Lawrence Berkeley National Laboratory
Recent Work

Title
FERREDOXIN: THE USES OF NATURAL AND MAGNETIC CIRCULAR DICHROISM IN A MULTI-CHROMOPHORIC SYSTEM

Permalink
https://escholarship.org/uc/item/7pb7m626

Author
Sutherland, John C.

Publication Date
1971-11-01

eScholarship.org Powered by the California Digital Library
University of California

Submitted to Biochimica et
Biophysica Acta

LBL-542
Preprint

c. 1

FERREDOXIN: THE USES OF NATURAL AND MAGNETIC CIRCULAR DICHROISM IN A MULTI-CHROMOPHORIC SYSTEM

John C. Sutherland, Irving Salmeen,
Alexander S. K. Sun and Melvin P. Klein

RECEIVED
LAWRENCE
RADIATION LABORATORY

FEB 22 1972

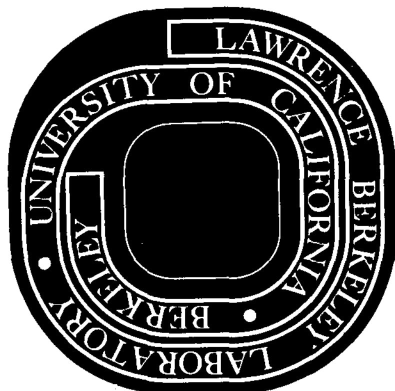
LIBRARY AND
DOCUMENTS SECTION

November 1971

AEC Contract No. W-7405-eng-48

For Reference

Not to be taken from this room



LBL-542

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

0 0 0 0 3 7 0 4 7 3 6

FERREDOXIN; THE USES OF NATURAL AND MAGNETIC CIRCULAR DICHROISM IN A MULTI-CHROMOPHORIC SYSTEM[#]

J. C. SUTHERLAND*, IRVING SALMEEN* **, ALEXANDER S. K. SUN***, and M. P. KLEIN

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory,
University of California, Berkeley, California 94720

[#]Research sponsored by the U. S. Atomic Energy Commission.

*Postdoctoral Fellow of the National Institute of General Medical Sciences, National Institutes of Health.

**Present Address: Scientific Research Staff, Ford Motor Company, Dearborn, Michigan.

***Present Address: Department of Physiology and Anatomy, University of California, Berkeley, California.

ions and sulfur atoms. The CD shows considerable structure throughout the spectrum. On the basis of absorption and CD alone, however, it would be extremely difficult to distinguish the origin of the absorption band at 277 from that at 313 nm. However, the MCD of the 277 band differs strikingly from the rest of the spectrum.[3,4] For wavelengths greater than 300 nm, the MCD is relatively broad and unstructured, while between 250 and 300 nm it exhibits a sharp "S" shaped spectrum indicative of an orbitally degenerate ground or excited state. The chromophore responsible for the distinctive "S" shaped MCD is the aromatic amino acid tryptophan whose lowest energy singlet excited state is known to be doubly degenerate.[5] The presence of tryptophan is indicated by the MCD even though the other aromatic amino acids and the iron-sulfur complex also contribute to the MCD spectrum at 280 nm. Thus, MCD can detect the presence of certain special molecules in systems which contain overlapping absorption bands of several chromophores. We shall call these chromophores <u>MCD markers</u>. We shall outline the properties of a chromophore which make it an MCD marker, after discussing the CD of ferredoxin.

Comparison of the spectra of the reduced and oxidized protein (Fig. 2) shows much larger changes in CD than in absorption. These visible changes and ultraviolet in the CD are very difficult to interpret in terms of the conformation of the active site.[6,7] Note, however, that the CD of the 275 nm band changes sign when the protein is oxidized. The position and width of these bands in the reduced and oxidized states suggest that they are mainly due to the aromatic amino acids and the sign reversal of the CD suggests that the environment of

0 0 0 0 3 7 0 4 7 3 7

Many biological macromolecules and subcellular particles contain more than one component which absorbs visible or ultraviolet light. For example, proteins may contain the aromatic amino acids tryptophan, phenylalanine and tyrosine, which absorb below 315 nm, and one or more prosthetic groups or cofactors which absorb visible and ultraviolet light. Among subcellular particles, chloroplasts possess especially complex absorption spectra due to chlorophyll, carotenoids, cytochromes, non-heme iron proteins, flavoproteins, quinones, and plastocyanin. It is often desirable to study such systems by a variety of spectroscopic techniques, each of which provides a different type of information. We shall discuss the interpretation of the natural and magnetic circular dichroism (CD and MCD) of multichromophoric systems. We wish to emphasize the complementary nature of these techniques.

