VOLTMETRIC STUDY OF CONFORMATIONAL
CHANGES AND PROPERTIES OF A PROTEIN MONOLAYER
ADSORBED ON A MERCURY ELECTRODE

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New directions are developing at the present time in bioelectrochemistry: bioelectrocatalysis [1] and bioelectrosynthesis [2], the primary problem of which is determination of the feasibility of direct electron transfer from electrodes to the enzyme. These processes are accomplished most efficiently only for direct transfer [3]. Determination of these possibilities and the study of the electrochemical characteristics of proteins, the carriers of the electrons and the enzymes taking part in the oxidation-reduction reactions, could be easily accomplished by the methods of polarography, potentiometry, and electrolysis with spectrophotometric monitoring. However, until the present time the state of the protein at the phase interface and the mechanisms of electron exchange at the interface have not been studied in detail, which makes the results of work on direct electrochemical investigation of proteins insufficiently conclusive. It has been shown that there are many complicating factors. In [4-7] it was established that at the water-mercury interface, fast surface denaturation and flattening of the protein occurs down to a thickness comparable with the thickness of the polypeptide chain. Under these conditions, certain electron-active groups, located within the globule, emerge onto the electrode and enter into the electrochemical reaction [5]. It has been proven that in the native state the proteins are also capable of exchanging electrons on different metallic electrodes [1, 5, 7-9]. In this case, a monolayer of the flattened protein on the one hand prevents denaturation of subsequent portions of protein diffusing toward the electrode, and on the other hand facilitates electron transfer. It has been proposed that the discharge of the native molecules occurs within pores of the monolayer of flattened protein [9]. By independent methods it has been demonstrated that proteins of various origins and natures form porous monolayers [6]. Thus, a monolayer of adsorbed protein plays a substantial role in the discharge mechanism for native molecules, and more detailed knowledge of its structure may explain processes occurring at the electrode.

In this paper we determine by the electrochemical method the surface concentrations of proteins in a monolayer on a Hg electrode (lysozyme, beef serum albumin, ribonuclease, xanthine oxidase, phosphorylase, and alcohol dehydrogenase), and we determine some general regularities in the surface denaturation of globular proteins and the formation of films of flattened protein.

EXPERIMENTAL

The surface concentration of the protein was determined from the catalytic current of the protein which is obtained in the presence of Co(iII) salt in ammonia buffer, pH 9.3, the so-called Brdicka waves [10]. It is known [4, 10] and later will be shown that only the irreversibly adsorbed protein is polarographically active, and that for low degrees of coverage there is a linear relationship between the height of the catalytic wave (I) and the amount of adsorbed protein (Γ):

\[ I = jΓ \]  

The surface concentration Γ at the beginning of the accumulation may be calculated independently from the diffusion equation, since the adsorption is irreversible and its rate is limited only by diffusion. From this we may determine the value of j. Its physical meaning is the catalytic current which provides one molecule or one mole of protein adsorbed on the electrode. Then the concentration of protein in the monolayer Γ∞ would be easily determined by dividing the limiting current I∞, obtained upon coverage of the monolayer, by the value of j. However, it was determined that the coefficient j appreciably decreases for high degrees of coverage, when the high surface pressure promotes displacement of some segments of the polypeptide chain of the protein from the active electrochemical zone. So for a correct determination of the amount of adsorbed protein, we need a method which would allow us to transfer the adsorbed protein monolayer to conditions of low pressures.
Fig. 1. Method for calculating the catalytic wave (1) of the protein. The current-voltage curve for cathodic (1) and cathodic-anodic polarization (2) with cusp A, at which $\tau_1 = \tau_2$. The explanation is given in the text. The height of the wave is $I = H - I_{Co}$.

Fig. 2. Dependence of the current of the catalytic wave $I$ on the adsorption time (on a static Hg electrode, $S_0 = 2.0 \times 10^{-2}$ cm$^2$): 1) lysozyme; 2) RNase; 3) BSA; 4) ADH; 5) xanthine oxidase; 6) phosphorylase. Protein concentration 10 $\mu$g/ml, hexaminecobaltichloride $4 \times 10^{-4}$ M, 0.1 M ammonia buffer, pH 9.3.

and low degree of coverage, when there is a linear relationship between $I$ and $\Gamma$. This is achieved by an increase in the surface of the Hg electrode with a previously adsorbed monolayer, after transfer to a buffered Co$^{3+}$ solution which does not contain protein.

The investigation was carried out on a static Hg electrode of the Kemula type with stationary drops delivered by a micrometer device for dispensing the Hg. At first, the electrode was placed in a cell containing a solution of protein (20 $\mu$g/ml in 0.1 M ammonia buffer (pH 9.3) or phosphate buffer (pH 7.0). After formation of the mercury drop, adsorption and accumulation of protein on the surface of the electrode occurred; after 2 min, in practice a monolayer was formed [4-6]. Then, after washing in a second cell in 0.1 M ammonia buffer (pH 9.3) for 1 min, the electrode was transferred to a third polarographic cell containing a solution of hexaminecobaltichloride ($4 \times 10^{-4}$ M) in the same buffer. In this cell, the mercury drop was increased, and after holding for 30 sec the normal current-voltage curves were recorded on a TsLA-03 oscillographic polarograph. The sweep rate was 1 V/sec; the potential at which we carried out adsorption in the first cell and washing in the second, and the initial potential in the third measurement cell, was $-0.8$ V relative to a saturated calomel electrode (SCE). All the cells were thermostated at 8°C, and the solutions were deaerated.

By virtue of the fact that on the oscillographic current-voltage curves obtained, the catalytic wave $I$ is located close to the double Co reduction wave, its correct measurement is hindered since the level of the Co reduction current at the potential of the catalytic wave varies depending on the degree of coverage of the surface of the electrode by protein. Therefore, we used a special method for finding this level, based on the fact that the Co reduction current after attainment of the potential of the second peak does not depend on the potential, and is determined by the transport of Co$^{3+}$ to the electrode. For each protein accumulation time we recorded two voltammograms: one with the cathode sweep rate in the range from $-0.8$ to $-1.7$ V, the second with a triangular cathode-anode sweep with initial potential $-0.3$ V; the cusp was chosen so that the time $\tau_1$ from this point to the middle of the Co-wave plateau on the anode sweep was equal to the time $\tau_2'$ from this same point to the peak of the catalytic wave $I$. In Fig. 1 we show the method of determining the height $I$ of the wave.

We used the following commercial preparations: beef serum albumin (BSA) (Koch Light Company); ribonuclease (RNase) from cattle pancreas and alcohol dehydrogenase (ADH) from horse liver (Reanal); lysozyme from egg albumin and xanthine oxidase from milk (Olainskii chemical reagent manufacturer). Phosphorylase from rabbit muscle, purified to a state homogeneous with respect to electrophoresis, was kindly supplied by N. B. Livanov. The purity of the preparations was monitored by the gel filtration method; BSA, ADH, and