CIRCULAR DICHROISM STUDIES OF THE COMPLEX BETWEEN FERREDOXIN AND FERREDOXIN-NADP REDUCTASE

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SUMMARY

Circular dichroism (CD) spectra are presented of ferredoxin, ferredoxin-NADP reductase and their complex. A change in CD occurs on complex formation which is consistent with a decrease in the Cotton effects due to the ferredoxin. This change is interpreted as due to a decrease in interaction in ferredoxin between the iron-sulphur chromophore group and the protein.

No significant changes in the EPR* signal of reduced ferredoxin were detected in the presence of the reductase.

The iron sulphur protein ferredoxin and the flavoprotein ferredoxin-NADP reductase are two consecutive carriers at the reducing end of the electron transport chain from oxygen to NADP in plant photosynthesis (1). The isolated proteins have been shown to combine in their oxidized forms to form a 1 : 1 complex (2-5). The reductase from a higher plant will form complexes with ferredoxins from other species of plants and from bacteria (4,5). Formation of the complex is accompanied by a change in the optical absorption spectrum, with increases in absorption around 395 and 465 nm, which correspond roughly with the absorption bands of the flavoprotein. It was suggested by Foust, Mayhew and Massey (5) that these changes were due to changes in the environment of the flavin chromophore in the reductase.

This paper reports CD* measurements of spinach ferredoxin, swiss chard

*Abbreviations: CD, circular dichroism.
EPR, Electron paramagnetic resonance.

ferredoxin-NADP reductase and the complex of these two proteins; it is suggested that, during complex formation, changes may also occur in the environment of the iron-sulphur chromophore of the ferredoxin.

EXPERIMENTAL

Circular dichroism measurements were made on a Jouan Dichrographe II instrument, which was made available to us by Dr. P.M. Scope and Professor W. Klyne, through the University of London Intercollegiate Research Service. EPR measurements were made on a Varian E4 spectrometer, using a liquid nitrogen insert dewar for the sample.

Spinach ferredoxin was prepared by the method of Rao, Cammack, Hall and Johnson (6), and had a ratio $A_{420}:A_{275}$ of 0.46. Swiss chard ferredoxin-NADP reductase was prepared by the method of Shin, Tagawa and Arnon (1), and had a ratio $A_{456}:A_{275}$ of 0.11. Measurements of circular dichroism of the proteins were made on 25.5 μM solutions in 5 mM Tris-Cl, pH 8.0.

Complex formation between the proteins was confirmed spectrophotometrically; the increase in absorption spectrum was very similar to that shown by complexes of the proteins from other plants (4,5).

RESULTS AND DISCUSSION

Fig. 1 shows the CD spectra of (a) spinach ferredoxin, (b) swiss chard ferredoxin-NADP reductase, and (c) a mixture of equimolar quantities of the proteins. Curve (d) is the calculated difference between curve (c) and curves (a) plus (b), and therefore represents the change in CD occurring on complex formation. This difference is somewhat small, and subject to cumulative errors in measurement of the three spectra, and replotting. It was found that a more accurate method of measuring its shape was to use the fact that high concentrations of salt will split the complex (4,5). After the CD spectrum of the complex, (Fig. 1c) had been measured, solid sodium chloride was added to bring the concentration to 0.3 M NaCl, which is sufficient to split the complex almost completely (4); the CD spectrum was then measured again. Curve (e) is the average of four

