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Enzyme based Amperometric Biosensors: From Direct Electron Transfer to Chimeric Enzymes

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Current research on enzyme based amperometric biosensors deals essentially with the same target analytes as was at focus in the early days of biosensor research, that are those within the clinical/medical, food/agriculture, and environmental fields. However, there has been substantial progress through the years and progress continues [1].

Communication between a redox enzyme and an electrode has been a central theme and continues along the traditional three major electron transfer (ET) routes, that are 1st, 2nd (mediated electron transfer) and 3rd generation biosensors (direct electron transfer, DET). DET consists in the direct electronic connection between the redox center of the enzyme and the electrode surface, which is working as a signal transducer. DET has been the target for many investigations both as a scientific challenge but also for practical reasons as a DET approach would simplify the construction of a biosensor and minimize the influence of other possible interfering components in the sample as well as allow mechanistic studies of the enzyme [2].

Beyond DET, another important topic within enzyme based amperometric biosensors is the possibility to target analytes that are not involved in ET chains by using chimeric/allosteric enzymes.

In the last decade, the rise of synthetic biology has driven the efforts to construct artificial allosteric protein switches to detect such target analytes. Typically, the construction of chimeric enzymes occurs via insertion of a regulatory receptor domain into the biocatalytic reporter domain. Construction of such chimeric enzymes utilizes the recombinant DNA technology that is a core technology of protein engineering [3].

In this regard, we have investigated the bioelectrocatalytic properties of pyrroloquinoline quinone-dependent glucose dehydrogenase fusion with calmodulin (PQQ-GDH-CaM). This protein is catalytically inactive in its ground form but can be activated by the addition of calmodulin binding peptide that induces its conformational transition and activation. This system was practically utilized to realize multipurpose biosensors platforms (e.g., glucose detection, peptide detection, rapamycin etc.) [4].

References

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