Lawrence Berkeley National Laboratory
Recent Work

Title
PHYSICAL PROPERTIES OF CHLOROPLAST LAMELLAR PROTEINS

Permalink
https://escholarship.org/uc/item/9pm8f2dx

Authors
Biggins, John
Park, Roderic B.

Publication Date
1965-05-01

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

UCRL-16118

University of California

Ernest O. Lawrence Radiation Laboratory

PHYSICAL PROPERTIES OF CHLOROPLAST LAMELLAR PROTEINS

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

Berkeley, California

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.

Submitted to Plant Physiology

UCRL-16118

UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

PHYSICAL PROPERTIES OF CHLOROPLAST LAMELLAR PROTEINS

John Biggins and Roderic B. Park

May 1965

UCRL 16118

Physical Properties of Chloroplast Lamellar Proteins[1,2]

John Biggins[3] and Roderic B. Park

Lawrence Radiation Laboartory

and

Department of Botany and Laboratory of Chemical Biodynamics

University of California, Berkeley, California

The chlorophyll-containing lamellae within higher plant chloroplasts are the site of the light reactions and associated electron transport reactions of the photosynthetic process (12,19). Investigations on the chemical composition of purified lamellae have shown that they are 50% protein and 50% lipid (13). Of the two fractions, the lipid has been studied the most extensively owing to the considerable interest in the photosynthetic pigments, and the majority of the fraction can be accounted for by known compounds (8,11).

---

[1]Received ______________________.

[2]This work was sponsored, in part, by the United States Atomic Energy Commission, Contract No. W-7405 - eng - 48.

[3]Present address: Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania.

The protein fraction contains the two cytochromes f and b_6 and the transition metals iron (non-heme), manganese and copper (13). This fraction has received very little direct attention, presumably owing to the extreme insolubility of the material at physiological pH and the lack of appropriate enzymological tools to cope with the problem.

Davenport and Hill (3) purified cytochrome f from an ammoniacal ethanolic extract of fresh parsley leaves by ammonium sulfate fractionation and calcium phosphate gel adsorption. The pure protein was shown to be MW = 110,000 and contained two hemes. The heme-protein linkage was found to be similar to that of cytochrome-c and the redox potential was estimated as $E_0' = 0.365V$.

Indirect evidence concerning the properties of the protein fraction comes from work primarily designed to investigate the nature of the chlorophyll-protein link in attempts to elucidate the state of chlorophyll *in vivo* (14,15). For this purpose a wide variety of detergents and organic solvents have been used to solubilize the membranes of chloroplasts. It was decided that a similar use of detergents for the removal of lipid from the lamellae and solubilization of the protein would be a fruitful approach to the study of the chloroplast protein fraction. These methods have also been shown to be of great value in the study of the mitochondrion where similar problems of protein insolubility prevail (5).

This report describes the preparation and some physical properties of the protein fraction of chloroplast lamellae in an attempt to derive information pertinent to the molecular architecture of the photosynthetic membrane.

Methods

Preparation of chloroplast lamellar protein fraction: Chloroplasts were prepared from fresh *Spinacia oleracea* and the lamellae were isolated by a procedure according to Park and Pon (13). The final sediment after ultracentrifugation at 145,000 g for 60 minutes was used. This purified preparation contains very little of the chloroplast stroma fraction.

Lamellae equivalent to 500 mg dry weight were solubilized in 20 ml 0.2% sodium dodecyl sulfate (SDS), pH 7.1, and allowed to stand at 4° C for one hour with gentle stirring. During this process a clear solution was obtained. Lipid was most effectively removed from such a detergent-dispersed solution by the slow addition of sufficient butanol at -5° C to give a final volume 20% greater than the original detergent solution. After rapid stirring for about 30 minutes the solution was centrifuged at 1000 g for 15 minutes and the upper organic layer removed by careful aspiration. Complete removal of lipid was achieved by one or more repetitions of this procedure. The aqueous phase was then dialyzed exhaustively against 10^{-2} M Tris chloride, pH 8.0, to remove as much butanol and detergent as possible.

An additional method of extracting lipid from the detergent-treated lamellae was by -10° C acetone precipitation. The protein precipitated at 80 to 85% acetone and was collected by centrifugation. It was shown by spectrophotometry to be free of the photosynthetic pigments after several washes with cold -10° C acetone. After removal of the acetone from the precipitate by evacuation, it was possible to dissolve about 75% of the protein (as determined by Kjeldahl analysis) in 0.05% SDS,

0.05 M Tris-Cl, pH 8.0, by extraction of the residue overnight. During this procedure the washing and removal of acetone was conducted as rapidly as possible and at -10° C to minimize protein denaturation.

The protein was concentrated by ammonium sulfate precipitation or lyophilization after dialysis against 10^{-4} M Tris-Cl, pH 8.0. The protein was yellow-brown in color and was stored at 4° C.