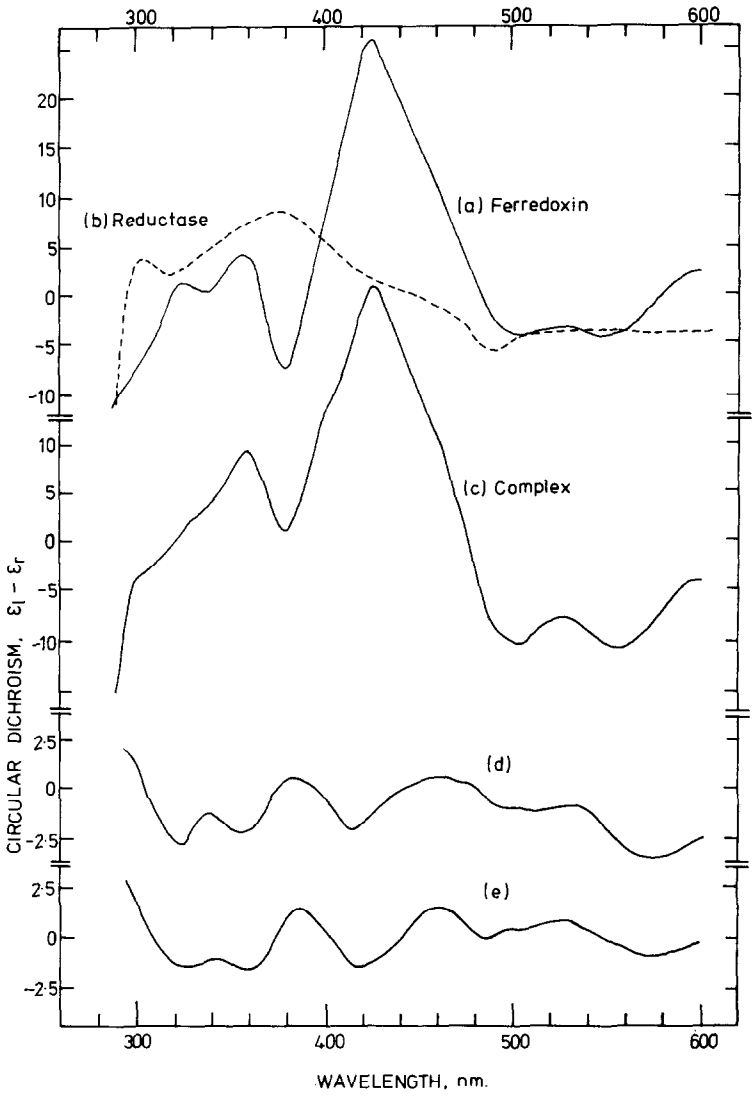

Fig. 1. (a) CD of spinach ferredoxin; (b) CD of swiss chard ferredoxin-NADP reductase; (c) CD of an equimolar mixture of ferredoxin and reductase; (d) calculated difference between curve (c) and the sum of curves (a) and (b); (e) calculated difference in CD of complex on adding 0.3M NaCl.

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difference spectra obtained in this way, with two different samples of ferredoxin and two of reductase. It can be seen that its amplitude and shape are substantially the same as that of curve (d).

The change in CD spectrum between 300 and 600 nm is about 10% of

the amplitude of the CD spectrum of the complex, so that it is comparable in magnitude with the change in absorption spectrum (there is an 8% increase in A_{469} on formation of the complex between these two proteins). Maximal positive changes in CD occur at about 385 and 460 nm and maximal negative changes at 326, 356 and 420 nm. These are close to the major Cotton effects of the ferredoxin and are all consistent with a decrease in CD of ferredoxin on complex formation.

Since there are changes in absorbance in the regions of flavin absorption, it might be expected that there would be corresponding CD changes. However the CD of the flavoprotein is weak compared with that of ferredoxin (see Fig. 1(a) and (b)) and in fact it is not possible to resolve such changes in Fig. 1(d) and (e).

Thus it appears that during formation of the complex there was a marked decrease in the CD of ferredoxin which was not accompanied by a correspondingly large change in optical absorption. The CD of ferredoxin might arise from an asymmetric iron-sulphur chromophore, or from the interaction between a symmetric chromophore with neighbouring asymmetric groups in the protein, or from a combination of both effects. Garbett et al. (7) showed that in the presence of urea, the CD of ferredoxin changed to a different, much weaker spectrum, while the absorbance spectrum remained almost unchanged. Padmanabhan and Kimura (8) demonstrated similar effects in spinach ferredoxin and adrenal iron-sulphur protein, in the presence of urea or guanidinium chloride, and concluded that the CD of the protein arose at least partially from the interaction of the chromophore with the protein. It appeared that the effect of the denaturing agents was to decrease this interaction.

By analogy with the effects of urea, it might be proposed that a similar effect, though less drastic, was occurring in the complex between ferredoxin and reductase. The forces involved in formation of the complex could result in a distortion of the ferredoxin protein so that the interactions

between the iron-sulphur group and asymmetric groups on its protein were weakened.

Urea and guanidinium chloride are known to cause considerable changes in the EPR signal of plant ferredoxins, in addition to their effects on the CD (9). Experiments were carried out to determine whether the reductase had a similar effect. Since oxidized ferredoxin has no EPR signal, it was necessary to study the reduced form. The sample used contained 0.24 mM ferredoxin and 0.30 mM reductase (an excess to ensure that most of the ferredoxin was complexed). As controls, samples were also prepared of ferredoxin alone and ferredoxin + reductase + 0.3 M NaCl. The samples were reduced with 2 mM sodium dithionite at 20°C. It was noted that ferredoxin in the complex was reduced much more slowly than free ferredoxin; the reduction required 10 min for completion, whereas ferredoxin alone was reduced in 30 seconds. This was partly due to the fact that the reductase flavin had first to be reduced to its fully reduced form, possibly *via* the semiquinone form, but was probably also due to steric effects.

These experiments indicated that the EPR spectra of the reduced ferredoxin at -77°K showed no significant changes in shape in the presence of reductase, nor in the presence of reductase plus 0.3M NaCl. Thus, assuming that the complex was not split by reducing the proteins, the reductase was not producing effects on the EPR signal analogous to those of guanidinium chloride and urea.

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