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# Electric Field-induced Conformational Transition of Bovine Serum Albumin from α-helix to β-sheet

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**Abstract:** The irreversible conformational transition of bovine serum albumin (BSA) from α-helix to β-sheet, induced by electric field near the electrode surface, was monitored by circular dichroism (CD) with a long optical path thin layer cell (LOPTLC).

**Keywords:** Bovine serum albumin, conformational transition, circular dichroism.

The conformational change of a biomolecule plays an important role in biological activity. Electric field induced conformational transitions of helix-coil have been reported for the proteins directly exposed to a high electric field, which is the results of electric birefringence and the electrooptical Kerr effects[1,2]. The adsorbed proteins at electrode interfaces[3,4] also show conformational changes. One of the reasons for this is that electric double layer near electrode surface offers a very strong non-uniform electric field. For instance, in 10 mM electrolyte solution a $3\times10^{-6}$ cm of an electric double layer[5] will form and an average 33 kV $cm^{-1}$ of electric field across electric double layer will exist if 0.10 V of potential is applied. If a parallel incident spectro-electrochemical method, such as a long optical path thin layer cell[6] (LOPTLC), the conformational changes can be monitored in the thin layer solution without influence of states of electrode and adsorption proteins. The time interval can be estimated from the thickness of solution and diffusion coeficient of protein[5]. If the diffusion coefficient is about $8\times10^{-8}$ cm $s^{-1}$ and thickness of solution is 0.02 cm then the time interval is about 140 s. Among several methods of conformational measurement, circular dichroism (CD)[7] is a very useful one, especially for determining the fraction of each conformation of a biomolecule in solution. The combination of thin-layer electro-chemistry with CD spectrometry, called thin layer CD spectroelectrochemistry, is a suitable method to study the conformational change of proteins induced by electric field in an electrochemical process. Bovine serum albumin[8] (BSA) is a small protein with a single polypeptide chain of molecular weight 69000 and diffusion coefficient of $8.3\times10^{-8}$ cm $s^{-1}$. BSA can be reduced in negative potential range, but in the positive potential range from 0.0 to 0.60 V (*vs.*Ag/AgCl), BSA is an electroinactive protein. Its conformation and conformational

change or denaturation in solutions of different pH have been well studied by optical rotation[9], intrinsic viscosity[10], fluorescence depolarization[11] and acid-base titration method[12] in early days.

This letter reports, for the first time, an electric field-induced conformational transition from α-helix to β-sheet of BSA in aqueous solution by CD spectroelectro-chemistry with a LOPTLC.

The experiment was carried out in a LOPTLC made in our laboratory[5]. The working electrode was a piece of glassy carbon, which was inserted into one of the thin layer walls opposite to a platinum auxiliary electrode and Ag/AgCl (KCl saturated) reference electrode connected to a solution with a 0.1 cm hole at the middle of another thin-layer wall. A common quartz cuvette served as an optical window. The polarized light passed through the thin layer being parallel to the working electrode, so that only the biomolecule in the solution layer could be monitored during the experiment. 0.125 mg/ml BSA in 10 mM phosphate buffer solution (pH 7.12) was put into a cuvette and deaerated with high purity nitrogen for 1 minute prior to use. The CD spectra with different applied potentials (shown in **Figure 1**) were recorded by using an AVIV 62A DS circular dichroism spectrometer (made in USA) and a PAR-370 electrochemical instrument (made in USA). The temperature in the sample compartment was kept at 25.0±0.1 C° during the experiment. From the CD spectrum at open circuit (**Figure 1**, a), it can be seen that the negative Cotton peaks at 212 and 222 nm are the symbol of α-helix [13] for the secondary structure of BSA under the experimental condition. It contains about 52% of α-helix (calculated by SELCON program[14]). As the applied potential shifting to positive, the peak at 212 nm is decreased and the peak at 222 nm is also decreased with a blue shift. This CD spectrum change implies that there exists a conformational transition from α-helix to β-sheet[7]. When the applied potential returning to 0.0V even to open circuit, the CD spectrum keeps no change. So the conformational transition induced by electric field is an irreversible process.

The CD spectra in **Figure 1** were analyzed by using self-consistent program, SELCON14. The fraction of each component (correlation coefficient is better than R=0.97 and standard deviation is less than 0.05) was plotted against applied potentials, as shown in **Figure 2**. When applied potential shifts to positive direction, the fraction of α-helix decreases while the fraction of β-sheet increases accompanied by slightly increasing the fractions of β-turn and random coil. In this process, the fraction of α-helix decreases by about 0.167, indicating about 70% of which changes into β-sheet. This result confirms that the conformational transition is mainly from α-helix to β-sheet.

The peak area of CD spectrum is commonly proportional to the intensity of all components in the system[15]. During the conformational transition, the peak area of the CD spectrum reduces with applied potential shifting to positive direction. So the plot of peak area against applied potential can serve as the total conformational transition curve, which is an S-shaped curve with a midpoint at 0.30 V, as shown in **Figure 3.**

**Figure 1.** CD spectra of BSA with different applied potentials. 0.125 mg/ml BSA in 10 mmol.$L^{-1}$ phosphate buffer solution(pH 7.12); Time interval:240s; temperature:25$^0$C; Applied potential: 1,open circuit; 2, 0.00; 3, 0.20; 4, 0.30; 5, 0.40; 6, 0.50; and 6, 0.60 V.

