Surface Enhanced Spectro-Electrochemistry in Electrocatalysis

Combining Electrochemistry and Surface Enhanced Raman Spectroscopy

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The mechanism of an electrochemical sensor and a fuel cell is in principle the same but the reaction is proceeded in reverse direction. In sensors an external voltage is applied on the working electrode and the resulting current is measured. If a certain molecule ("substrate") is present it will be transformed catalytically and the current density is increased. In this respect the high substrate specificity of enzymes is of great advantage, as very small amounts of a certain target molecule can be detected. In fuel cells, molecules are transformed on the two electrodes respectively into energetically lower products. The reaction energy that is released in these exothermic reactions can directly be used to build up an electrical voltage. In electrocatalysis one distinguishes generally between heterogeneous, molecular and enzymatic catalysts (fig. 1 left). The highest intrinsic catalytic efficiency and selectivity is achieved in general by enzymes. Therefore they should be ideal candidates for sensors and fuel cells. However, enzymes tend to denature on metal surfaces and loose therefore their high catalytic activity in the immobilized state. Their applicability is further limited by eventual bad electrical communication between electrode and catalytic center. To prevent denaturation the metal electrode has to be coated with a biocompatible material. Binding of the enzyme to the surface can be tuned by the choice of the coating’s functional groups and by the composition of the surrounding solution. It is therefore necessary to find the optimum surface modification for each enzyme individually. It would be thus of great advantage to know the orientation of the enzyme on the surface and to be able to track its electron transfer pathways during catalysis. Furthermore it is of great importance to identify structural changes or partial inactivity of the enzyme after immobilization. This requires experimental methods that can give simultaneously information about structure and functionality of the enzyme on the electrode surface.

**Combination of Electrochemistry and SERS**

Electrocatalytic activity is in general determined by electrochemical measurements.
This technique yields valuable information about the average performance of the system, but only limited insight is given on the structure of the surface bound catalyst. This insight, however, is largely needed in order to find general patterns that control the efficiency of the device. Spectroscopic methods on the other hand are very suitable for structural investigations. Raman spectroscopy for example detects the inelastically scattered light by a molecule and gives therefore indirect information on the molecular vibrational modes. This method is very suitable to analyze structural changes i.e. of the oxidation state or bond grade. Unfortunately Raman spectroscopy is not sensitive enough to detect the very small amount of molecules that are attached to the surface. A local enhancement in sensitivity for surface bound molecules is exploited in surface enhanced Raman spectroscopy (SERS). Here it is made use of the fact that nanostructured noble metals like gold or silver can increase the light intensity at the metal surface if they are illuminated with laser light, which again enhances the Raman signal of adsorbed molecules. The surface morphology determines the frequency dependent intensity of the local light enhancement. Calculations show hereby that highly anisotropic nanostructures with small gaps between them create the highest light enhancement (fig. 2). For very complex molecules, such as proteins, SER spectroscopy has an additional advantage as here the Raman signal of certain parts of the molecule can be selectively enhanced by appropriate choice of the laser wavelength. This so called resonance Raman effect occurs when the frequency of the incoming light matches an electronic transition of the target molecule. Enzymes that exhibit metal centers often show absorption of the metal complex in the visible light region whereas the protein backbone exhibits electronic transitions only in the UV region. The combination of surface enhanced Raman spectroscopy with resonance Raman spectroscopy (SERRS) is thus capable to investigate selectively certain active centers of surface bound enzymes.

Example: Cellobiose Dehydrogenase
The insights gained by combined electrochemistry and SERS shall be illustrated on the example of the enzyme Cellobiose dehydrogenase (CDH). This enzyme catalyzes the oxidation of cellobiose to cellobiono-1,5-lactone. Since CDH reacts also with a variety of other carbohydrates, it is a very interesting catalyst for biomass fuel cells. During substrate conversion two electrons are created that can be used to build up an electrical voltage. A problematic key step for the functionality of such a biofuel cell is establishing an efficient electron transport from the catalytic Flavin center to the electrode. However, as this problem also exists under natural conditions in solution, CDH exhibits a flexible subunit that harbors a heme center. The heme takes up transiently one electron after the other and transfers it to an external electron acceptor molecule. Because of this flexible “shuttle-service” good
electrocatalytic activity of CDH can be achieved on electrodes. Addition of Calcium ions has shown to further increase the efficiency of this system, the reason for this, however, remain unclear.

In our setup CDH was adsorbed on a polymer coated electrode. Electrochemical measurements show a catalytic current upon addition of lactose that shifts to more negative potentials upon further addition of calcium (fig. 3 top right). As this curve corresponds to an anodic process (electrons are donated to the electrode), the observed shift corresponds to a higher efficiency of a CDH based fuel cell. SERRS measurements on the same system using violet laser light show selectively the porphyrin vibrations of the heme (fig. 3 left). Addition of lactose leads to structural changes of the heme indicating that it participates in the electron transfer pathway. Upon further addition of calcium the structure of the heme remains the same but its intensity is decreased. As the light enhancement is a strong function of the distance this intensity decrease indicates that the heme has moved farer away from the electrode. Furthermore a comparison between spectroscopy and electrochemistry shows that under certain conditions a closed catalytic cycle can be achieved that does not involve the heme as electron acceptor/donator. The combination of both methods thus allows tracking the movements of the enzyme on the surface and its consequences for the electron transfer pathway: In the case of CDH addition of calcium induces a reorientation of the heme domain farer away from the electrode but closer to the catalytic Flavin center (fig. 3 bottom left). As a consequence of this reorientation new electron transfer pathways are established that are activated at lower potentials.

**Conclusion**

The combination of surface enhanced Raman spectroscopy with electrochemistry allows correlating the efficiency of a surface bound reaction with structural changes of the molecule. We use this method to study electrocatalytic processes, especially of enzymes, on electrodes. With the insights gained from this technique it is possible to optimize electrochemical devices like sensors and fuel cells.

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References

https://dx.doi.org/10.1002/iub.1020

https://dx.doi.org/10.1002/cphc.201500112

More information on spectroscopy:
http://www.laboratory-journal.com/search/gitsearch/spectroscopy%20type:t...

More information on electrochemistry:
http://www.chem1.com/acad/webtext/elchem/