Spectrophotometry. A Cary Model 14R automatic recording spectrophotometer with a Scatter Transmission Attachment was used. Oxidation and reduction difference spectra were obtained using the 0 to 0.2 optical density slidewire on the spectrophotometer. In some cases, split compartment cuvettes obtained from Pyrocell Mfg. Co., New York, N. Y., were used (22). The use of these cuvettes permitted accurate, unambiguous measurement of the Soret region of the cytochromes in the protein fraction as the concentration of oxidant (ferricyanide) or reductant (dithionite) in both analyzing beams was identical.

Sedimentation Coefficient. A Spinco Model E analytical ultracentrifuge equipped with schlieren optics and phase plate was used in the sedimentation experiments. An AN-D rotor and standard single 4° sector cell with an aluminum centerpiece was used. The protein solution in 0.02% SDS was exhaustively dialyzed against 10^{-2} M Tris-Cl, pH 8.0, and then centrifuged at 110,000 g for 30 minutes in a Spinco Model L preparative ultracentrifuge. The supernatant was then dialyzed to equilibrium against 0.002% SDS, 5×10^{-3} M Tris-Cl, pH 8.0, and 0.1 M NaCl. The dialysate was subsequently used for dilution of the protein solution in order to produce varying concentrations of protein. Sedimentation velocity studies were carried out at 59,480 rpm at 20° C.

Sedimentation coefficients were determined at six concentrations spanning a tenfold range of dilution.

Diffusion Coefficient. The apparent diffusion coefficient of the protein fraction (6 mg protein/ml) was determined in the analytical ultracentrifuge by a procedure according to Ehrenberg (4). A capillary-type, double-sector, synthetic boundary cell (Spinco No. 306075, Epon-filled) was used. After careful layering of buffer (0.1 M NaCl, 0.002% SDS, 0.05 M Tris-Cl, pH 8.0) at low speed, the centrifuge was adjusted to 10,580 rpm and the experiment was concluded after one hour.

Results

Figure 1 shows the absorption spectrum (230-400 mμ) and a difference spectrum (400-600 mμ) of the lamellar protein fraction in 2 x 10^{-3}% SDS, 0.5 M Tris-Cl, pH 8.0. There is a large absorption band at 279 mμ and a shoulder at 290 mμ. The difference spectrum in the visible region shows the α, β and Soret absorption bands of ferrocytochromes f and b_6. The density of the absorption band in the ultraviolet is seven times greater than that of the combined Soret bands, indicating that the greater proportion of the protein fraction is not hemoprotein.

Figure 2 is a difference spectrum, untreated minus oxidized lamellar protein. This shows the absorption spectrum of ferrocytochrome f. The bands are α = 530 mμ, β' = 530 mμ, β = 524 mμ, and γ = 421 mμ. These maxima are in very good agreement with those reported by Hill and Bonner (6) for 80% acetone-extracted material. However, in this work, the β band is split whereas Hill and Bonner only found splitting at low temperature. The absorption ratios α/β and γ/α are also in good agreement with the data on highly purified ferrocytochrome f (3).

Figure 3 is a difference spectrum of reduced lamellar protein minus untreated protein and shows the absorption spectrum of ferrocytochrome b_6. The absorption maxima are α = 561 mμ, β = 530 mμ and γ = 431 mμ. This result is in agreement with the values given by Hill and Bonner (6) for 80% acetone-extracted lamellae although it appears that in the isolated protein the bands are shifted slightly to shorter wavelengths.

Figures 4 and 5 are difference spectra of the region of the α and β bands of an 80% acetone powder of chloroplast lamellae and of lipid-free lamellae solubilized in cholate (2 mg/mg). The absorption band maxima are in excellent agreement with those reported by Hill and Bonner (6).

The cytochrome content of the chloroplast lamellar protein was determined from these spectrophotometric measurements and knowledge of the weight of protein in solution. Using the α-band extinction coefficients of 2.5×10^4 for cytochrome f and 2.0×10^4 for cytochrome b_6, the cytochrome b_6/f ratio is then 0.92 and 1.1×10^6 g of the protein would contain molar quantities of the two cytochromes. Hence, there is one of each cytochrome per 230 chlorophylls or one quantasome (11).

These values for the cytochrome concentration are higher than the concentration measured by Davenport and Hill (3) for cytochrome f in parsley and elder, and Lundegårdh (9) for spinach. However, the agreement with Sironval and Engelert-Dujardin (18) and Hill and Bonner (6) is good. Table I summarizes the data in this study and the literature values for comparison. The variation in values is quite high, and it is suggested that differences in the physiological condition of the starting materials could contribute to such discrepancies.

Our results show that the lamellar protein fraction contains the two cytochromes f and b_6 in about equimolar amounts. The absorption spectra of the ferrocytochromes are very similar to those described previously for lamellae extracted with acetone. It also appears that very low concentrations of SDS and proportionately high concentrations of sodium cholate do not radically modify the absorption spectra.

