Electrochemical monitoring of biotin binding to avidin. Electroactivity of avidin and streptavidin.

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Avidin is a basic glycoprotein of molecular weight approximately 66 000, with an isoelectric point of 9.5 [1] and exceptionally high affinity for the low-molecular weight vitamin biotin[2, 3]. Similarly high affinity for biotin is exhibited by a bacterial protein streptavidin. In difference to avidin, streptavidin does not contain any sulfur-containing amino acid residues [4]. The (strept)avidin-biotin system has been extensively used in biotechnologies. The general idea of the system use is that biotin, coupled to target molecule, is linked to (strept)avidin labelled with some reporter groups.

Voltammetric methods have been used to study avidin and its biotin binding by several authors using solid, mainly carbon electrodes [5-7]. In these papers the avidin – biotin interaction was mainly monitored using a derivatized biotin. Buckley et al. [4] investigated adsorption behavior of both avidin and streptavidin by means of voltammetry at mercury electrodes. They observed reduction signal of avidin close to -0.5 V at neutral pH, reportedly due to the reduction of the -S-S- group and characteristic for cystine-containing proteins. Streptavidin (which does not contain cystine/cysteine residues) produced the same signal. In spite of the Buckley et al. results, up to now avidin and streptavidin have been considered to be electroinactive [6-8].

We studied avidin and streptavidin by phase-sensitive a.c. and cyclic voltammetry as well as by constant current chronopotentiometry at mercury (in alkaline media) and square wave voltammetry at carbon electrodes (in acid medium). Contrary to widely held understanding about electroinactivity [6-8] of avidin and streptavidin, both proteins produced peaks due to oxidation of tyrosine and tryptophan residues at carbon electrodes and a catalytic peak H at a hanging mercury drop electrode (HMDE). At the HMDE avidin produced in an alkaline medium phase-in a.c. voltammetric and cyclic voltammetric peaks close to -0.6 V (peak S) which were assigned to Hg-S interactions, involving cystine/cysteine residues. In cobalt containing solution avidin produced a characteristic catalytic double wave requiring presence of cystine/cysteine residues in the protein molecule. Streptavidin, which does not contain these residues, yielded neither the catalytic double wave nor peak S. All the above avidin signals responded to biotin binding; peak S increased (up to 4 biotin molecules bound) while other avidin signals decreased as a result of biotin binding.

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