MAGNETO-OPTICAL ROTATORY DISPERSION (MORD) STUDIES ON SPINACH FERREDOXIN

D.I. Marlborough, D.O. Hall and R. Cammack,
Chemistry Department, Imperial College, London
and Botany Department, King's College, London.

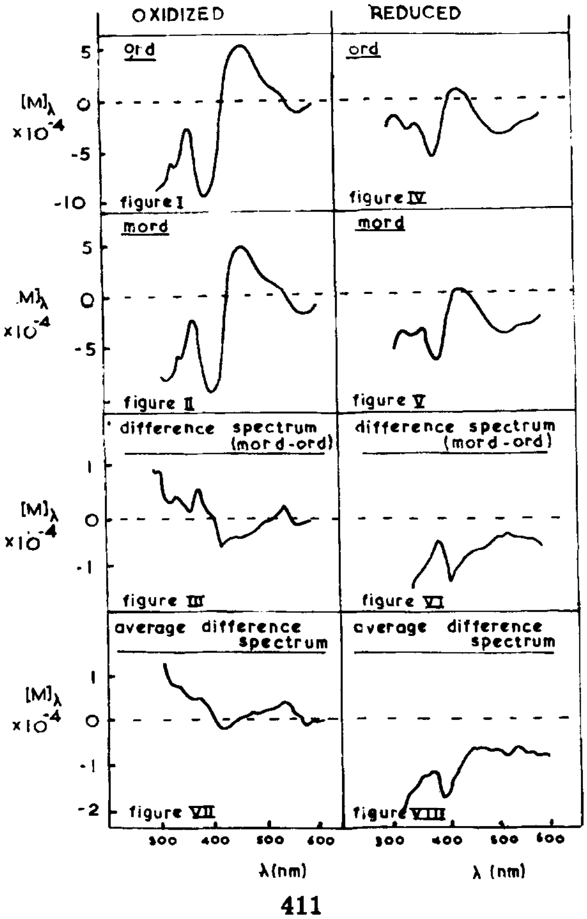
Received April 7, 1969

Summary. Optical rotatory dispersion (ORD) measurements have been carried out on spinach ferredoxin both in the presence and absence of a 3 kilogauss magnetic field. The transitions observed in the absence of the magnetic field are not materially altered when the magnetic field is applied. However, additional rotations are induced; a positive rotatory dispersion in the case of the oxidized form of the spinach ferredoxin and a negative rotatory dispersion in the reduced form. In addition it was found that the ORD spectrum, particularly of the reduced form of the protein, was sensitive to the decomposition of the protein.

Optical rotatory dispersion (ORD) studies have been carried out over the last few years on a number of iron-sulphur proteins[1-5]. These show the influence of the iron-sulphur chromophore on the ORD spectra between 600 and 300 nm. wavelength. ORD and circular dichroism (CD) spectra have revealed differences between the oxidized and reduced forms of these proteins; here we wish to report the application of the somewhat more recent technique of MORD[6] to the study of one member of this class of proteins, viz. spinach ferredoxin, which show further differences between the oxidized and reduced forms.

The spinach ferredoxin was isolated by the method of Tagawa and Arnon[7], as modified by Evans _et al_[8]. The ferredoxin was made up as a 0.3% solution in 20 _mM_ phosphate buffer, pH 7.5. The reduced form was obtained by addition of an excess of crystalline sodium dithionite. The spectra were run at 15°C with a 20 minute scanning time on a FICA machine kindly made available for our use by Applied Research Laboratories of Luton. This machine has the cell compartment adapted for use with a 3 kilogauss magnetic field for measuring the MORD spectra. For determining the specific molar rotations and for comparability a molecular weight of 13,000 was assumed for the spinach ferredoxin[4].

The spectra obtained in a representative run are shown in figures I to VI. Figure I shows the ORD spectrum for oxidized spinach ferredoxin, figure II the MORD spectrum for oxidized spinach ferredoxin and figure III the difference spectrum obtained by subtracting the ORD values from the MORD values. Figures IV, V and VI are the corresponding curves for reduced spinach ferredoxin. Figures III and VI thus represent the additional rotation induced by the magnetic field. Since the values are small, and may therefore be subject to some random error, average difference spectra (from 3 different samples) are shown in figures VII and VIII for oxidized and reduced spinach ferredoxin, respectively. As can be seen the latter two graphs show substantially the same trend as in the spectra shown with a single sample in figures III and VI.



The values for the principal peaks and troughs from spectra I, II, IV and V are shown in the table below:

OXIDIZED				REDUCED			
ORD		MORD		ORD		MORD	
(nm)	[M] x10^{64}	(nm)	[M] x10^{-4}	(nm)	[M] x 10^{-4}	(nm)	[M] x10^{-4}
575	-2.25	573	-2.47	520	-4.07	520	-4.60
470	+4.98	473	+4.71	432	+0.98	432	+0.08
396	-10.00	398	-10.00	385	-7.04	387	-7.75
368	-2.55	369	-2.28	357	-2.64	359	-3.62

It is obvious from this table that the most striking effect of applying a magnetic field is in the reduced ferredoxin in the visible and ultraviolet regions. This is probably due to a change in magnitude of rotations and/or red shift in one or more of the transitions. This change may be indicative of the active site or chromophore of the ferredoxin.

In terms of Shashoua's[6] classification of MORD spectra, the MORD-ORD difference spectrum for oxidized spinach ferredoxin is a type Va curve (positive rotation towards the UV) while that for reduced spinach ferredoxin is a type Vb curve (negative rotation towards the UV). At wavelengths lower than 370 nm a positive contribution of added dithionite in the reduced spectra has not been taken into account since a non-stoichiometric excess of solid dithionite was added. If this were taken into account it would only serve to accentuate the negative dispersion. It was also noted in running the spectra that the reduced form underwent a progressive change presumably due to decomposition of the protein. The peaks in the ORD and MORD spectra between 600 and 400 nm. broadened, and the 432 nm, peak fell to negative values. This is consistent with the loss of structure around the iron-sulphur chromophore, with the spectrum approaching that for a normal polypeptide. The spectra of the reduced form were therefore measured as soon as possible after reduction.

We are at present investigating whether other iron-sulphur proteins with different numbers of paramagnetic centres[8] show similar reversals of sign of their MORD spectra accompanying electron transfer. The measurement of MORD spectra of other metalloproteins is also likely to be important.

This work was supported by a Science Research Council grant to D.O.H. and a fellowship to D.I.M.

References

1. D.D. Ulmer and B.L. Vallee. Biochemistry, 2, 1335, (1963).
2. R.D. Gillard, E.D. McKenzie, R. Mason, S.G. Mayhew, J.L. Peel and J.E. Stangroom, Nature, 208, 769, (1965).
3. N.M. Atherton, K. Garbett, R.D. Gillard, R. Mason, S.J. Mayhew, J.L. Peel and J.E. Stangroom, Nature 212, 590 (1966).
4. K. Garbett, R.D. Gillard, P.F. Knowles and J.E. Stangroom, Nature, 215, 824, (1967).
5. G. Palmer, H. Brintzinger and R.W. Estabrook, Biochemistry, 6, 1658, (1967).
6. V.E. Shashoua, J. Am. Chem. Soc. 86, 2109 (1964).
7. K. Tagawa and D.I. Arnon, Nature, 195, 537 (1962).
8. M.C.W. Evans, D.O. Hall, H. Bothe and F.R. Whatley, Biochem. J. 110, 485 (1968).