The protein fraction was studied by analytical ultracentrifucation to ascertain the extent of the heterogeneity and to gain information concerning the molecular size and properties of component species. Figure 6 shows a schlieren photograph of the protein fraction during a sedimentation velocity determination. The material appears as a single broad boundary and the color of the two cytochromes is clearly associated with the boundary. Furthermore, the color remained associated with the boundary throughout the sedimentation indicating that the cytochromes are of very similar sedimentation coefficient to that of the major component in the boundary.

Figure 7 shows the dependence of sedimentation coefficient of the lamellar protein on concentration. At high concentrations there appears to be a linear dependence, the higher the concentration, the lower the coefficient. However, at lower concentrations, the reverse behavior is apparent. Schwert (17) noted this type of anomaly for α-chymotrypsin and suggested that the system was undergoing a rapid association-dissociation equilibrium. It is likely that this interpretation could apply here for the lamellar protein system, as the tendency for aggregation is very high. It is suggested that these physical interactions between the protein species are instrumental in maintaining the structure of the membrane _in vivo_.

By extrapolation of the linear portion of the sedimentation data to infinite dilution, the sedimentation coefficient of the apparent "dimer" is 4.5×10^{-13}s. Extrapolation of the lower concentration points to infinite dilution permits an estimate of the sedimentation coefficient of the "monomeric" form as 2.3×10^{-13}s. Owing to this anomalous behavior of the protein fraction, both these extrapolations are hazardous and the results are considered with caution.

The apparent diffusion coefficient of the protein fraction was determined in the ultracentrifuge by a layering experiment. The data were evaluated by the maximum ordinate method (4), and the diffusion coefficient was determined as 9.1×10^{-7} cm^2 sec^{-1}. It was noted that during the experiment the area under the gradient curve remained constant indicating that no large aggregates formed and sedimented from the boundary.

Discussion

From the measured physical constants of the chloroplast lamellar protein fraction bear a striking similarity to the structural protein of mitochondria described by Criddle, *et al.* (1). However, they found that although the aggregation tendency is high, as evidenced by its insolubility at physiological pH, the sedimentation coefficient of the protein is independent of concentration. This is in contrast to the result found here, and a possible explanation of this discrepancy is that the two studies were conducted in different concentrations of detergent. Criddle, *et al.* (1) used a hundredfold higher concentration of SDS, and it is likely that this high concentration sufficiently eliminated interaction of the protein even at high protein concentrations. Low detergent concentrations were used in this study to avoid

possible ambiguities arising from operating above critical micellar concentrations.

As only one boundary was detected in the sedimentation velocity studies, it can be concluded that all the molecular species in the lamellar protein fraction, including the two cytochromes, have similar sedimentation coefficients. Assuming that other hydrodynamic parameters are also similar, then the proteins are of approximately the same molecular weight--i.e., about 22,000. Assuming the proteins to be spherical, their diameters are about 40 Å. If the molecular weight of a quantasome is 2×10^6 and is 50% protein (11), then there are about 40 of the lamellar proteins per quantasome, two of them being cytochromes f and b_6.

There have been other reports of preparations of proteins from the lamellae of chloroplasts. Criddle and Park (2) prepared a protein from an acetone powder of lamellae by solubilization with bile salts followed by salt fractionation. The protein is heme-free, the sedimentation coefficient is 2.2 S and MW = 23,000. By analogy to the protein isolated from mitochondria (1) it was termed "structural protein".

Weber (21) and Thornber, et al. (20) solubilized lamellae in acid and found a 6 S component. The 6 S component was resolved into six protein species by polyacrylamide gel electrophoresis, but neither relative concentrations of the components nor detailed behavior of the protein in the ultracentrifuge were given (20).

Pertinent information concerning lamellar sub-structure comes from observations on the effect of detergents on the chloroplasts and the

formation of pigment-protein complexes. Treatment of chloroplast lamellae with digitonin or bile salts (2-3%) leads to the formation of a particle with a sedimentation coefficient of 13.8 S and a molecular weight of about 265,000 (16). Itoh, et al. (7) showed that treatment of grana with 3×10^{-3} M dodecylbenzene sulfonate formed a pigment-protein with a sedimentation coefficient of 2.9 S, and higher concentrations led to the formation of 1.2 S component. This effect is very similar to that induced by dodecyl sulfate where a 2.5 S component is formed with 0.25% detergent, and higher concentrations yield a 1.69 S component (16). Furthermore, in both cases the pigment remains bound to the protein in the presence of these anionic detergents, whereas bile salts and digitonin extract the pigments (15).

Thus, it appears from this study of the properties of the protein fraction of chloroplast lamellae, other reports in the literature and the effect of detergents on lamellae, that the principal protein in the membrane in the chloroplast is in the order of 22,000 molecular weight and 40 Å in diameter. That this is a fundamental repeating sub-unit is also suggested by the work of Kreutz and Menke (10). They studied the low angle X-ray scattering of chloroplast lamellae and found a 36 Å to 40 Å periodicity in the plane of the lamellae.

