

A STUDY OF THE MECHANISM OF THE ELECTROCHEMICAL
OXIDATION OF CHLOROPHYLL *a* IN AQUEOUS ACETONIC
SOLUTIONS. I

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The study of the mechanism of the reversible oxidation of chlorophyll *a* (Chl^0) at an electrode and the nature of the products formed in this process is important for explaining the mechanism of the sensitizing action of this pigment in photosynthesis. Until now, the reaction center of the chlorophyll molecule has not been known, in spite of the numerous experimental results obtained in the investigation of its photochemical reactions in solutions.

The polarographic method has come to be used for this study of the reversible oxidation of chlorophyll comparatively recently [1]. Nevertheless, important results have been obtained: the possibilities have been shown of the successive double oxidation of chlorophyll and the redox potentials E_0 of the reversible systems $\text{Chl}^+[\text{Chl}^0]$ and $\text{Chl}^{++}[\text{Chl}^+]$ have been determined [2-4]. However, this information was obtained only for dilute solutions of chlorophyll (10^{-6} – 10^{-4} M). Concentration of the chlorophyll solutions led either to the loss of the reversibility of electron transfer [2] or to a change in the number and nature of the polarographic waves [3, 4], which was connected, as it was found, with the presence of the chloride ion in the support.

Nevertheless, the electrochemical behavior of chlorophyll in more concentrated solutions ($>10^{-3}$ M) is of great interest, since its concentration in chloroplasts is 10^{-2} M. The behavior of the additional polarographic waves when the solution is concentrated [4] could be connected with the association of the pigments that has been detected by spectral methods. Then to the processes of the oxidation of the associates should correspond their anodic waves, i.e., in contrast to dilute solutions, where a mechanism of the successive oxidation of the monomeric chlorophyll molecules (more accurately, their solvates) takes place, in solutions containing 10^{-3} M of the pigment the parallel oxidation of different forms (monomers and associates) of chlorophyll is possible. However, in the investigations mentioned above there are no direct proofs of the existence of a successive or a parallel mechanism of the oxidation of the pigment in concentrated solutions.

Consequently, we have studied the mechanism of the electrochemical oxidation of chlorophyll in a wide range of concentrations of 10^{-6} to 10^{-3} M taking two factors into account. In the first place, in order to exclude the influence of chloride ions a normal mercury sulfate electrode was used for the comparison electrode instead of a silver chloride electrode; in the second place, to determine the lifetime of the products of the oxidation of chlorophyll and their possible adsorption on the electrode we used a rotating disc electrode with a ring in association with oscillopolarography.

The chlorophyll was isolated from nettles and was purified by a method adopted in our laboratory [5]. The concentration of water in the acetone solutions of the chlorophyll varied from 0.5 to 5% by volume. Smooth platinum was the material used for the disc and ring electrodes. In order to record the reaction products, in addition to the disc electrode with a ring we used the method of triangular voltage pulses. The solutions were polarographed in an atmosphere of air, since the polarization curves measured in an atmos-

* Deceased.

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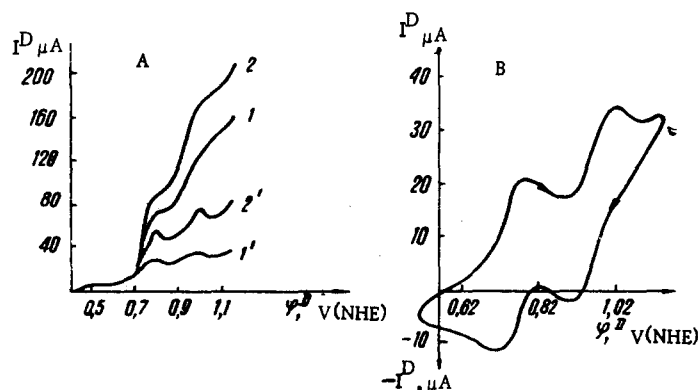


Fig. 1. Polarization I^D versus φ^D curves for the oxidation of chlorophyll a: A. 1, 2) at a rotating disc electrode with speeds of rotation of 69.1 and 79.6 sec^{-1} and scan rates of 40 mV/sec; 1', 2') at a stationary disc electrode at scan rates of 40 mV/sec and 167 mV/sec, respectively. B. Triangular form of the polarizing voltage.

phere of helium did not differ in any way from those obtained in the air. The potentials given here have been recalculated from the normal mercury sulfate electrode to the normal hydrogen electrode (NHE).