Spinach ferredoxin (Fd) is a good example of a protein with more than one chromophore. Ferredoxins are redox carriers found in the electron transfer systems of a wide variety of organisms (for a review see Ref. 1). Depending on the source, ferredoxins contain from two to eight iron ions and an equal number of inorganic sulfur atoms. The iron ions are complexed with the inorganic sulfurs and the sulfur atoms of cysteine residues. The iron-sulfur complex gives rise to the broad absorption bands which extend throughout the visible and UV spectral regions. Spinach ferredoxin contains two iron ions. In addition, there are also four tyrosine, two phenylalanine, and one tryptophane residues[2] which absorb between 250 and 315 nm.

Figure 1 shows the absorption spectrum, CD and MCD of reduced spinach ferredoxin from 250 to 630 nm. The visible and near UV absorption is presumably due to charge transfer transitions between the iron

0 0 0 0 3 7 0 4 7 3 8

one or more of the aromatic acids has changed as a result of oxidation. One obvious explanation is that oxidation changes the tertiary structure of the protein which may be associated with its binding specificity or the conformation of the iron-sulfur complex. The aromatic amino acids are thus acting as probes of the conformation of the protein or "CD reporters".

To qualify as an MCD marker, a chromophore must possess a 3-fold or higher axis of symmetry.[8] The symmetry need not be exact, however, since small perturbations have little effect on the MCD.[9] In addition to tryptophan, purines (but not the pyrimidines)[6] and some metallopor-phyrins are good MCD markers. Cytochromes are particularly interesting, since in their reduced state they are excellent MCD markers while in the oxidized form their MCD is decreased by about a factor of 20. In a solution of oxidized cytochrome c which contains a few percent of the reduced form, the MCD clearly indicates the presence of the reduced material, while no indication of reduced protein is obvious in the absorp-tion spectrum.[10] Chlorophyll molecules are not good MCD markers, since the degeneracy of their excited states has been lifted by the 5 member isocyclic ring. Thus, in chloroplast preparations one can detect reduced cytochromes even though they represent a small fraction of the absorption of the system.[11]

MCD markers often have a plane or center of symmetry and thus have no CD of their own. These molecules or groups make excellent reporters since their CD is caused by interactions with their surroundings.

The experimental techniques for measuring CD and MCD are very similar. However, the physical basis of the two phenomena are quite

different, and, as we have shown, they provide complementary information about complicated biochemical systems.

REFERENCES

1 R. MALKIN AND J. C. RABINOWITZ, Ann. Rev. Biochemistry, 36 (1967) 113.

2 H. MATUUBARA, R. M. SASKI AND R. K. CHAIN, a) Proc. Nat. Acad. Sci. U.S., 57 (1967) 439; b) J. Biol. Chem., 243 (1968) 1725.