It also appears that various complexes of the lamellar protein can be isolated. Unfortunately, there are no analytical data on the 13.8 S complexes formed by treatment of the lamellae with digitonin or bile salts, but they represent one-fourth of the quantasome protein. Park and Biggins (11) showed electron micrographs of shadowed lamellar sonicates which clearly indicated that the quantasome could be comprised

of four sub-units. It is possible that these may represent specific *in vivo* functional protein complexes of which the cytochromes and the, as yet unknown, enzymic apparatus responsible for oxygen evolution are a part.

Summary

Treatment of purified chloroplast lamellae with dodecylsulfate and butanol effectively strips the protein free of lipid. The lipid-free protein fraction was studied by spectrophotometry and analytical ultracentrifugation.

The protein fraction was found to consist of a large quantity of heme-free protein and the two photosynthetic cytochromes f and b_6. The absorption maxima of the α and β bands of the ferrocytochromes do not appear to be significantly modified by low concentrations of detergent.

In the analytical ultracentrifuge, the protein fraction appeared as a single broad boundary exhibiting an association-dissociation equilibirum. The mean sedimentation coefficient at infinite dilution was found to be 2.3×10^{-13}s and the diffusion coefficient 9.1×10^{-7} cm^2 sec^{-1}. Hence, the average molecular weight of the proteins is about 22,000 g·mole^{-1}. This value can only be considered approximate, owing to the uncertain extrapolation of the sedimentation velocity data and to the assumed partial specific volume of the proteins. Assuming the proteins to be spherical, the diameter of the proteins is about 40 Å. It is suggested that these units comprise a fundamental repeating structural unit in the lamellae.

Acknowledgments

The authors wish to express their indebtedness to Dr. D. P. Hackett for his interest and helpful advice throughout the course of the investigation.

Literature Cited

1. Criddle, R. S., R. M. Bock, D. E. Green, and H. Tisdale. 1962. Physical characteristics of proteins of the electron transfer system and interpretation of the structure of the mitochondrion. Biochemistry 1: 827-842.

2. Criddle, R. S., and L. Park. 1964. Isolation and properties of a structural protein from chloroplasts. Biochem. Biophys. Res. Commun. 17: 74-79.

3. Davenport, H. E., and R. Hill. 1952. The preparation and some properties of cytochrom f. Proc. Roy. Soc. London B 139: 327-345.

4. Ehrenberg, A. 1957. Determination of molecular weights and diffusion coefficients in the ultracentrifuge. Acta. Chem. Scand. 11: 1257-1270.

5. Green, D. 1962. Structure and function of subcellular particles. Comp. Bioch. Physiol. 4: 81-122.

6. Hill, R., and W. D. Bonner, Jr. 1961. The nature and possible function of chloroplast cytochromes, in "Light and Life", 424-435. W. D. McElroy and B. Glass, ed. Johns Hopkins Press, Baltimore.

7. Itoh, M., S. Izawa, and K. Shibata. 1963. Disintegration of chloroplasts with dodecylbenzene sulfonate as measured by flattening effect and size distribution. Biochim. Biophys. Acta 69: 130-142.

8. Lichtenthaler, H. K., and R. B. Park. 1963. Chemical composition of chloroplast lamellae from spinach. Nature 198: 1070-1072.

9. Lundegårdh, H. 1962. Quantitative relations between chlorophyll and cytochromes in chloroplasts. Physiol. Plantarum 15: 390-398.

10. Kreutz, W., and W. Menke. 1962. Strukturuntersuchungen an plastiden, III. Röntgenographische untersuchungen an isolierten chloroplasten und chloroplasten lekender zellen. Z. Naturforsch. 17b: 675-683.

11. Park, R. B., and J. Biggins. 1964. Quantasome: Size and composition. Science 144: 1009-1011.

12. Park, R. B., and N. G. Pon. 1961. Correlation of structure with function in Spinacea oleracea chloroplasts. J. Mol. Biol. 3: 1-10.

13. Park, R. B., and N. G. Pon. 1963. Chemical composition and the substructure of lamellae isolated from Spinacea oleracea chloroplasts. J. Mol. Biol. 6: 105-114.

14. Smith, E. L. 1940. Chlorophyll as the prosthetic group of a protein in the green leaf. Science 91: 199-200.

15. Smith, E. L. 1941. The chlorophyll-protein compound of the green leaf. J. Gen. Physiol. 24: 565-582.

16. Smith, E. L., and E. G. Pickles. 1941. The effect of detergents on the chlorophyll-protein compound as studied in the ultracentrifuge. J. Gen. Physiol. 24: 753-764.

17. Schwert, G. W. 1949. The molecular size and shape of the pancreatic proteases. I. Sedimentation studies on chymotrypsinogen and on α- and γ-chymotrypsin. J. Biol. Chem. 179: 655-664.

18. Sironval, C., and E. Engelert-Dujardin. 1963. Sur les cytochromes des chloroplastes: Variations de leur teneur en rapport avec l'âge foliaire et le contenu en chlorophylle. Physiol. Vég. 1: 191-201.

