Electrochemical regeneration of oxidised nicotinamide cofactors in a scalable reactor

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ABSTRACT

An efficient and scalable NAD(P)$^+$ regeneration system to promote alcohol dehydrogenase-catalysed oxidation reactions is reported. Indirect electrochemical oxidation of NADH was established with 2,2′-azino-bis-(3 ethyl-benzo-thiazoline-6-sulfonic acid) (ABTS), being the most efficient mediator amongst the candidates screened. ABTS exhibited very high catalytic performance of 1200 catalytic turnovers per hour. In a three-dimensional electrochemical cell with a high working electrode surface area of 24 m^2 and optimised concentrations of substrate, enzyme, cofactor and mediator, TTNs of 1860 for the mediator and of 93 for the cofactor were measured. Besides, a maximum STY of 1.4 g l^{-1} h^{-1} was determined. Here we show the highest TTN ever reported for a mediated NAD$^+$ regeneration in an electro-enzymatic process. The use of the three-dimensional electrochemical reactor led to an 8-fold improvement of the STY compared to a published system, based on a two-dimensional cell.

1. Introduction

The selective oxidation of alcohols to the corresponding carbonyl group is one of the basic transformations in organic synthesis. Next to the established transition metal-catalysed methods [1,2], biocatalysis [3,4] is receiving increasing interest. Using isolated alcohol dehydrogenases bears the promise of clean and efficient reaction procedures, but is still hampered by some practical drawbacks impairing their application at preparative scale. Amongst them, efficient regeneration of the oxidised nicotinamide cofactors (NAD(P)$^+$) remains an issue of intensive research [5–11]. Most popular is the so-called substrate-coupled approach, wherein the production enzyme also serves as regeneration catalyst. The reduction equivalents liberated in the oxidation reaction are transferred to a sacrificial electron acceptor, typically a ketone. Problematic about this approach are the huge molar surpluses of the cosubstrate sometimes needed to achieve full conversion; even though Kroutil and coworkers have achieved some drastic improvements here [12,13]. From an environmental point of view, molecular oxygen represents an attractive cosubstrate for oxidations due to the high thermodynamic driving force. Furthermore, either water or hydrogen peroxide (which is easily dismutated into water and molecular oxygen) is formed as byproduct [14]. By using a laccase mediator system (LMS) or NADH oxidase (NOX), molecular oxygen acts as

terminal electron acceptor. In these approaches the use of an additional enzyme remains an important drawback. Furthermore, the limited stability of the NOX under reaction conditions is a major disadvantage [15].

Even more elegantly, electrochemical regeneration (i.e. transfer of the reduction equivalents to an anode) would result in a cosubstrate- and coproduct-free reaction setup [16–18]. As this system does not need a cosubstrate no byproducts are produced, which facilitates the recovery of the desired product. Nevertheless, some drawbacks have to be considered: specially designed bioreactors are required for the synthesis reactions and the enzymes are not optimised by nature for electrochemical conditions. Therefore, only a few examples exist where electrochemistry and enzyme catalysis are used in preparative synthesis [18]. On the one hand different results from the research on biosensor can be used as a basis for the development of electroenzymatic production processes [19–24]. On the other hand different requirements, e.g. catalytic performance and scalability, of these applications must be considered. Direct oxidation of NADH, despite its simplicity, is not feasible since with unmodified anode materials electron transfer is kinetically impeded. As a result, high overpotentials, significantly beyond the equilibrium potential of the NAD(P)H/NAD(P)$^+$-couple of −320 mV (vs. normal hydrogen electrode), are required to attain acceptable reaction rates [18]. Under these conditions undesired side reactions such as oxidation of the biocatalyst, direct anodic oxidation of the reactants and electrode fouling occur [20]. Therefore, mediated electrochemical oxidation of NAD(P)H is frequently used to circumvent the above-mentioned challenge. In such systems a redox

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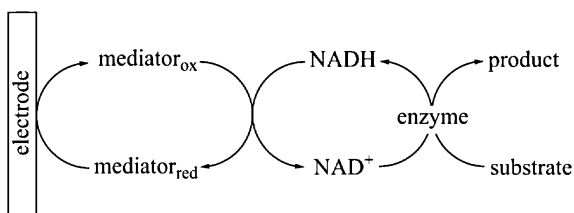
Scheme 1. Mechanism of mediated electrochemical cofactor regeneration coupled to an enzymatic oxidation reaction.

active molecule, a so-called mediator, catalyses the electron transfer between NAD(P)H and the anode (Scheme 1).

