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Adduct formation of electrochemically generated reactive intermediates with biomolecules

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ABSTRACT

Electrochemistry (EC) has turned out to be a valuable tool for the elucidation of reactive oxidation products of pharmaceuticals and ingredients of personal care products when investigating the affinity towards biomolecules, such as peptides or proteins containing nucleophilic groups. The combination of EC with liquid chromatography (LC) and mass spectrometry (MS) allows investigation of adduct formation, identification of reactive metabolites prior to binding, localization of active binding sites in a biomolecule and monitoring of isomer formation. The first part provides an overview on studies on formation of covalent conjugates. The second part presents several methods, which are dedicated to tagging cysteine moieties in biomolecules. The generation of electrophilic intermediates enables the selective reaction of these species with the nucleophilic thiol group in cysteine. Thus, counting of cysteines in proteins and mass fingerprinting of cysteine containing tryptic peptides is accomplished.

Contents

1. Introduction

The combination of electrochemical methods with mass spectrometry (MS) is of great interest in analytical chemistry. Electrochemistry (EC) can be used to study the redox behavior of organic compounds, metal chelates or biomolecules. MS has proved to be a very powerful detection method for this purpose, due to its broad range of applicability and its high information content. However, it is possible to obtain molecular information about compounds of interest, thus elucidating both elemental composition and molecular structure. It is therefore possible to detect exact masses and isotope patterns by means of high-resolution MS (HR–MS) and to investigate the fragmentation behavior by MS/MS experiments. Predominantly, electrospray ionization (ESI) is the ionization method of choice. Alternatively, desorption electrospray ionization (DESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) can be used. By using inductively-coupled plasma MS (ICP-MS), quantitative data for heteroatom-containing oxidation products can be obtained, since ICP-MS is an element-specific, matrix-independent method. In recent reviews, the developments and various applications in coupling EC to different ionization methods were summarized with the focus on ionization methods for molecular MS [1,2].

By applying an oxidative potential in an EC cell, the oxidation behavior of organic compounds, such as pharmaceuticals or pesticides, can be studied by means of MS detection. Jurva et al. demonstrated the potential of this technique for the *in vitro* simulation of the oxidative drug metabolism [3,4]. Several oxidation reactions, which are catalyzed by cytochrome P450 enzymes in the organism, were successfully mimicked. Some of these reactions led to the formation of reactive drug metabolites, which may contain electrophilic groups that are reactive towards specific nucleophilic groups in the human body, such as amino acid residues or DNA [3,4]. The electrochemical generation of such reactive

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metabolites in order to study the *in vivo* formation of adducts with biomolecules is of great importance, because it allows the easy, fast prediction of possible toxic side-effects of a drug substance. Within this review, we summarize the electrochemical simulation of the oxidative metabolism of xenobiotics with the focus on conjugation adducts with nucleophiles. For this purpose, we consider not only pharmaceuticals, but also pesticides, allergens (e.g., skin sensitizers) and endogenous compounds. We especially focus on the formation of protein adducts. Furthermore, we present a different aspect of adduct formation with reactive intermediates, dealing with the specific tagging of a certain amino acid, namely cysteine, in peptides and proteins. There, we demonstrate the great potential of EC-MS as a tool in proteomic research. This is the first review to focus on protein-adduct formation and tagging by electrogenerated reactive metabolites.

As initially mentioned, the purely instrumental EC-MS approach is a good alternative to common studies for the investigation of drug metabolism, as reviewed previously [5–11]. Due to the absence of complex biological matrices and the short delay time between EC oxidation and MS detection, the generation and the detection of reactive and/or short-lived intermediates is possible. If further characterization of the oxidation products is required, a liquid chromatography (LC) separation can be carried out prior to MS detection. This delivers additional information regarding the formation of possible isomers and the polarity of the generated products. The LC system can be integrated in an on-line EC-LC-MS set-up or performed off-line with a common LC-MS instrument. Baumann et al. summarized the advantages and the disadvantages of EC-LC-MS systems and also compared different EC-cell types used in drug-metabolism studies [8]. Furthermore, the miniaturization of EC cells in order to reduce the sample volume for electrochemical investigations is another aim in EC-MS-based methods. Odijk et al. developed several microfluidic EC cells for drug metabolism studies [12–14].

Also, there are numerous further applications of EC-MS for analytical purposes. Electrochemical oxidation can be used to selectively cleave peptide bonds at the C-termini of tryptophan and tyrosine, thus enabling a purely instrumental digest of proteins [15–18]. By applying negative potentials, disulfide bonds in peptides, proteins and even antibodies can be reduced electrochemically. As reduction is commonly performed in a chemical approach and as the sample has to be purified afterwards, this is a laborious and expensive step in typical proteomics experiments. Hence, EC-MS is currently of major interest in this area [19–25]. Also, there are several other applications of EC, which are summarized in other review articles in this Special Issue on the application of EC-MS in modern analytical chemistry.

