STUDY OF TWO FORMS OF FERREDOXIN FROM DESULFOVIBRIO GIGAS BY DIFFERENTIAL PULSE POLAROGRAPHY

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SUMMARY : The two forms of ferredoxin from Desulfovibrio gigas, Fd I and Fd II, are studied by differential pulse polarography*. Fd I and Fd II give one well defined peak corresponding to $E_{1/2}$ = - 0.33 and - 0.35 V (vs. the hydrogen electrode) respectively, at c > 5 μM. The influence of the concentration on the peak potentials E_p and the peak heights i_p is examined. The denaturation of the two forms of ferredoxin is studied by polarography in conjunction with UV spectrophotometry. Two new peaks at negative potentials before the reduction of the solvent are observed in denaturated proteins.

Some properties of ferredoxin from the sulphate reducing bacterium Desulfovibrio gigas have been reported recently (1, 2, 3). This ferredoxin consists of three different oligomers, termed Fd I, Fd I' and Fd II. Oxidation-reduction titrations coupled with EPR* measurements have been carried out (3) with the platinum electrode using various mediators. The three types of ferredoxin have different midpoint potentials ; in each case, two values have been determined for C^{-} / C^{2-} and C^{2-} / C^{3-} couples [in the terminology of the "three-state" theory of Carter et al. (4)].

The purpose of the present work is a direct determination of the redox potentials of ferredoxin Fd I and Fd II by means of dropping mercury electrode. To our knowledge, only one study seems to have been previously reported concerning the polarographic behaviour of ferredoxins, by Weitzman et al. (5), who have found that C. pasteurianum ferredoxin gives a polarographic reduction wave at about - 0.32 V (vs. the hydrogen electrode). It seems that this technique may provide an advantageous means of studying iron-sulphur proteins, but the very low concentrations of the solutions ordinarily used (< 20 μM) make this investigation difficult by classical DCP*, specially because the limiting current is poorly defined. This is the reason why DPP has been chosen in the present work. No other study on iron-sulphur proteins seems to have been previously reported with this recent technique.

In addition, the polarographic study as been performed in connection

* Abbreviations : DPP, differential pulse polarography ; EPR, electron paramagnetic resonance ; DCP, direct current polarography.

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with measurements of the respective absorption spectra to follow the rate of Fd I and Fd II denaturation.

EXPERIMENTAL

Fd I and Fd II samples from Desulfovibrio gigas were prepared and purified in the Laboratoire de Chimie Bactérienne du CNRS-Marseille (1), and were supplied in 0.01 M Tris-HCl buffer (pH = 7.6). All experiments were performed in this medium which served as supporting electrolyte.

Polarograms were recorded on a Sefram XY recorder coupled with a PAR 174A polarographic analyzer equipped with a M 174/10 drop timer. The counter electrode was a platinum one. The reference electrode was a Metrohm silver-silver chloride (saturated KCl solution) electrode (symbolized by Ag/AgCl). The sample solutions (5 ml) were deaerated by bubbling U nitrogen for 1/2 hr. before recording.

Cathodic DP polarograms were made at 22 °C from 0 to - 1.5 V at a scan rate of 2 mV/s using a pulse amplitude of 50 mV, a drop time of 0.5 s and a mercury flow of about 1 mg/s. The peak currents i_p were measured with correction of the blank relative to the supporting electrolyte.

Optical absorption spectra were recorded on a Cary 14 spectrophotometer with Hellma quartz cells.

In order to verify that the observed peaks were indeed due to ferredoxin, the absence of inorganic impurities was investigated by subjecting the samples to oxidation by inorganic acids and recording polarograms after mineralisation. A first oxidation of the samples containing 0.1 to 0.3 mg of ferredoxin by the mixture HNO_3 + H_2SO_4 was achieved after adding $HClO_4$ and warming at 270 °C for 1/2 hr. All inorganic acids were Ultrapure Normatom Prolabo reagents. The same procedure was applied to the blanks of the various reagents used for mineralisation and to the blanks of the supporting electrolyte. These experiments have been repeated on several samples from various preparations and have shown that no inorganic impurities could interfere with the observed results.

In order to ensure that samples tested by polarography were undenaturated, absorption spectra were compared before and after each experiment by measuring the characteristic absorbance A_{405}/A_{300} ratio for Fd I and A_{415}/A_{305} ratio for Fd II (1).

RESULTS AND DISCUSSION

The DP polarograms of Fd I and Fd II are shown in figures 1 and 2 for undenaturated and denaturated proteins. It is found that the two forms give one well defined peak. For c > 5 μM, the E_p values are :

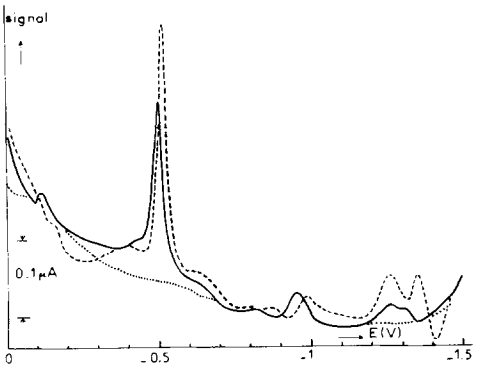
Fd I :	E_p = - 0.50 V	E_p = - 0.30 V	[vs. the hydrogen electrode (6)]
Fd II :	E_p = - 0.52 V	E_p = - 0.32 V	
	(vs. Ag/AgCl)		

By application of the Parry and Osteryoung relationship (7) $E_p = E_{1/2} - \Delta E/2$ relating the peak potential E_p to the half-wave potential $E_{1/2}$ for a given pulse amplitude ΔE, the following half-wave potentials are available : Fd I : $E_{1/2}$ = - 0.33 V

Fd II : $E_{1/2}$ = - 0.35 V (vs. the hydrogen electrode)

These values are in good agreement with the $E_{1/2}$ of C. pasteurianum ferredoxin determined by classical DCP (5).