Another challenge of the electrochemical NAD(P)$^+$ regeneration en route to preparative-scale application is the heterogeneous nature of the reaction. As a result, diffusion limitations may impair the overall rate of the envisioned electro-enzymatic reaction. Efficient electrochemical processes can be achieved by the use of a three-dimensional electrode, e.g. a cell with a packed bed of graphite particles, which was already used to remove dissolved oxygen from water [25,26]. A comparable cell was applied for the electro-generation of H_2O_2 as substrate of a peroxidase [27] and the reduction of oxidised nicotinamide cofactors [28]. Here we describe the application of a packed bed electrochemical cell for the oxidation of NADH coupled to an enzymatic oxidation.

2. Experimental

If not stated otherwise, chemicals were purchased from Sigma–Aldrich in analytical grade quality. Methylene blue, tris(hydroxymethyl)-aminomethane (TRIS) and hydrochloric acid

(HCl) were purchased from Carl Roth. Meldola's Blue was purchased from Chemos GmbH (Regenstauf, Germany). GDH-03L was kindly provided by C-LEcta (Leipzig, Germany). The purity of NADH was 95%. Mediators used in this study are listed in Table 1. Reaction solutions were prepared in 50 mM TRIS/HCl (pH 8). The reaction solution was used without degassing or additional aeration.

Cyclic voltammetric experiments were performed in an electrochemical cell consisting of three electrode system and a total volume of 5 mL. The working electrode was a glassy carbon plate ($A = 2.4\,cm^2$), a platinum wire was used as counter electrode and all potentials were measured and quoted vs. Ag/AgCl (3 M KCl) functioning as the reference electrode. In between all experiments, the working electrode was polished mechanically with alumina powder, rinsed twice and sonicated for 5 min with de-ionised water. Experiments were performed in 50 mM TRIS/HCl buffer solution (pH 8) containing 0.12 mM mediator. The buffer was chosen as a compromise between the requirements for electrochemical investigations (e.g. high conductivity), ecological and economic topics and in particular the enzyme activity. For studies pointing to the NADH oxidation, NADH (1.5 mM) was added to the reaction solution containing the different mediators. In case of ABTS, different concentrations of NADH (0.115, 0.43, 1, 1.5 mM) were added to the reaction solution. Cyclic voltammetry experiments were performed with a scan rate of 25 mV s^{-1} between 200 mV and 700 mV vs. reference potential. The applied potentials were adjusted for the different mediators. Experiments for the determination of the electrochemical reversibility were done at different scan rates (10, 15, 25, 50, 100 mV s^{-1}). To examine the electrochemical stability of the mediator 500 cyclic voltammograms (CV) were recorded at a scan rate of 10 mV s^{-1}.

Chronoamperometric experiments for the determination of the turnover frequency (TF) for the mediators were performed in a spectro-electrochemical cell ($V = 1\,mL$) with a path length of 1 mm and a gold gauze as working electrode. For the characterisation

Table 1
Mediators used in this study.

No.	Mediator	Structure	No.	Mediator	Structure
1	Azure B		6	2,6-Dichloro-phenol-indophenol (DCPIP)	
2	Methylene green		7	Phenazinemetho-sulfate (PMS)	
3	Methylene blue		8	Toluidine blue	
4	Chlorogenic acid		9	Meldola's Blue	
5	Caffeic acid		10	2,2′-Azino-bis-(3 ethyl-benzo-thiazoline-6-sulfonic acid) (ABTS)	