19. Trebst, A. V., H. Y. Tsujimoto, and D. I. Arnon. 1958. Separation of light and dark phases in the photosynthesis of isolated chloroplasts. Nature 182: 351-355.

20. Thornber, J. P., J. L. Bailey, and S. M. Ridley. 1964. The relationship of Fraction I protein to the proteins of the lamellae of spinach chloroplasts. Biochem. J. 92: 18P.

21. Weber, P. 1963. Über gelöstes lamellares strukturproteid aus chloroplasten von Antirrhinum majus. V. Mitteilung über lamellares strukturproteid. Z. Naturforsch. 18b: 1105-1110.

22. Yankeelov, J. A. 1963. Split-compartment mixing cells for difference spectroscopy. Anal. Biochem. 6: 287-289.

Table I. Cytochrome content of chloroplasts--this study and literature values

Plant	cytochrome ratio b_6/f	molar ratio $\frac{\text{chlorophyll}}{\text{cytochromes f and } b_6}$	Author
parsley	--	380 (f only)	Davenport and Hill (3)
elder	--	430 (f only)	
spinach	1	200 to 300	Sironval and Engelert-Dujardin (18)
spinach	--	400	Lundegårdh (9)
spinach	1.3	--	Hill and Bonner (6)
spinach	0.92	about 230*	this work

*calculated on the basis of the composition of a quantasome of 50% protein and containing 230 chlorophyll molecules (11).

Legends for figures

Fig. 1. Absorption spectrum of the chloroplast lamellar protein fraction. The spectrum in the visible region (400 - 600 mμ) is a difference spectrum, reduced minus oxidized.

Fig. 2. Chloroplast lamellar protein fraction. Untreated (ferricyanide) minus oxidized. This shows the absorption spectrum of cytochrome f.

Fig. 3. Chloroplast lamellar protein fraction. Reduced (dithionite) minus untreated. This shows the absorption spectrum of cytochrome b_6.

Fig. 4. Difference spectra of an 80% acetone powder of pure chloroplast lamellae. Powder suspended in 10^{-1} M Tris-Cl, pH 8.0. A, base line. B, dithionite - reduced minus untreated. C, untreated minus ferricyanide - oxidized. D, dithionite - reduced minus ferricyanide - oxidized.

Fig. 5. Difference spectra of lipid - free chloroplast lamellae solubilized in cholate (2 mg/mg protein). A, untreated minus ferricyanide - oxidized. B, dithionite - reduced minus untreated. C, dithionite - reduced minus ferricyanide - oxidized.

Fig. 6. Schlieren photograph of the chloroplast lamellar protein fraction during a sedimentation velocity determination. The color due to the cytochromes is clearly associated with the boundary. Protein 7 mg/ml in 0.1 M NaCl, 0.002% Na-dodecyl sulfate, 5×10^{-2} M Tris, pH 8. Rotor speed 59,780 rpm, 4° C. Phase plate 60°. Photograph taken 12 minutes after reaching speed.

Fig. 7. Dependence of sedimentation coefficient on concentration of the lamellar protein fraction. All experiments conducted in 0.1 M NaCl, 0.002% Na-dodecyl sulfate and 5×10^{-2} M Tris, pH 8.

