PROBING THE SURFACE PROPERTIES OF CYTOCHROMES *C*/GOLD NANOPARTICLES COMPLEXES

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Gold nanoparticles (AuNPs) present unique optical, electronic and chemical properties and can be stabilized by proteins, generating bio-nanoprobes with wide applications in bioassays and cell targeting[1]. Spherical gold nanoparticles were prepared and coated with two natural cytochromes of the *c* type, namely, horse heart cytochrome *c* (HHCC) and yeast iso-1-cytochrome *c* (YCC), each at a ratio of 250 proteins per AuNP, assuring complete AuNP surface coverage. According to previously published models, HHCC is bound to the gold surface through electrostatic interactions while YCC is covalently bound to the gold surface *via* the thiol group of its single cystein-102 residue[2]. Upon pH variation of solutions containing the AuNP-protein complexes, a UV/vis-detectable change occurred as the solution shifted from the original color (red) to blue. This shift was sharper for the HHCC-AuNP complex and occurred around pH 6.2, whereas for the YCC-AuNP complex was more gradual and the color change occurred around pH 9.2. The bare gold nanoparticles and the cytochrome *c* solutions alone showed no change from the original color upon pH variation. The conformational changes occurring on the protein secondary structure upon interaction with the gold nanoparticles were investigated by Circular Dichroism (CD) spectroscopy, by monitoring the "far-UV" spectral region (190-250 nm). Preliminary experimental results at several pH values showed conformational changes of little significance in HHCC alone and a gradual increase of β-sheet from pH 6 for the HHCC-AuNP complex. In YCC alone, CD spectrum at pH 11 showed predominance of random coil. Comparing these results with the CD spectrum of the YCC-AuNP complex, an increase of random coil around 10% is observed from pH 10 and a gradual increase of β-sheet is observed from pH 9. ζ-Potential measurements obtained for AuNP-complexes with different protein:nanoparticles ratio, showed that the ζ-potential of the complexes increases as more protein binds to the AuNPs, until a protein:nanoparticles ratio of 250, stabilizing for larger ratios. For YCC-AuNP complexes, the ζ-potential values were more negative than for the HHCC-AuNP complexes. Taken together, the experimental data seem to indicate that the surface charge distribution is very different for both complexes, indicating that natural derivatives of cytochromes can be used to control properties at the nanoscale for these bio-nanoprobes.

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