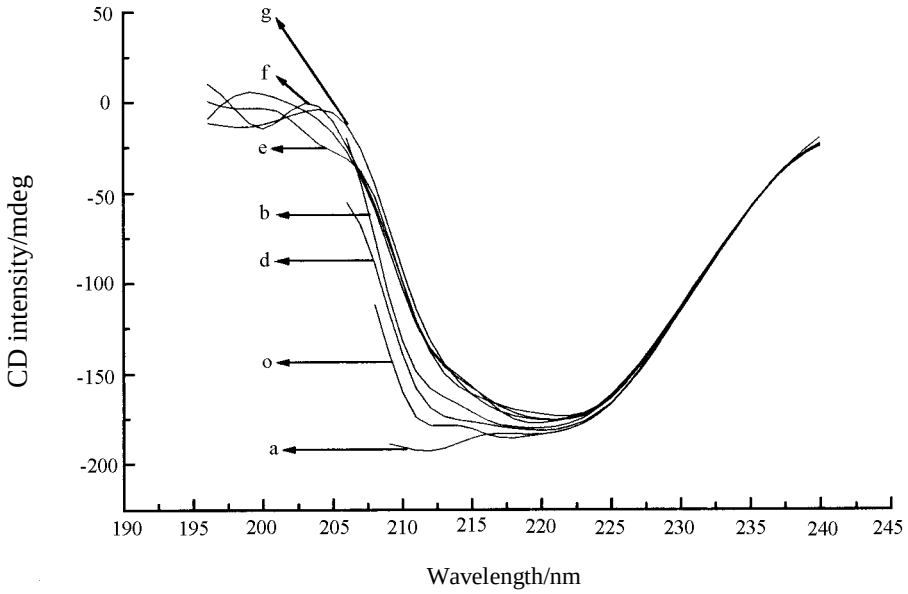



**Figure 2.** Fraction change of each formation of BSA with applied potentials. a, α helix; b, β sheet; c; β turn; d, remainder. (calculated by SELCON program, the correlation coefficient is better than 0.97; deviation is less than 0.05; ↑ indicates the open circuit case).

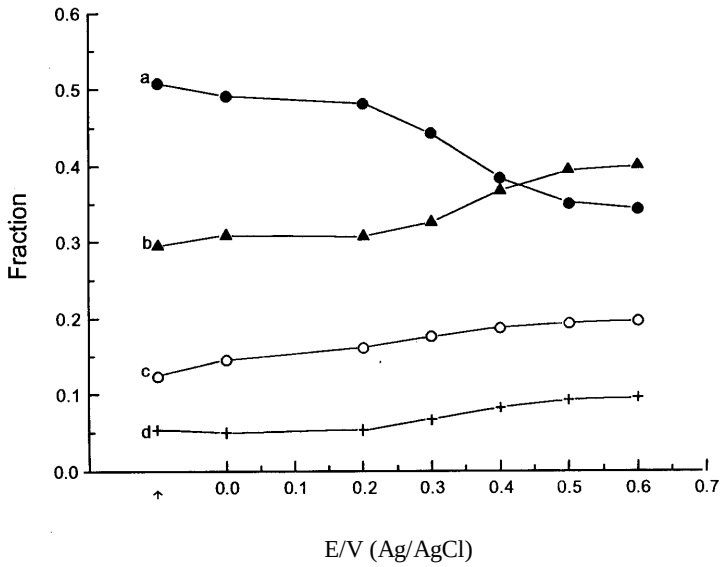
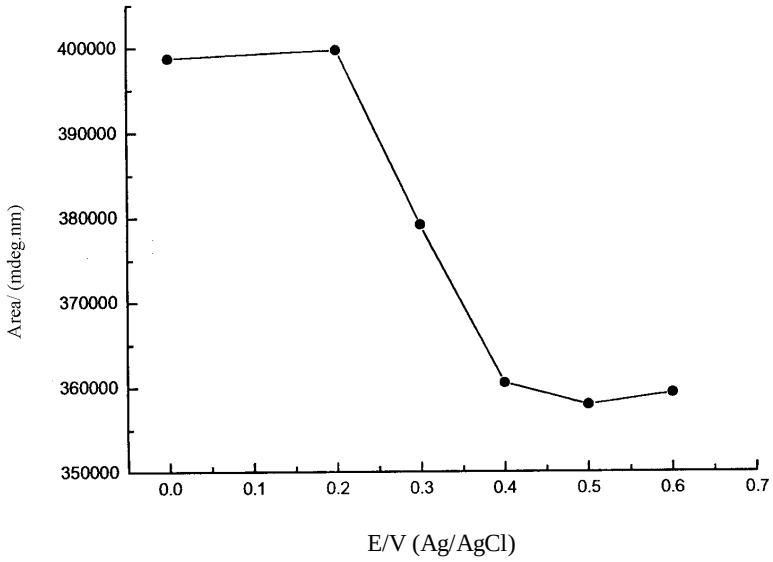


**Figure 3.** Plot of area of CD spectra against applied potentials

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