Enzyme Biosensors for Plasma Histamine Assay

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Histamine is one of the most important human mediators and is mostly found in the initial phase of an anaphylactic reaction ("immediate type" allergy). In the organism, histamine is present in nearly all tissues, and it is mainly stored in the metachromatic granula of mast cells and the basophilic leukocytes. It is present in an inactive bound form and is only released as required.

Quantification of the histamine release and its concentration in various body fluids (plasma, urine, cell culture supernatants) after allergen administration is in the centre of the clinical interest. The direct detection of mediator substances like histamine during an allergic reaction is not only of scientific interest but possibly also of practical importance in connection with a specific antagonistic therapy.

We prepared an amperometric biosensor for sensitive detection of histamine in body fluids and tested it in the flow injection analysis (FIA) system. The biosensor was based on mediated carbon paste electrode with immobilised pea seedlings amine oxidase (PSAO) on its surface. We synthesised two inorganic matrixes based on triazine (2-[4,6-bis(2-aminoethylamine)-1,3,5-triazine]-Silasorb SPH; *BAT-Silasorb* and 2,4,6-tris(2-aminoethylamine)-1,3,5-triazine; *TAT-ligand*) which very tightly bind PSAO in parallel maintenance of its catalytic activity and used them as immobilising species for entrapment of the enzyme on the electrode surface giving rise to the reaction layer of biosensor. Amperometric signal results from horseradish peroxidase catalysed turnover of H_2O_2, a secondary product of the oxidative deamination of histamine catalysed by PSAO. Electrochemical measurements were made by FIA using a three-electrode system (working electrode, saturated calomel electrode as auxiliary and platinum wire as helping electrodes). The working electrode operated at formal potential 0.0 V vs. SCE. The low working potential of the biosensor gave advantage for eliminating the influence of interfering species and amplifying the current signal without significant increasing of noise.

During our study we tested different immobilisation techniques of amine oxidase at the electrode surface, including both affinity carriers and copolymerisation with glutaraldialdehyde, and effects of various covering membranes to stability of resultant amperometric signals. Subsequently, the most optimal variant of sensor was further characterised (influence of flow rate, concentration of mediator, concentration and pH of used buffers, calibration curves).

The sensor shows good reproducibility (RSD = 2.1%, n = 15), high sensitivity to histamine (44.7 $mA.l.mol^{-1}.cm^{-2}$) with linear range of current response to 0.01–100 $\mu mol.l^{-1}$ and limit of detection 0.2 $\mu mol.l^{-1}$ at steady state arrangement or 9.55 $\mu mol.l^{-1}$ in FIA system. Interferences of electrochemically active compounds presented in materials of biologic origin are an obvious problem in amperometric analysis. Our sensor shows no interferences to usual compounds including ascorbate, urate, glutamate, creatine, creatinine, urea and paracetamole at their expected concentrations in the human plasma. These results confirm the advantage of low polarisation potential used for measurements.