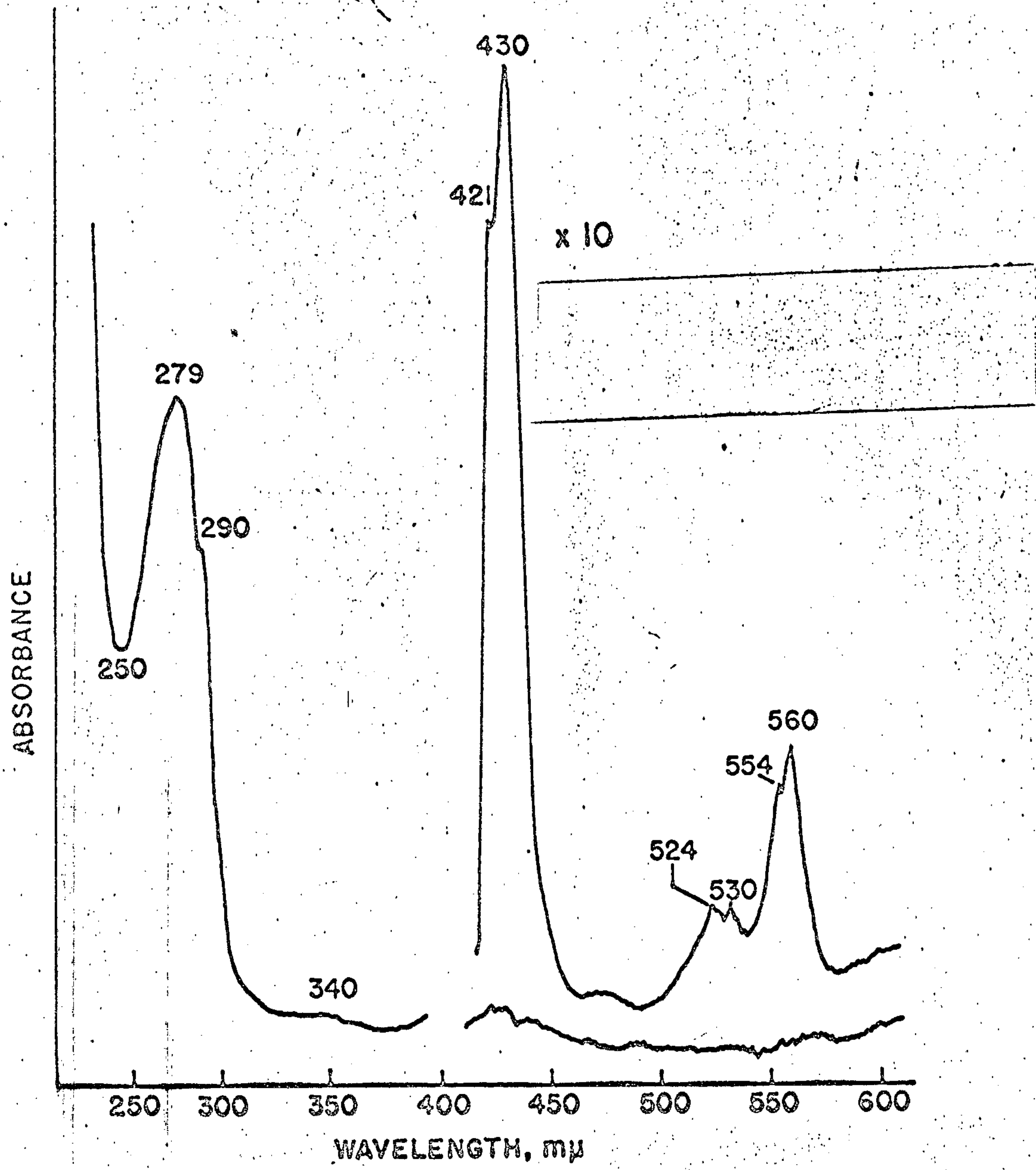


Fig. 1

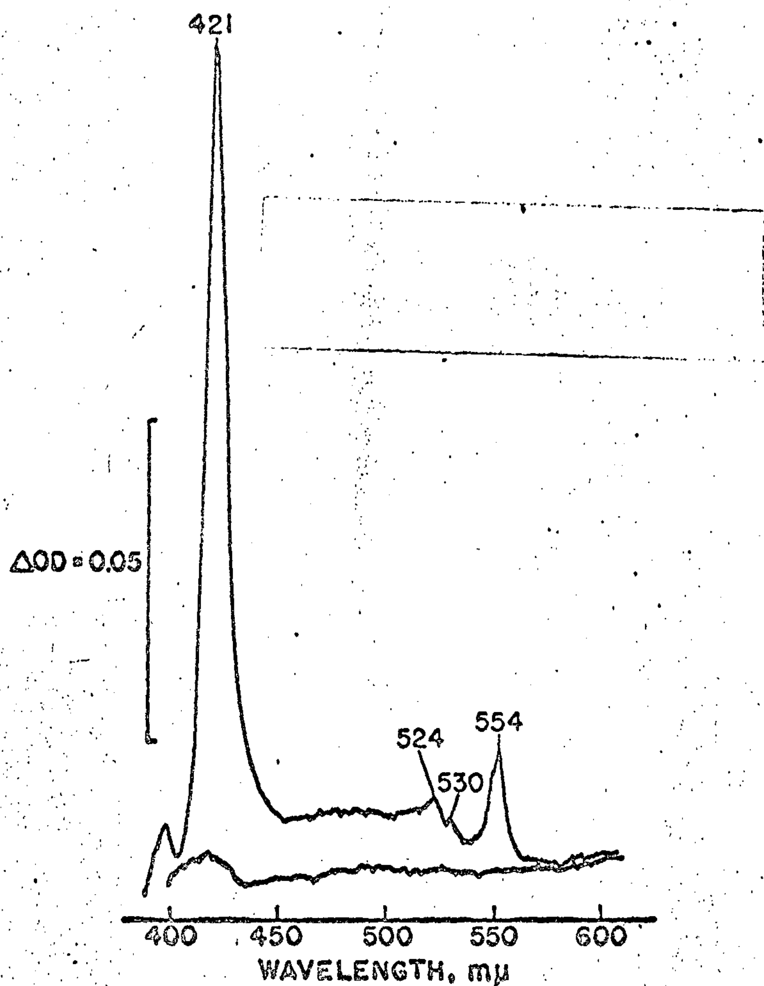


