

## Supplementary Data

# **Ionic strength-dependent structural transition of proteins at electrode surfaces**

Emil Paleček\* and Veronika Ostatná

Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i.,  
Královopolská 135, 612 65 Brno (Czech Republic)

### **Hydrogen evolution catalyzed by proteins at mercury electrodes**

In the catalytic process responsible for peak H labile protons are split off from some amino acid residues and reduced at the electrode. This process is followed by fast recombination of protons from the background electrolyte<sup>1,2</sup>. Recent data suggest that the ability of the protein to catalyze hydrogen evolution is closely related to its conformation<sup>3-8</sup>. The participation of amino acid residues in the electrocatalysis depends on their accessibility and ionic conditions of the experiment. Under the given conditions (Fig. 1A) lysine and cysteine residues were probably the most important proton donors in the BSA electrocatalysis<sup>2,4</sup>. In difference to the intensively studied redox processes in some conjugated proteins<sup>9,10</sup>, no typical metal or organic (e.g. flavin) redox centers are necessary for the protein catalyzed hydrogen evolution.

### **Peak H of native and denatured BSA**

In BSA many amino acid residues are buried in the protein structure while in denatured (fully or partially unfolded) BSA a lot of these groups are solvent accessible. Generally, in native proteins hydrophobic amino acid side chains (which are very important for the protein adsorption at the hydrophobic mercury surface) are buried in the interior of the protein molecule<sup>11</sup>. As a result of the protein denaturation these groups become accessible. Similar situation can be expected in the case of cysteine residues, due to their high affinity of sulfur to mercury<sup>12,13</sup>. Thus, at least in the initial stage of the adsorption, denatured BSA offers a larger number of groups with high affinity for the mercury surface than native BSA. Therefore, if no significant unfolding of native BSA takes place at the surface, this BSA should produce differently structured adsorbed layer, with different numbers of accessible catalytically active amino acid residues than denatured BSA.

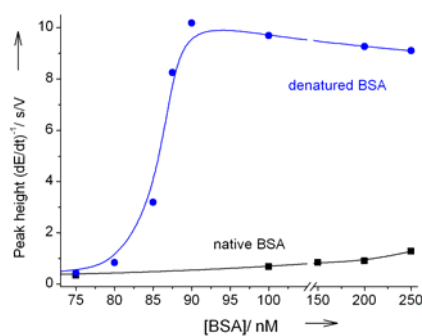
### **Effect of pH**

Large differences between the heights of peak H of native and denatured BSA and of other proteins were observed at alkaline<sup>3,5-8,14-19</sup> and neutral pH's (Fig. 1A), but as a result of changing pH in acid direction these differences became smaller. It was not possible to detect any transition at pH 4.5, where native and denatured BSA produced roughly the same peaks. More details will be published elsewhere. Here we prepared urea-denatured BSA and measured peaks of native and denatured BSA in 50 mM sodium phosphate with 56 mM urea, pH 7.0. 100 nM urea-denatured BSA yielded a

symmetric peak H at -1.81 V, which was almost 10-fold higher than that of native BSA under the same conditions (Fig. 1A). Peak potential,  $E_p$  of the latter peak was by about 10 mV more negative than  $E_p$  of denatured BSA and about by 30 mV less negative than  $E_p$  of native BSA in absence of urea. Peak H of 100 nM urea-denatured BSA obtained in neutral sodium phosphate was substantially larger (~ten-fold) than peak of the same BSA in 50 mM sodium borate, pH 9.5 (not shown) in agreement with the electrocatalytic nature of these peaks.

### Dependence on BSA concentration

We run the dependence of peak H on concentration of urea-denatured and native BSA in 50 mM phosphate with 111 mM urea, pH 7.0. Peak H grew gradually with concentration of native BSA between 50 nM and 500 nM (Fig. ESI-1). On the other hand peak H of urea-denatured BSA after some lag at low concentrations grew with BSA concentration and then it leveled off. Even at 500 nM BSA the ratio of peak heights of denatured to native BSA,  $h_{\text{BSA}_{\text{den}}}/h_{\text{BSA}_{\text{nat}}}$  was about 6 (not shown). The results suggest that full electrode coverage is obtained at much lower solution concentration of denatured BSA than with native BSA probably due to different adsorption of these two BSA forms.

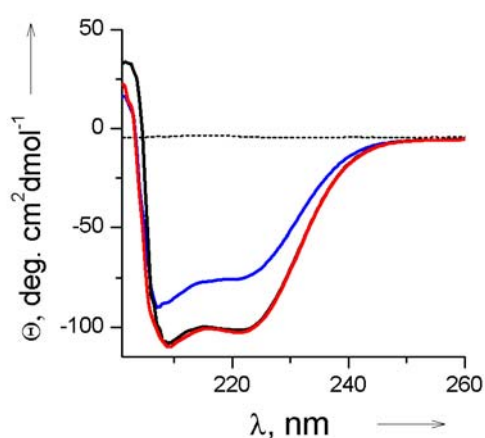


**Figure ESI-1**

Dependence of peak H height on concentration of native and denatured BSA in 50 mM sodium phosphate, pH 7 in presence 111 mM urea. 100 nM BSA was adsorbed on hanging mercury drop electrode (HMDE) for accumulation time  $t_A$ , 60 s, at accumulation potential  $E_A$  -0.1 V with stirring at 1500 rpm.  $I_{\text{str}}$  -30  $\mu\text{A}$ . Denaturation of 14.4  $\mu\text{M}$  BSA in 8 M urea was performed overnight at 4 °C. AUTOLAB Analyzer (EcoChemie, Utrecht, The Netherlands) and VA-Stand 663 (Metrohm, Herisau, Switzerland); HMDE was used as working electrodes in a three-electrode system. Reference electrode: Ag/AgCl/3M KCl; auxiliary electrode: platinum wire. After accumulation, stripping current ( $I_{\text{str}}$ ) was applied to measure the E-t curve (which was automatically converted to  $(dE/dt)^{-1}$ -E curve). Experiments were carried out at room temperature open to air. Other details as in Fig. 1A.

### Solution circular dichroism spectra of BSA

CD spectra of native BSA in 50 and 200 mM sodium phosphate, pH 7 did not show any difference (Fig. ESI-2). These spectra significantly differed from that of denatured BSA. Similarly, no difference was observed in (a) fluorescence spectra and (b) gel mobilities (in native polyacrylamide gel) of native BSA in 50 and 200 mM sodium phosphate, pH 7 (not shown). These results are in agreement with our suggestion that the changes in the protein structure signaled by peak H (Fig. 2) took place at the electrode surface and not in solution.



**Figure ESI-2**

Circular dichroism (CD) spectra of 1  $\mu\text{M}$  native BSA in 50 (red) and 200 mM (black) sodium phosphate, pH 7 and of 1  $\mu\text{M}$  denatured BSA (blue) in 200 mM sodium phosphate, pH 7. CD spectra were recorded using Jasco J-720 spectropolarimeter (Easton, MD) equipped with a thermoelectrically controlled cell holder. The cell pathlength was 1cm. Isothermal CD spectra were recorded from 200 to 320nm in 1-nm increments with an averaging time of 5 s. See Fig. 1 for more details.

### Materials

Bovine Serum Albumin (BSA), Human Serum Albumin (HSA), Albumin from chicken egg white, Grade V,  $\gamma$ -Globulin, cytochrome c from bovine heart and urea were purchased from Sigma-Aldrich. All other chemicals were of analytical grade; solutions were prepared from triply distilled water.

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