3 B. HOLMQUIST, Annual Meeting of the American Society of Biological Chemists, San Francisco, Calif., 1971.

4 J. C. SUTHERLAND, Pacific Conference on Chemistry and Spectroscopy, Anaheim, Calif., 1971.

5 G. BARTH, R. RECORDS, E. BUNNENBERG, C. DJERASSI AND W. VOELTER, J. Amer. Chem. Soc., 93 (1971) 2545.

6 G. PALMER, H. BRINTZINGER AND R. W. ESTABROOK, Biochemistry, 6 (1967) 1658.

7 K. GARBETT, R. D. GILLARD, P. F. KNOWLES AND J. E. STANGROOM, Nature, 215 (1967) 824.

8 C. DJERASSI, E. BUNNENBERG AND D. L. ELDER, Pure and Applied Chemistry, 25 (1971) 57.

9 J. C. SUTHERLAND, DANIEL AXELROD AND M. P. KLEIN, J. Chem. Phys., 54 (1971) 2888.

10 J. C. SUTHERLAND AND M. P. KLEIN, in preparation.

11 J. BRETON AND M. HILAIRE, C. R. Acad. Sci., Paris, Series D, in press.

12 K. TAGAWA AND D. I. ARNON, Nature, 95 (1962) 537.

0 0 0 0 3 7 0 4 7 3 9

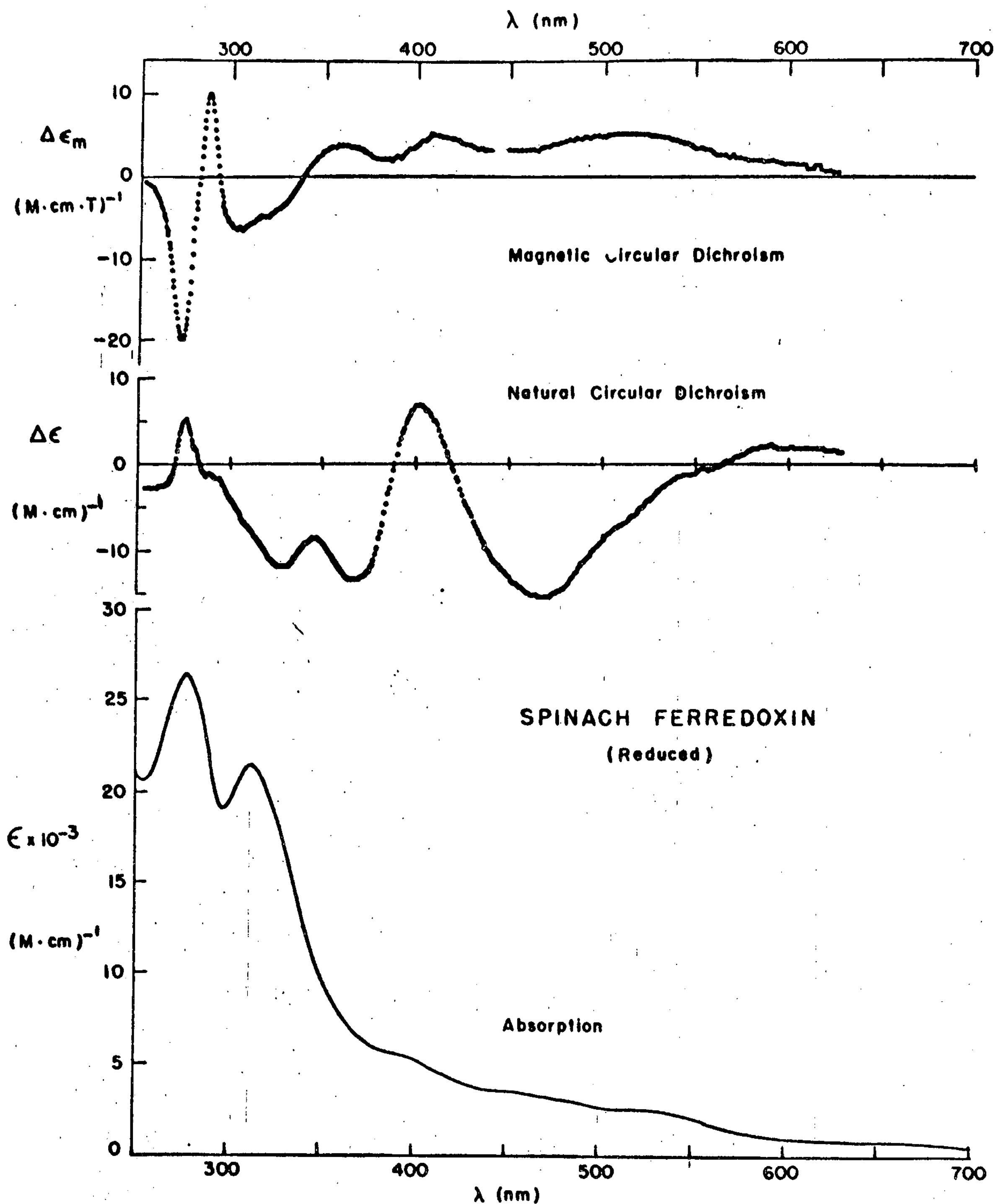


Fig. 1. The absorption, CD and MCD of reduced spinach ferredoxin in the visible and UV. The protein was extracted by the method of Tagawa and Arnon[12] and was dissolved in .15 M phosphate buffer, pH 7.3. It was reduced with sodium dithionite which contributes slightly to the UV absorption spectrum. Measurements were made with a spectrometer which has been described elsewhere.[10]