Fig 2

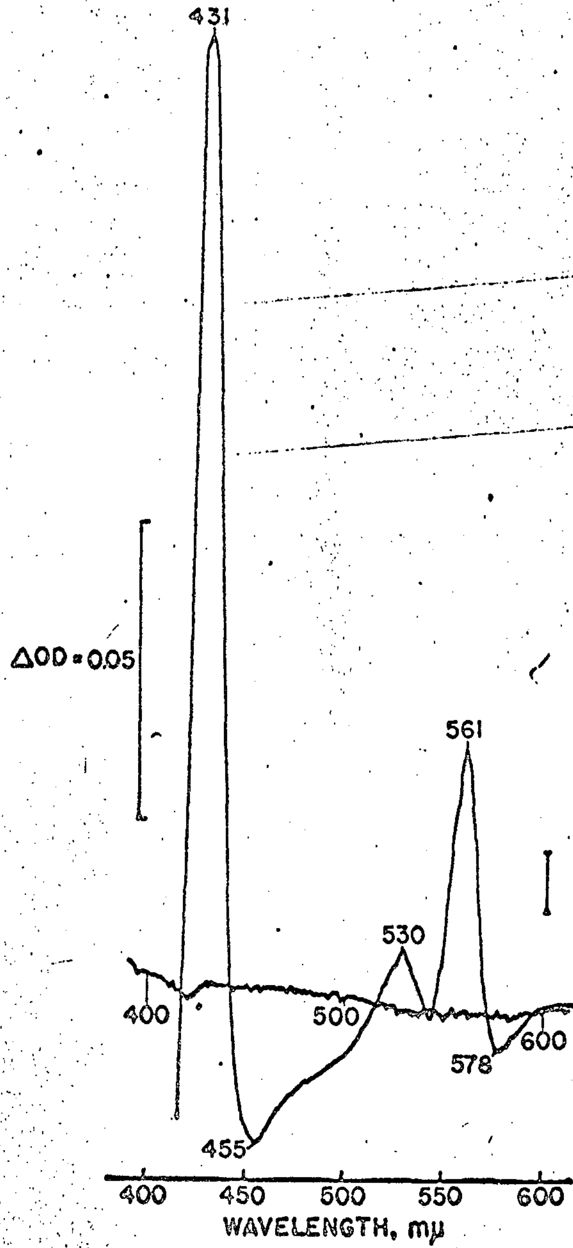


Fig 3

