. This approach was based on the patterning of gold electrodes with a mixed self-assembled monolayer of perthiolated β-cyclodextrin and 1-octanethiol. The proper functionalization of β-cyclodextrin with primary amino groups allowed it to act as amino-donor substrates for transglutaminase and molecular nanopores for the enzyme-controlled diffusion of $Fe(CN)_6^{3-/4-}$ to the electrode surface. Voltamperometric measurements allowed detection of transglutaminase in the range of 1.9–37 mU/mL with a sensitivity of 1.42 nA μL U^{-1} s^{-1}.

1. Introduction

Quantification of enzyme activity is relevant for clinical analysis, microbiological characterization, food quality assessment and the control of many industrial and biotechnological processes. Enzymes have been largely used in electroanalytical chemistry as catalytic bioreceptors and labels for the construction of a great variety of biosensors [1–4]. However, few attempts have been made to develop electrochemical methods to determine enzymatic activity [5–8]. Such approaches have been mainly based on the enzyme-catalyzed transformation or production of electroactive compounds, and have been mainly focused on redox enzymes [5]. For this reason, the establishment of electroanalytical methods to estimate the activity of non-redox enzymes deserves considerable attention.

In this context, transglutaminases (TGases, protein-glutamine γ-glutamyl transferases, EC 2.3.2.13) are particularly interesting enzymes. TGases are transaminases that catalyze the formation of ε-(γ-glutamyl)-lysine isopeptide cross-links into proteins via an acyl transfer reaction, where the γ-carboxyamide group of glutamine serves as the acyl donor and the ε-amino group of lysine serves as the acyl acceptor [9]. These enzymes are biologically important for the protection and prevention of body injury, tissue assembly and repair, and have also relevant pathophysiological role in several diseases [10]. TGases are also valuable biotechnological tools for food processing, neoglycoconjugates synthesis and edible films preparation [9,11–13]. In general, TGases are assayed by optical and radioactive methods [14], but to the best of our knowledge, electrochemical approaches have not been previously employed to quantify this enzyme.

In this communication we describe for the first time a voltammetric method to determine TGase activity. This approach is based on the patterning of a gold surface with molecular nanopores by coating with a mixed monolayer of perthiolated β-cyclodextrin (CD) and 1-octanethiol. Proper functionalization of the secondary face of β-CDs with primary amino groups allows them to act as amino-donor substrates for TGase as well as molecular nanopores for the enzyme-controlled diffusion of an electroactive probe to the electrode surface in the presence of a glutamine-donor substrate (Scheme 1).

2. Materials and methods

2.1. Reagents

TGase from *Streptoverticillium* sp. was purchased from Ajinomoto. *N*-Benzyloxycarbonyl-L-glutaminylglycine (CBZ), βCD and the other reagents were from Sigma.

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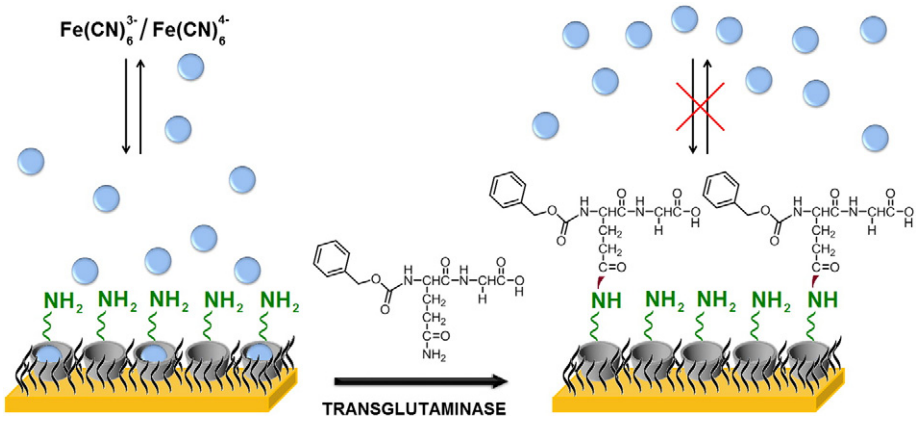

Scheme 1. Schematic display of the processes involved in the voltammetric determination of TGase activity.

2.2. Preparation of electrodes

Per-6-thio-6-deoxy-βCD was synthesized as described earlier [15]. The mixed βCD/1-octanethiol monolayers were assembled by successive immersion of the gold disk electrode (2.0 mm diameter) in 1.0 mM per-6-thio-6-deoxy-βCD in DMSO/H_2O (3:2, v/v) solution for 3 days, in an aqueous 60 μM ferrocene solution over 1 h, and in a 2.5 mM ferrocene + 0.5 mM 1-octanethiol solution in 1:1 v/v EtOH/H_2O overnight [16]. The electrode was washed with DMSO and EtOH.