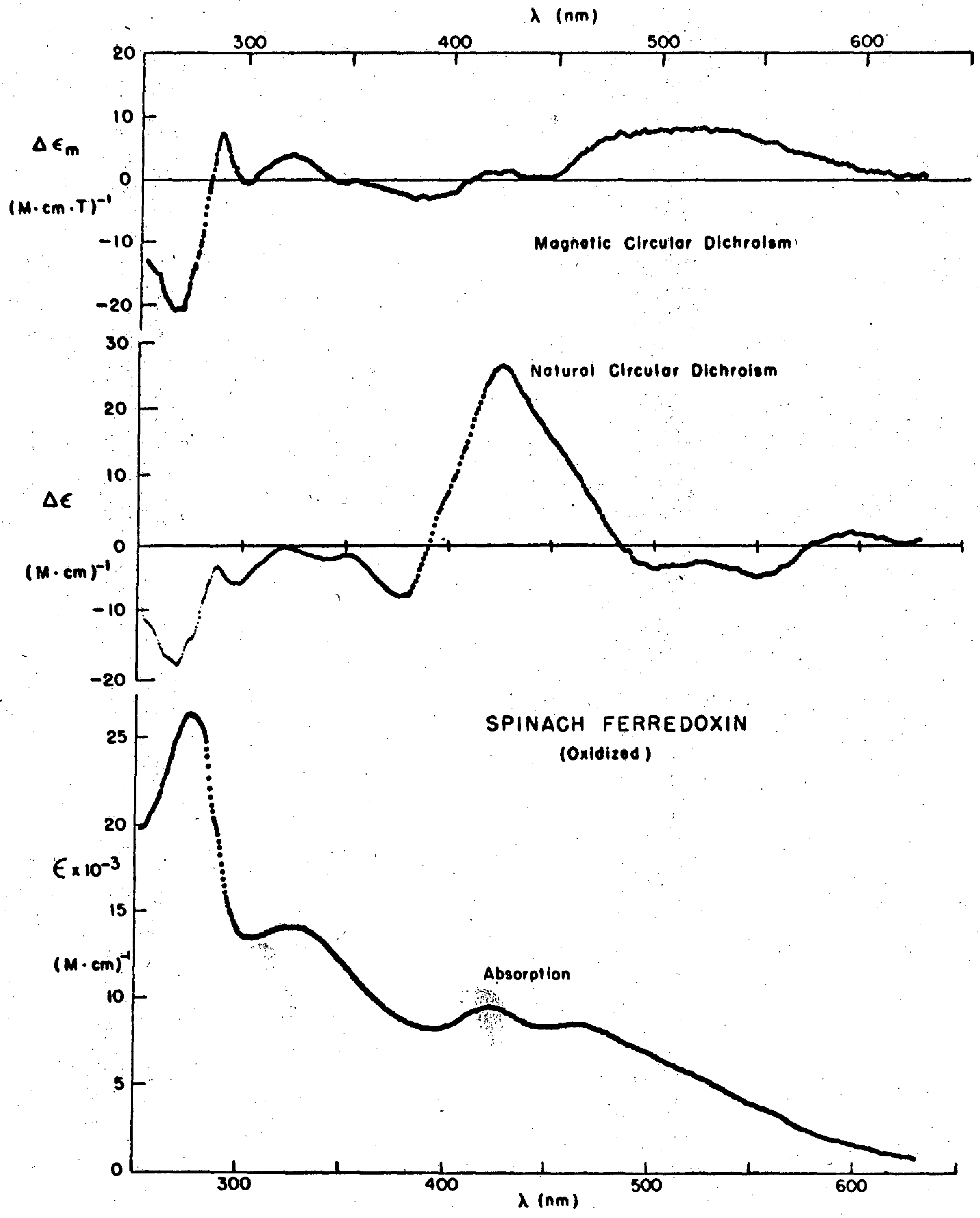


Fig. 2. The absorption, CD and MCD of oxidized spinach ferredoxin. The protein was in .15 M phosphate buffer, pH 7.3, and was autooxidized.

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720