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Short communication

Development of an enzyme-modified carbon paste electrode for determining inhibitors of lipoxygenase

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Abstract

In this study, a 15-lipoxygenase-modified carbon paste electrode (15-LOX-MCPE) was developed in connection with the help of voltammetry, which can be used as an assay system for screening drugs with inhibiting lipoxygenase (LOX) activity. The influence of different experimental conditions (LOX loading of carbon paste, pH, type of buffer system etc.) was investigated in order to optimise the biosensing device. The best composition of the biosensor is 30% paraffin oil, 68% graphite powder and 2% LOX. The optimised voltammetric measurement medium is Sörensen/NaOH (0.1 M, pH 9.0) using linoleic acid as a substrate. Under these conditions the hydroperoxy linoleic acid is formed, which can be oxidised at a potential of +0.9 V versus Ag/AgCl/3M KCl. The applicability of the LOX biosensor as assay of lipoxygenase inhibitors was successfully tested with nordihydroguaiaretic acid, zileuton and fenleuton, which are well known inhibitors of LOX.

Keywords: Modified carbon paste electrode; Lipoxygenase; 15-LOX; Voltammetry

1. Introduction

Lipoxygenases (LOX) are non-heme iron enzymes found in plants and mammals and also occur in algae and fungi (Gardner, 1991; Schewe et al., 1986; Siedow, 1991; Veldink and Vliegenthart, 1984). They selectively catalyse the introduction of molecular dioxygen in polyunsaturated fatty acids like linoleic acid and arachidonic acid. The typical products are hydroperoxy fatty acids containing a *Z,E*-conjugated diene system (De Groot et al., 1975). In mammals, the various lipoxygenases (5-, 12- and 15-LOX) convert arachidonic acid (5,8,11,14-eicosatetraenoic acid, ETE) in accordance with their positional specificity for the dioxygenation into the appropriate arachidonic acid hydroperoxides (5-, 12- and 15-HPETE). 5-HPETE is the substrate for the biosynthesis of leukotrienes, which are an important species in the genesis of inflammatory reactions and diseases, such as rheumatoid arthritis, or psoriasis (Lewis et al., 1990). Furthermore, cysteinyl-containing leukotrienes are regarded as key mediators of bronchial asthma (Drazen et al., 1999). Hence drugs that inhibit the biosynthesis of leukotrienes have been discussed as novel medications in treating asthma and allergic rhinitis. Assay systems based on spectrophotometry (Auerbach et al., 1992; Yagi et al., 1986; Thomas and Poznansky, 1990), voltammetry (Perusse and Leech, 2003) and on polarography (Pereira and Das, 1991; Daniel et al., 1994) have been applied for the screening and development of drugs with lipoxygenase inhibiting activity.

Furthermore, electrochemical biosensors have won considerable interest for monitoring analytes in pharmaceutical and environmental analysis. Various procedures are suggested for preparing reagentless biosensing devices in connection with electrochemical measurements (Thevenot et al., 2001). Carbon paste electrodes (CPE), for instance, are successfully used for immobilising biological components especially enzymes. They show low background current, give fast responses and they are inexpensive and easy to produce (Gorton, 1995).

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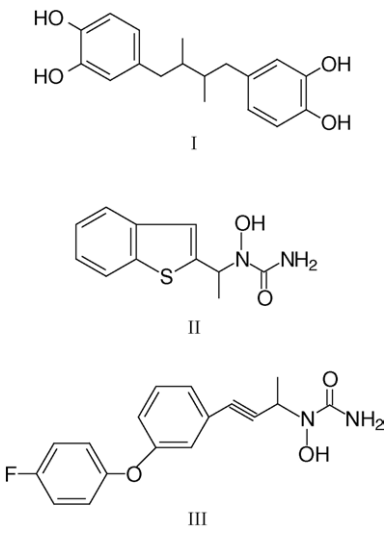

Fig. 1. Structural formulae of nordihydroguaiaretic acid I (NDGA), zileuton II and fenleuton III.

15-Lipoxygenase has already been successfully used as an enzymatic component in amperometric biosensors for the determination of polyunsaturated fatty acids (PUFA) (Schoemaker et al., 1997). This sensor is based on the Clark oxygen electrode covered – sandwich-like – with a polyethylene membrane, the enzyme being entrapped in gelatine and covered with a dialysis membrane. The LOX-based reaction was electrochemically followed by monitoring the decrease of oxygen in the enzyme layer.