2. Adduct formation between reactive intermediates and biomolecules

As shown in Fig. 1, when a xenobiotic enters the human body, it is metabolized in order to convert the mostly lipophilic compounds into hydrophilic products that can be excreted via the kidneys.

Metabolism can be divided into Phase I and Phase II metabolism. In Phase I, polar groups are introduced into the compounds and, in Phase II, some of these functionalized compounds are conjugated to endogenous nucleophiles (e.g., glutathione, glucuronic acid or sulfate via transferases, such as glutathione-S-transferase). Apart from reductions, isomerizations and hydrolysis, oxidations are the main Phase I reactions, which are typically catalyzed by enzymes of the cytochrome P450 family. Besides detoxification and excretion of xenobiotics, it is also possible that reactive intermediates are formed. They can directly bind to biomolecules (proteins, DNA) and thus cause toxic side-effects. Commonly applied metabolism

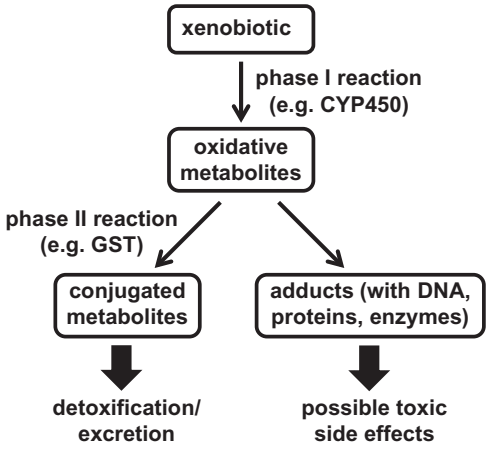

Fig. 1. The biotransformation of xenobiotics after uptake in the organism. CYP450, Cytochrome P450; GST, Glutathione-S-transferase.

studies are mainly based on the biomatrix with the disadvantage that reactive metabolites can directly bind to peptides or proteins, so these species may not be detected.

Since EC-MS is a purely instrumental approach, it can serve as a tool for generation and detection of reactive metabolites [8]. Furthermore, the reactions of the generated reactive intermediates with biomolecules, such as peptides and proteins, can be investigated under selected conditions. In this part of the review, we summarize instrumentation applied and studies aiming at the conjugation of reactive species to biomolecules. Table 1 lists all compounds investigated, their reactive metabolites and corresponding adducts.

Electrochemical cells used for the simulation of the oxidative metabolism commonly consist of a three-electrode set-up containing a working electrode (WE), an auxiliary/counter electrode (CE) and a reference electrode (RE). The cells can be distinguished according to their geometry. Fig. 2 (A, B) shows commercially available cells. Coulometric flow-through cells (A) typically contain a porous glassy carbon (GC) WE, a Pd CE and a Pd/H_2 RE. The major advantage of this configuration is the high conversion rate due to the comparatively large electrode surface. However, since the WE is porous, the application of different electrode materials is limited to porous materials. In amperometric thin-layer cells (B), the WE material can easily be exchanged due to the non-porous nature of the WE and the easy disassembly of the cell compartments. In state-of-the-art thin-layer cells, the auxiliary electrode is made of graphite-doped Teflon and Pd/H_2 is used as the RE. Beyond the exchangeable WE, another advantage of this cell type and its geometry is that adsorption of non-polar compounds is less.

In recent studies, thin-layer cells were predominantly used, since they allow the use of boron-doped diamond (BDD) as WE material. As BDD has a higher overpotential regarding the generation of oxygen in aqueous solutions, higher oxidation potentials can be applied. Thus, it is possible to obtain OH radicals, which allow a greater variety of reactions to be mimicked electrochemically.

Fig. 2(C) displays different instrumental on-line set-ups to carry out adduct-formation experiments with electrochemically generated reactive intermediates. The compound of interest is continuously pumped through the EC cell, where a potential ramp or a fixed potential is applied. To the cell effluent, nucleophiles, such as amino acids, peptides and proteins, are added. After a defined reaction time, the reaction mixture can be directly analyzed by MS in case of the addition of amino acids, small peptides or other small nucleophiles, as shown in Fig. 2.C.1. If proteins are added, an LC separation has to be integrated prior to MS detection (Fig. 2.C.2.) in order to separate salts and small molecules from the protein fraction. With