To introduce a primary amino group at the secondary face of βCD, the electrode was immersed in DMF (15 ml) at room temperature and NaH (60% in mineral oil, 70 mg) was then added [17]. The mixture was stirred for 2 h at this temperature under argon atmosphere, and 15 ml 1-(*p*-tolylsulfonyl)-(1-*H*)-1,2,4-triazole (0.392 g) [18] in DMF was added dropwise. The mixture was further stirred for 12 h at room temperature, and the electrode thus modified with the 2^I-*O*-*p*-tolylsulfonyl-βCD derivative was exhaustively washed with H_2O/EtOH and then immersed in a solution of 100 mg of hexylenediamine and 500 μL Et_3N in 15 mL of DMSO. The mixture was stirred during 12 h and the modified electrode was finally washed with EtOH.

2.3. Voltamperometric TGase assay

The modified electrodes were dipped into 5 mL of 15 mM CBZ in 50 mM sodium phosphate buffer, pH 7.0 at 25 ºC. The mixture was stirred and 200 μL of TGase solution at different concentrations in the same buffer was added. The electrodes were removed at scheduled

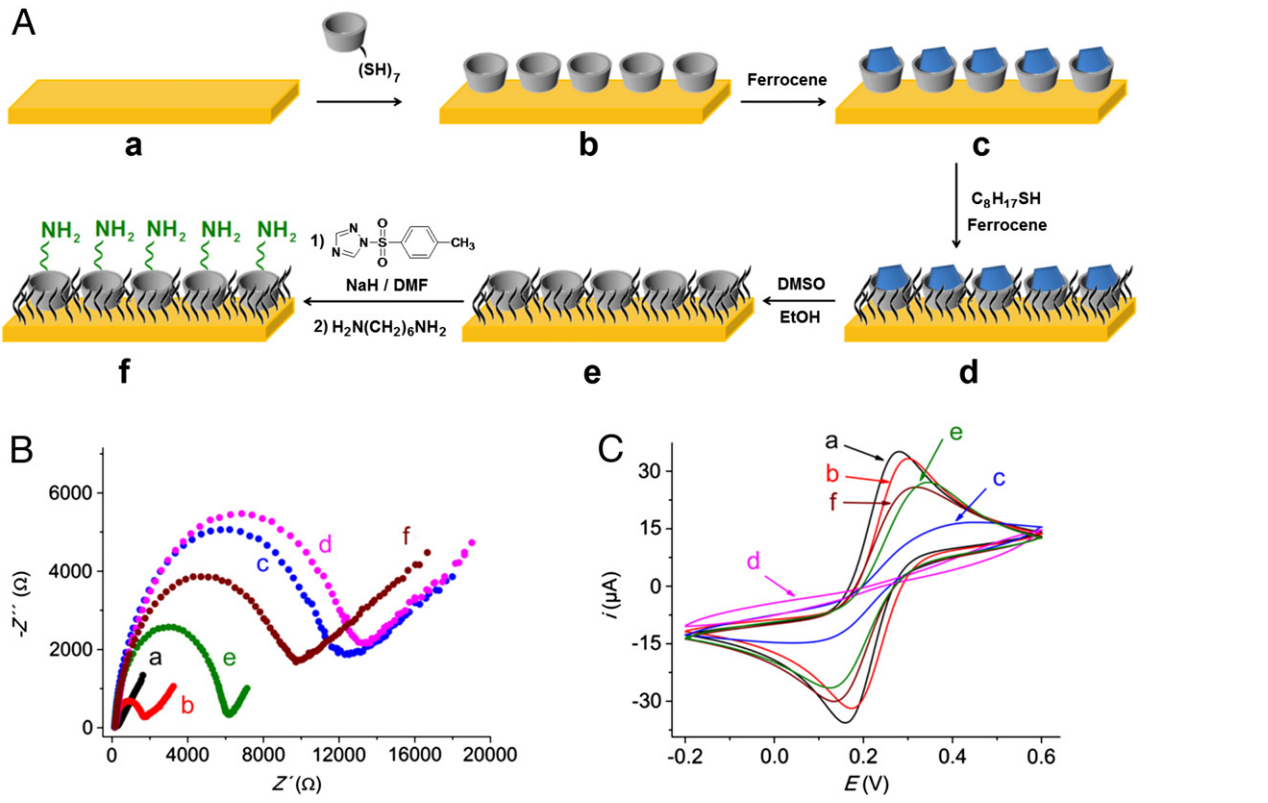

Fig. 1. A) Preparation of the Au electrode patterned with the βCD-based molecular pores. B) Nyquist plots and C) cyclic voltammograms recorded with the Au electrode in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) before (a) and after modification with perthiolated βCD (b), ferrocene (c), 1-octanethiol (d), washing with DMSO/EtOH (e) and attachment of hexylenediamine (f).