Figure 1 shows the polarization curves of the oxidation of chlorophyll in aqueous acetic solution containing $1 \cdot 10^{-3}$ M of the pigment and 0.5% of water (by volume). Similar curves were obtained over the whole range of concentrations of 10^{-5} to 10^{-3} M of the pigment. It was established that the relationship between I_{lim}^D and $I_{\text{lim}}^{II,D}$ was rectilinear over the whole range of concentrations and, in addition, the linear relationship $I^D - \sqrt{\omega}$ (Levich's relationship) was satisfied, which shows the diffusion nature of the currents of both polarographic waves. The degree of reversibility of the processes was judged from the slope of the straight line of a plot of $\ln i/(i_\alpha - i)$ versus φ and from the shape of the oscillographic curve of I versus φ obtained with a triangular form of the polarizing voltage (see Fig. 1B). As can be seen from Fig. 1, the anodic branch of the oscillopolarographic curve lies in the same region of potentials as the cathodic branch, and the difference of the corresponding anodic and cathodic peaks is 60 mV for the first wave and 70 mV for the second. This result, together with the shape of the oscillopolarographic curves — their curvature, the correspondence of the peaks (curves 1' and 2' in Fig. 1A) and the existence of plateaus of the limiting currents of the anodic waves (curves 1 and 2 in Fig. 1A) — and also the equality of I_{lim}^D and $I_{\text{lim}}^{II,D}$, shows the reversibility of the electrode processes under consideration [6]. The slope of the straight line corresponds to a value of 58 mV for the first anodic wave. Consequently, the oxidation of chlorophyll takes place by the detachment of one electron. This is valid both for the first and for the second wave, since the currents I_{lim}^D and $I_{\text{lim}}^{II,D}$ are equal and both electrode processes are reversible.

To determine the mechanism of the reaction and the properties of the chlorophyll oxidation products formed, solutions of chlorophyll after electrolysis at the potentials of the first and second oxidation waves were investigated in an ESR spectrometer. It was found that the chlorophyll oxidation products possessed unpaired electrons; i.e., the oxidation of chlorophyll even at the potential of the first wave forms a radical cation. Furthermore, the successive nature of the oxidation of chlorophyll was established by the recording of the polarization curves at the ring with a fixed value of the potential on the disc electrode. As shown in Fig. 2, at three fixed values of the potential on the disc the polarization curves obtained at the ring consisted of two waves of similar height which shifted with an increase in the positive values of φ^D in the direction of cathodic currents while remaining parallel with one another (curves 1-3, Fig. 2A). The first waves in the curves 2 and 3 are single anode-cathode curves, the maximum values of the limiting cathodic currents in both cases differing by a factor of precisely two.

The dependence of the limiting currents at the ring measured at three fixed values of the potentials on the ring φ_i^R on the potential of the disc electrode (I^R versus φ^D) are shown in Fig. 2B. Curve 4, obtained

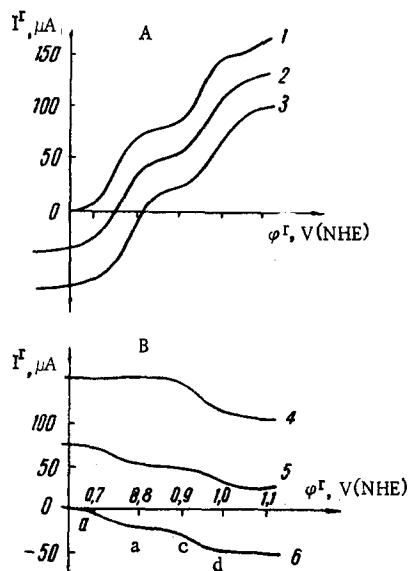


Fig. 2. Polarization curves of chlorophyll a at a rotating disc electrode. A. I^r versus φ^r curves at the following values of φ at the disc: 1) +0.55 V; 2) +0.85 V; 3) +1.07 V. B. I^r versus φ^D curves at the following values of φ at the ring: 4) +1.07 V; 5) +0.85 V; 6) +0.55 V.

