DETERMINATION OF THE LIFETIME OF THE RADICAL CATIONS OF CHLOROPHYLL AND ITS DOUBLY OXIDIZED FORM UNDER THE CONDITIONS OF THEIR SYNTHESIS AT AN ELECTRODE. II

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We have previously [1] reported that the oxidation of chlorophyll a (Chl^0) at an electrode in aqueous acetonic solution takes place in two successive stages. At the potential of the first wave radical cations of chlorophyll ($Chl^{\cdot+}$) arise, and the second anodic wave on the polarogram of chlorophyll corresponds to the further oxidation of $Chl^{\cdot+}$ to the doubly oxidized form of $Chl^{\cdot++}$. There is no accurate information in the literature on the stability of the radical cations and of the doubly oxidized form of chlorophyll formed in the dark at an electrode. There is only a paper by Stanienda [2] in which it was shown by the method of triangular voltage pulses that the particles considered are fairly stable.

The present paper gives the results of a determination of the true lifetime of radical cations of chlorophyll a and its doubly oxidized form under the conditions of their synthesis at an electrode in the dark in aqueous acetonic solutions.

In the previous investigation [1] we showed that if the potential of the synthesis of the radical cation or its doubly oxidized form is imposed on the disc electrode, cathodic polarograms of these particles can be obtained at the ring electrode which repeat the polarization curves recorded at a rotating disc electrode (Fig. 1A). Figure 1B shows the dependence of the limiting currents of the reduction of the radical cations of chlorophyll (section OC on the curves 1'-3') and of its doubly oxidized form (section KM on the same curves) to chlorophyll measured at $\varphi^r = +0.4$ V. Similar results (I^r versus φ^D) were obtained at different rates of rotation of the electrode over the whole range of concentrations from 10^{-5} to 10^{-3} M of pigment and with a concentration of water in the acetone of 0.5-5% by volume. On the basis of the experimental results, the values of the current yield at the ring electrode of the radical cations of chlorophyll ($Chl^{\cdot+}$) and of its doubly oxidized form ($Chl^{\cdot++}$) were calculated from the formula

$$Q_i = \frac{I_i^r \cdot n^D}{I_i^D \cdot n^r \cdot N} \cdot 100\%,$$

where I_i^r and I_i^D are the limiting diffusion current of the i-th wave at the ring electrode and the current at the disc at a given potential; n^r and n^D are the numbers of electrons corresponding to the reactions at the ring and disc electrodes; and N is the constant in the Levich—Ivanov formula for a rotating electrode with a ring (see [3]).

For the electrode that we used, $r_1 = 2.5$ mm, $r_2 = 2.75$ mm, $r_3 = 3.55$ mm, N = 0.38, and n^r and $n^D = 1$. It was found that the current yields $Q_i\%$ for both reaction products (Q_1 and Q_2) were independent of the potential of the disc electrode, $\varphi^D = 0.75$ and 0.83 V and 0.95 and 1.05 V, respectively, independent of the rate of rotation ($\omega = 35$-154 sec^{-1}), and also independent of the amount of chlorophyll in the solution. In all cases, the current yield was 100%.

*Deceased.

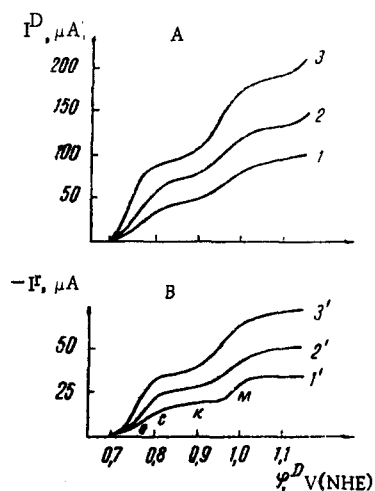Fig. 1   Fig. 2

Fig. 1. Polarization curves of chlorophyll a at a rotating disc electrode:
A. I^D versus φ^D curves for the following rates of rotation: 1) 5.9 sec^{-1},
2) 8.9 sec^{-1}, 3) 13.0 sec^{-1}; B. I^r versus φ^D curves at φ^r +0.4 V (the
same rates of rotation as for 1, 2, and 3).
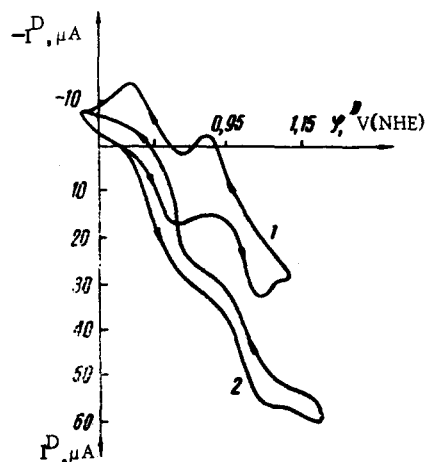
Fig. 2. Polarization curves I^D versus φ^D for the triangular form of the
polarization voltage with a rate of 40 mV/sec: 1) stationary disc elec-
trode; 2) rotating disc electrode.

This shows that $Chl^{\bullet+}$ and $Chl^{\bullet++}$ behave under the conditions of synthesis at the disc as stable reac-
tion products with respect to the ring electrode. Since it is known that the time of passage of the reaction
product from the disc to the ring electrode is 0.1 sec [4], it may be concluded that the lifetime of the radi-
cal cations and of the doubly oxidized form of chlorophyll must be not less than 0.1 sec. Since the method
of a rotating disc electrode with a ring was designed for the investigation of shorter-lived reaction products
(0.001 sec), it did not appear possible to determine the true lifetime of the particles under consideration.
With a sufficiently long lifetime of the radical cations and of the doubly oxidized form of chlorophyll the
possibility has also been shown of the appearance of cathodic reduction peaks of $Chl^{\bullet+}$ and $Chl^{\bullet++}$ on an
oscillogram measured with a triangular form of the polarizing voltage at a stationary disc electrode with
a low rate of scan of the potential (40 mV/sec) (Fig. 2, curve 1). This is confirmed by the fact that with
the rotation of the electrode, when the reaction products are transported by a flow of liquid from the sur-
face of the electrode, both reduction waves in the cathodic region disappear on the oscillogram of chloro-
phyll (Fig. 2, curve 2).