The height of the peaks i_p and the value of E_p were found to be dependent on concentration as shown in figures 3a and 3b. i_p values increase



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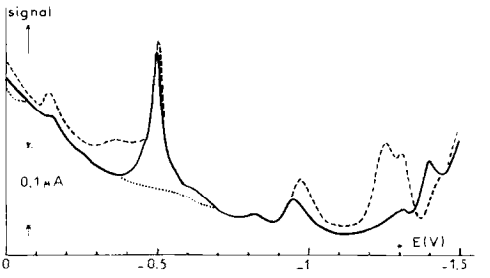

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Fig. 1. DP polarograms of ferredoxin Fd I (concentration = 10 μM) in 0.01 M Tris-HCl (pH = 7.6).
..... blank ; ——— undenaturated protein ; ---- denaturated protein after 14 days.
pulse amplitude = 50 mV ; drop time = 0.5 s ; scan rate = 2 mV/s ; reference electrode : Ag/AgCl (sat. KCl).

Fig. 2. DP polarograms of ferredoxin Fd II (concentration = 3 μM) in 0.01 M Tris-HCl (pH = 7.6).
..... blank ; ——— undenaturated protein ; ---- denaturated protein after 1 day.
pulse amplitude = 50 mV ; drop time = 0.5 s ; scan rate = 2 mV/s ; reference electrode : Ag/AgCl (sat.KCl).

linearly with the concentration until a limit is reached at about 5 μM. Above this concentration, i_p remains constant for Fd I whereas it increases more slowly for Fd II. Moreover, i_p values for Fd II are heigher than for Fd I. The constancy of i_p above a given concentration is explained by a saturation of the mercury drop surface with a layer of ferredoxin molecules ; this phenomenon is observed with some other proteins (5). The same explanation may be given for the variation of E_p which reaches limiting values at the same concentration as i_p.

The denaturation of the two forms of ferredoxin has been examined as a function of time by recording simultaneously DP polarograms (fig. 1 and 2) and absorption spectra. For this study, the samples were kept at room temperature (~ 22 °C).

For Fd I, denaturation causes an enhancement of the peak which is slightly shifted towards more negative values ; for Fd II, no shift of the peak is observed, but a very badly defined one develops between - 0.35 and - 0.45 V.

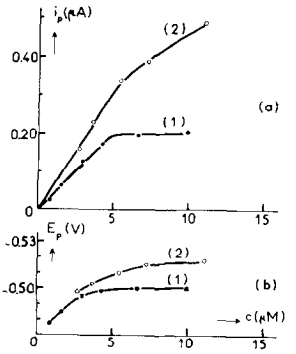

Fig. 3. - (a) Plot of peak current (ip) versus concentration of Fd I (1) and Fd II (2), after substraction of the blank.
- (b) Plot of peak potential (E_p vs. Ag/AgCl) versus concentration of Fd I (1) and Fd II (2).

In the two forms of ferredoxin, the denaturation process seems to be closely associated with the presence of two peaks at very negative potentials (- 1.2 to - 1.3 V). With some other proteins, a double polarographic wave near the reduction potential of the solvent has already been observed by Brdicka et al. (8) and it has been noted that the height of the double wave increased during the first part of the denaturation process. This catalytic double wave is influenced by several factors, for example the metal concentration. An analogous phenomenon could occur with ferredoxin and the two peaks could thus have a catalytic origin.

The two forms of undenatured ferredoxin Fd I and Fd II give a characteristic polarogram and the respective reduction peaks are probably associated with the Fe/S centre of this protein. Indeed, on studying the corresponding apoprotein obtained by classical treatment (3) no peak could be detected.

However, some divergences seem to appear between the polarographic behaviour described in the present work and the results from oxidation-reduction titrations using the platinum electrode, in conjunction with EPR measurements, of Cammack et al. (3). In particular, the resulting values are quite different. Such differences between the polarographic half-wave potentials and the redox potentials determined by other procedures have already been noticed by Weitzman et al. (5) in the case of C. pasteurianum ferredoxin. It is likely that adsorption phenomena occur during electrolytic reduction, as has been seen above when the protein concentration increases, and are probably not absent on the mercury electrode.

On the other hand, we have verified that direct voltammetry with the

platinum electrode gave no wave in the available range of potentials, so direct measurements cannot be carried out with this procedure. For this reason indirect determination by potentiometry requires mediators.

Finally the analytical interest of the DPP technique should be underlined as by this means ferredoxin concentrations as low as 1 μM can be determined.

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