Effect of architecture on the activity of glucose oxidase/horseradish peroxidase on carbon nanoparticle conjugates for sensing applications.

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The enzyme glucose oxidase (GOx) has been exploited for a wide range of industrial and medical applications, including electrochemical blood glucose sensors, food preservation, and biofuel cells. The majority of these applications require immobilization of GOx onto some sort of planar or nanostructured surface, where a suitable interface enzyme-surface is critical. Immobilization must be done in a way that maintains not only the protein structure, but also the protein microenvironment, orientation, and the ability of substrate and product to diffuse to/from the binding pocket and active site[1]. Nanoparticle (NP) or nanostructured surfaces are desirable for enzyme supports due to their high surface area and consequently high capacity for enzyme coupling, as well as their ability to be solubilized in the case of NPs.

Furthermore, most of the GOx activity measurements rely on the presence of a second enzyme, horseradish peroxidase (HRP) which utilizes the enzymatic product of GOx to induce a colorimetric change or a current generation in an electrode, thus amplifying the GOx activity into a measurable signal. Therefore, to construct a functional GOx-HRP complex, not only the interface of the GOx to the NP must be considered, but also to HRP and its role in the chain of activity[2]. It is expected that enzyme arrangement on the NP will influence overall activity[3]. However, how to optimize the system is not immediately clear, especially since they work in concert. Additionally, the enzymes differ in their surface charges and sizes, and may have different affinities for the nanoparticle surface or propensities towards denaturation. Because product of GOx is the substrate of HRP, diffusion of both substrate/product within the complex needs to be taken into account. Thus, constructing an optimal multi-enzyme-NP conjugate involves a broad range of issues that are made more complicated due to the intricacies of the nano-bio interface.

Even though the NP surface is often problematic for immobilized enzymes, its properties can be advantageous[4]. Carbon NPs (CNPs) are particularly attractive as solid supports for enzymes because they promote electron transfer due to their electrical conductivity, enabling applications in which the glucose is detected electrochemically. Also, production of CNPs is more affordable than production of NPs made of semiconductors, noble metals, metal oxide, and other crystalline or specialty materials. Furthermore, the carbon substrate is not inert and, depending on its form, can take an active role in redox activity.

Present work investigates how conjugate architecture affects enzymatic activity for GOx and HRP on CNPs in order to understand how best to co-immobilize both enzymes on NPs surfaces. GOx and HRP activity was measured via coupled colorimetric assays as a function of architecture of the NP-bienzyme complex. For this purpose, different functionalization approaches were performed (Scheme 1) and showed that changing how the complex is assembled affects overall activity. The results exhibit that GOx is more negatively impacted by the CNP surface than HRP. Thus, those arrangements where GOx is not directly on the surface of the CNP (simultaneous and sequential H) are more favorable for overall activity. Also, HRP activity is actually enhanced by the CNP, pointing that interfaces do not always have detrimental effects on enzymes. Other factors as coverage or the presence of glucose performing the conjugation were also studied.

Our results highlight issues that can critically affect glucose sensor, biofuel cells or any complex which involves multiple immobilized enzymes that work in concert.

References

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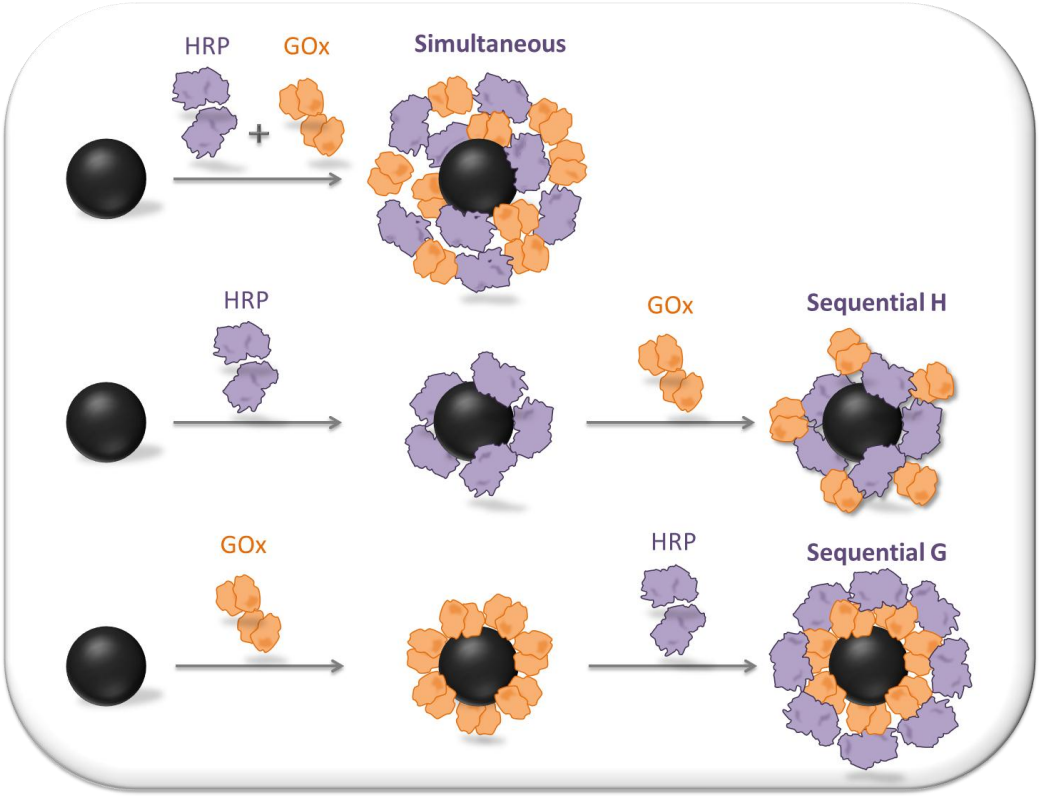
Figures



Scheme 1: Different coupling strategies for glucose oxidase (GOx, orange) with horseradish peroxidase (HRP, purple) to carbon nanoparticles (black).