To determine the true lifetime of the chlorophyll oxidation products, we used the method of prepara-
tive electrolysis at a controlled potential with the aid of a Bulgarian potentiostat of the IP-410 type. The
electrolysis of the chlorophyll solutions was performed in an atmosphere of helium in an electrochemical
cell with three separate spaces by two methods.

In the first variant, the chlorophyll oxidation products accumulated in the electrode layer through
the electrolysis of an aqueous acetonic solution of chlorophyll containing 10^{-3} M of the pigment and 0.5%
of water at the potentials of the limiting diffusion currents of the first and of the second anodic waves at a
stationary disc electrode. Then, after the switching off of the electrolysis current, the limiting cathodic
currents of the reduction of the oxidized products were recorded at the same electrode by means of a
TsLA oscillopolarograph working under potentiostat conditions at φ^D = +0.4 V. Having determined the
time at which the limiting reduction currents of the reduction of $Chl^{\bullet+}$ and $Chl^{\bullet++}$ to chlorophyll amounted
to half their initial values, we obtained the half-decomposition time of the particles, $\tau_{1/2}$. However, the
values of the half-decomposition time of the particles ($Chl^{\bullet+}$ and $Chl^{\bullet++}$) found in this way should be low,
since the decrease in the cathodic currents here is due not only to the destruction of the radical particles
in the electrode layer as the result of a chemical reaction but also to the increase in the thickness of the
diffuse layer with time.

In the second variant, electrolysis of the same chlorophyll solution was performed at a platinum gauze electrode with a visible surface of 10 cm^2 (the volume of the solution was 5 cm^3). The use for electrolysis of an electrode with a large surface in a small volume of solution enabled the time of electrolysis to be shortened with the oxidation of practically all the chlorophyll present in the solution; i.e., it enabled a concentration of oxidized products close to the initial concentration of the pigment to be obtained. In this variant, the lifetime of the $Chl^{\cdot+}$ and $Chl^{\cdot++}$ formed was judged from the change in the limiting currents for the reduction of the particles considered after the switching off of electrolysis, but the cathodic currents were recorded not at the Pt gauze but at the rotating disc electrode, which served simultaneously to stir the solution. Since a rotating disc electrode was used to record the cathodic currents, the thickness of the diffusion layer did not depend on the time, as is well-known [3], and the values $\tau_{1/2}^1$ and $\tau_{1/2}^2$ unambiguously characterized the stability of the particles. The time of electrolysis was selected so that it was twice the time of half-decomposition of the reaction products. During the experiment the electrolysis current varied from $(7-10) \cdot 10^3$ to 20-30 μA.

The values of the time of half-conversion of the radical cations of chlorophyll and its doubly oxidized form found by the first method were 17 and 13 sec at 25°C and of the order of 10 min at −60°C, and they increased to a day at −120°C (the aqueous acetonic solution had still not solidified).

As was to be expected, the values of $\tau_{1/2}^1$ and $\tau_{1/2}^2$ determined by the second method were considerably higher, amounting to 45 and 35 sec for $Chl^{\cdot+}$ and $Chl^{\cdot++}$, respectively. A lowering of the temperature of the solution led to a sharp rise in the stability of the particles. Thus, at −70°C the values of $\tau_{1/2}^1$ and $\tau_{1/2}^2$ were already tens of minutes.

Taking into account the extremely stable nature of the chlorophyll oxidation products under the conditions of their synthesis at an electrode, particularly at a reduced temperature, we performed special experiments on the accumulation in solution of these particles in order to detect their ESR signal and to obtain the absorption spectra of $Chl^{\cdot+}$ and $Chl^{\cdot++}$. Electrolysis was performed in an atmosphere of helium at −60°C in an electrochemical cell with three separated spaces and with a cock for running off the solution. The solution was stirred by means of the rotating electrode. The solutions were transferred to capillaries and cells for recording the spectra in air. The ESR spectra were taken on an ESR-2 instrument, and the absorption spectra on a Unicom SP-700 recording spectrophotometer.

The ESR signal consisted of a singlet at H 3300 Oe. The half-decomposition periods of $Chl^{\cdot+}$ and $Chl^{\cdot++}$ calculated from the kinetic indices of the ESR signal and from the change in the absorption spectrum of the oxidized products correlated fairly well with the values of $\tau_{1/2}^1$ and $\tau_{1/2}^2$ obtained in the electrolysis of a solution of chlorophyll at a Pt gauze electrode.

Thus, the results of the measurements performed at a rotating disk electrode with a ring in combination with the experimental results obtained by the method of preparative electrolysis and with spectroscopy have shown for the first time that the lifetimes of the radical cation and the doubly oxidized forms of chlorophyll under the conditions of their synthesis at an electrode in the dark are greater by at least a factor of 10^3-10^4 than the lifetime of the radical cation of chlorophyll formed in aqueous organic solutions in photochemical reactions. This difference in the lifetimes of the radical cations is due mainly to the absence, under the conditions of the electrochemical generation of the ions, of electron donors and acceptors, such as semiquinones, which lead to the rapid deactivation of the radical cations in photochemical reactions.

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