Specific Ion effects on the Electrochemical Properties of Cytochrome c

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Heme-containing proteins such as Cytochrome c have a range of uses and applications in biocatalysis and biosensors. The factors that can influence the electrochemical potential (E°) of heme proteins include the nature of the axial ligands, heme-protein interactions and the dielectric properties of the solvent [1]. The E° of the heme proteins can be estimated using voltammetric techniques as cyclic voltammetry (CV) and differential pulse voltammetry (DPV). In these techniques an electrolyte and an opportune electron mediator, such as 4,4′-bypiridyl [2], are used to provide an adequate electric conductivity and to ensure the electronic transfer between the protein and the electrode surface, respectively. In this condition the process is diffusion controlled and the peak current is proportional to the amount of the adsorbed protein on the electrode surface.

DPV’s of cyt c in a solution of potassium phosphate buffer (4.4 mM, pH 7.15) for NaCl (200 mM) as a function the electrolyte salt (A); Plot of E° (B) and peak current (C) of cyt c at 298K for a range of anions.

Here the effect of the salts of the Hofmeister series on E° and the peak current of the cyt c was investigated. The experiments showed that anions have a larger effect than cations. E° decreased in the order F- > Cl- > Br- > ClO₄⁻ > SCN-. A possible explanation is that kosmotropic anions tend to stabilize the reduced state of the iron in the heme, facilitating the redox reaction, so raising E°. The peak current showed a bell-shaped trend for the anions, with a maximum in correspondence of the sodium chloride. Anions-protein interactions affect the current intensity, since this last is proportional to the amount of protein interacting with 4,4′-bypiridyl mediator adsorbed on the electrode surface. A part the unexpected behaviour of F-, chaotropic anions can bind to chaotropic charged lysine residues on cyt c surface, according to Collins law of ‘Matching Water Affinities’ [3]. This may negatively affect the formation of hydrogen bonds between charged lysine residues and nitrogen atoms of the mediator, that are responsible of the current flow between cyt c and the electrode.

In conclusion, these results demonstrate the importance of the choice of the salt electrolyte in the set up of a bioelectrochemical experiment since it can strongly affect the redox behaviour of the investigated enzyme.