from chlorophyll: $\text{Chl}^0 - \bar{e} \rightleftharpoons \text{Chl}^{\cdot+}$. In the same region curve 3 corresponds to two parallel processes: $\text{Chl}^0 - \bar{e} \rightleftharpoons \text{Chl}^{\cdot+}$ and $\text{Chl}^{\cdot++} + \bar{e} \rightleftharpoons \text{Chl}^{\cdot+}$. The second limiting anodic currents for all three polarization curves 1-3 (see Fig. 2A) correspond to the same process of the oxidation of $\text{Chl}^{\cdot+}$ to the doubly oxidized form of chlorophyll; i.e., $\text{Chl}^{\cdot+} - \bar{e} \rightleftharpoons \text{Chl}^{\cdot++}$.

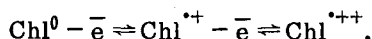
Curve 6 (see Fig. 2B) shows the reduction of the oxidized products of chlorophyll at the ring: sections a and b reflect the increase in the current of the reduction of the radical cations of chlorophyll with a rise in the current of the synthesis of these particles at the disc, and sections c and d the increase in the cathodic reduction current connected with an increase in the surface concentration of the doubly oxidized form of chlorophyll at the ring electrode in connection with the rise in φ^D at the disc. Consequently, in essence, curve 6 shows the dependence of the yield of $\text{Chl}^{\cdot+}$ and $\text{Chl}^{\cdot++}$ at the ring on the potential of the disc electrode.

Thus, in the absence of chloride ions under the conditions that we have selected, it is possible to observe the reversible nature of the twofold successive oxidation of chlorophyll a over the whole range of concentrations investigated. Our results show the fairly stable nature of the radical cations formed, their lifetime being 10^{-1} sec. This circumstance opens up a route for their further study.

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at $\varphi^r = 1.07$ V, unlike curves 5 and 6, has only one step: a decrease in the limiting anodic current is found only in the region of the potentials of the $\text{II} \varphi^D$. In the region of the potentials of the second wave $\text{I} \varphi^D$, the limiting current does not depend on the potential of the disc electrode. The constancy of the limiting current at the ring in this case can be connected only with the fact that the radical cation of chlorophyll ($\text{Chl}^{\cdot+}$) is formed at the disc at $\text{I} \varphi^D$ of the first wave by the reaction $\text{Chl}^0 - \bar{e} \rightleftharpoons \text{Chl}^{\cdot+}$, and after reaching the ring will be oxidized to the doubly oxidized form at the ring at a potential φ_4^r by the reaction $\text{Chl}^{\cdot+} - \bar{e} \rightleftharpoons \text{Chl}^{\cdot++}$. If $\text{Chl}^{\cdot+}$ were not oxidized at the ring at φ_4^r but some other particle (dimeric or trimeric form of chlorophyll) were oxidized, curve 4 in Fig. 2B, would also have a two-step nature. The fact that the second limiting anodic current at the ring is independent of the potential of the disc electrode in the range of potentials from +0.52 to +0.85 V unambiguously shows the successive mechanism of the oxidation of chlorophyll by the scheme



Consequently, the polarogram obtained at the ring electrode in the absence of a current at the disc (curve 1, Fig. 2A) is an ordinary polarization curve of the oxidation of chlorophyll analogous to that shown in Fig. 1A. The cathodic currents in curves 2 and 3, Fig. 2A, correspond to processes of the reduction of the radical cation to chlorophyll and of the doubly oxidized form to the radical cation. Consequently, in the region of the potentials of the first limiting anodic current, polarograms 1 and 2 correspond to the sole process of the synthesis of $\text{Chl}^{\cdot+}$.