The purpose of this paper is to develop a new electrochemical biosensor for screening drugs with lipoxygenase inhibition activity. The presented biosensor is made on basis of 15-LOX isolated from soybeans as a standard test enzyme. This lipoxygenase is frequently used as a prototype of its class because it is inexpensive and easily available. The 15-LOX was incorporated into a paraffin oil graphite particle matrix (carbon paste). The biosensor was characterised on the basis of the response of linoleic acid as a substrate. The influence of different experimental parameters like LOX loading of the carbon paste, type of the buffer system at various pH was investigated. Furthermore the selectivity and sensitivity of the 15-LOX-modified carbon paste electrode was investigated by using different substrates. Having established the optimised biocomposite and measurement conditions the inhibition of 15-LOX in the carbon paste electrode was tested with nordihydroguaiaretic acid I (NDGA, Fig. 1), which is well known as a strong redox inhibitor of LOX (Kemal et al., 1987). Also zileuton II and fenleuton III the iron chelating inhibitors of 5-LOX were screened concerning their effects onto the 15-LOX-MCPE. Zileuton was brought to market in 1996 and is used in the prevention and treatment of chronic asthma (Liu et al., 1996). Fenleuton, the phenol analogue, has been appreciated as a potential treatment for allergic and inflammatory disorders in horses (Annual Drug Data Report, 1995).

2. Experimental

2.1. Apparatus and voltammetric parameters

The voltammetric measurements were carried out using a 693 VA processor (Metrohm AG, Herisau, Switzerland) in combination with a VA stand 694 (Metrohm AG). This stand consists of a 15-LOX-MCPE as the working electrode, a silver/silver chloride/potassium chloride (3 M) reference electrode and a platinum wire as an auxiliary electrode. The analyser was operated under the following parameters: RDE; mode, DPP; potential ramp, +0.6 to +1.20 V; potential step, 2 mV/s; pulse amplitude, 50 mV. Evaluation was computer-assisted (Metrohm VA Database 2.0) using the tangents method.

The pH of the solutions was adjusted employing a Metrohm pH meter Model 632 and a combined glass electrode model 6.0232.100 (Metrohm AG). The measurements were carried out at various temperatures (20–40 °C).

2.2. Compounds and reagents

Soybean 15-lipoxygenase isoform 1 (EC 1.13.11.12; 11.2 U/mg), linoleic acid, linolenic acid, arachidonic acid, linoleic ethylester, arachidonic methylester and nordihydroguaiaretic acid (NDGA) were purchased from Sigma–Aldrich Handels GmbH (Vienna, Austria). Oleic acid and paraffin oil Uvasol were obtained from Merck (Darmstadt, Germany). Zileuton in the form of ZYFLO film-tablets (600 mg zileuton per tablet) were obtained by Abbott Laboratories (North Chicago, USA). Fenleuton was provided by Phenion GmbH & Co. KG (Frankfurt, Germany). Spectral carbon powder of the RW-B type was obtained from Rinsdorff-Werke (Bad Godesberg/Bonn, Germany).

All reagents were of Suprapur and/or ProAnalysis grade (Merck). Distilled water was purified with a Milli-Q Nanopure (Millipore, Bedford, MA, USA) system and stored in Nalgene containers.

Britton Robinson buffer (0.1 M), Sörensen buffer (0.1 M) and borate buffer (0.1 M) each in the pH range from 6.0 to 9.5 were used as supporting electrolytes for basic voltammetric tests.

Standard substrate solutions of linoleic acid, linolenic acid, arachidonic acid, linoleic ethylester and arachidonic methylester were prepared by transferring 10.0 mg of the appropriate compound to a 5 mL volumetric flask and dissolved in Sörensen/NaOH buffer pH 9.0 (0.1 M). All these solutions were freshly prepared. For the inhibition tests, 1×10^{-3} M stock solutions of NDGA, zileuton (the proper amount of the tablets) and fenleuton were prepared by using methanol as solvent.

2.3. Preparation of carbon paste electrode and 15-LOX-modified carbon paste electrode

A rod of synthetic material (length: 50 mm; diameter: 7 mm) with a hole at one end (diameter: 4 mm, depth: 2 mm)