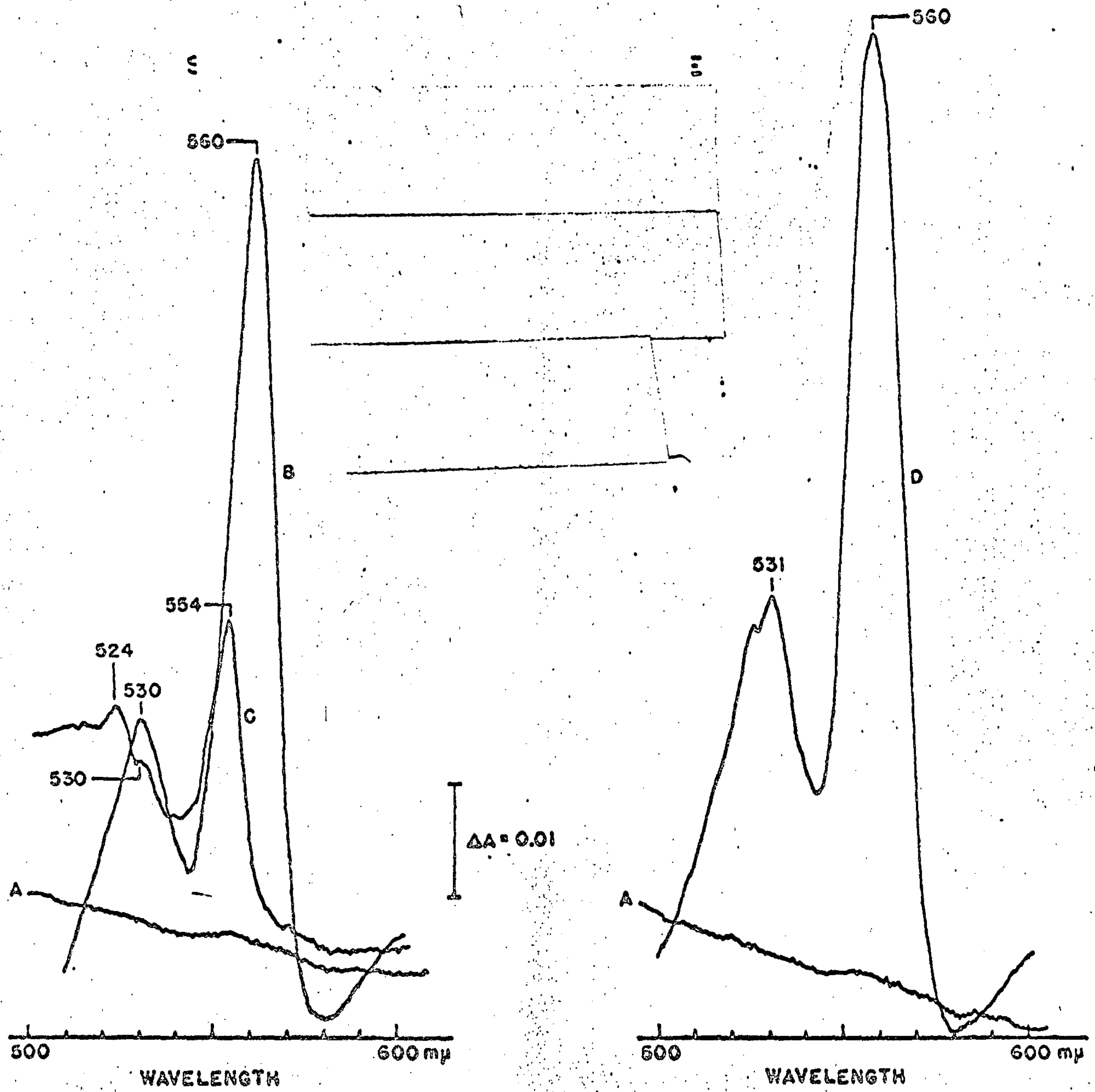


Fig 4

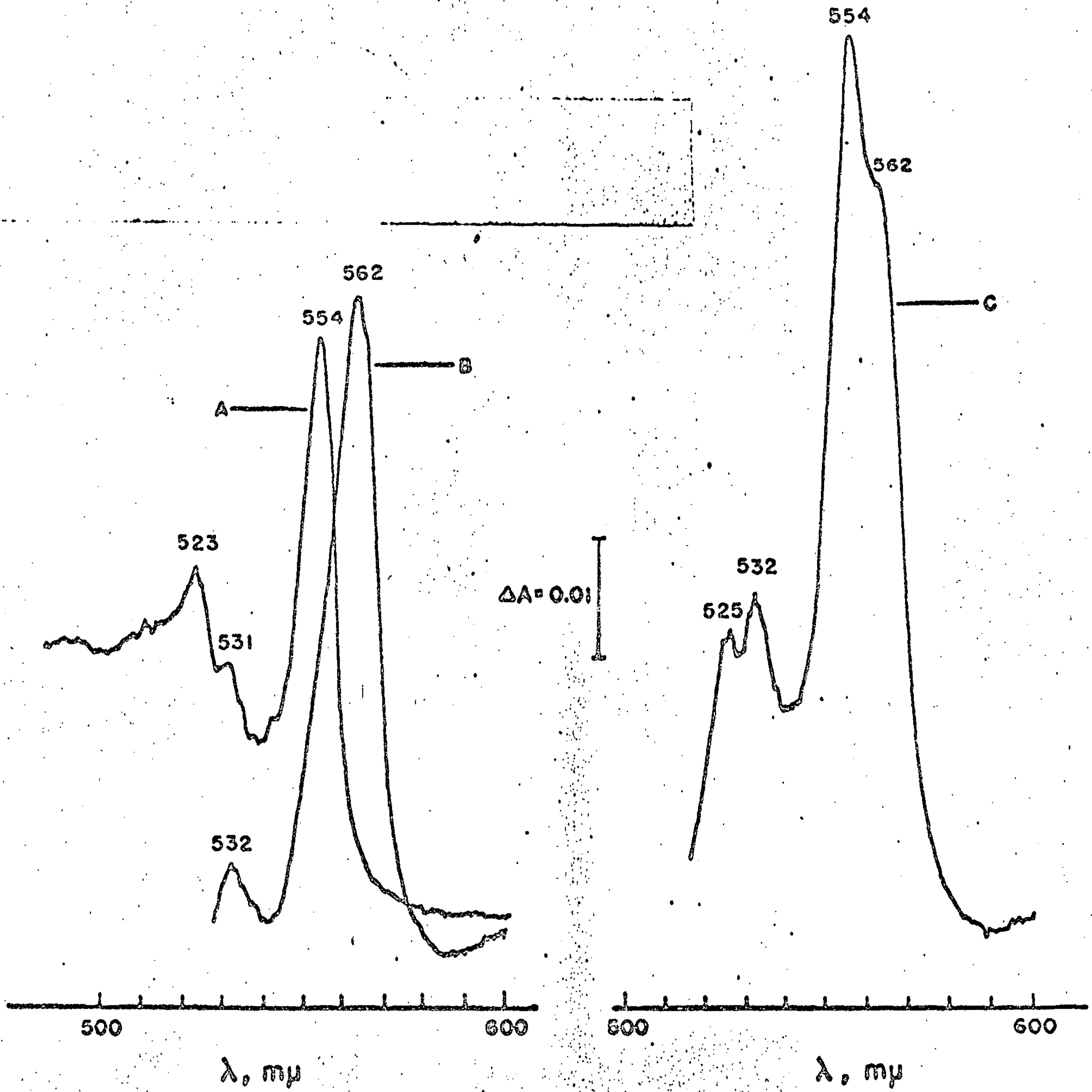
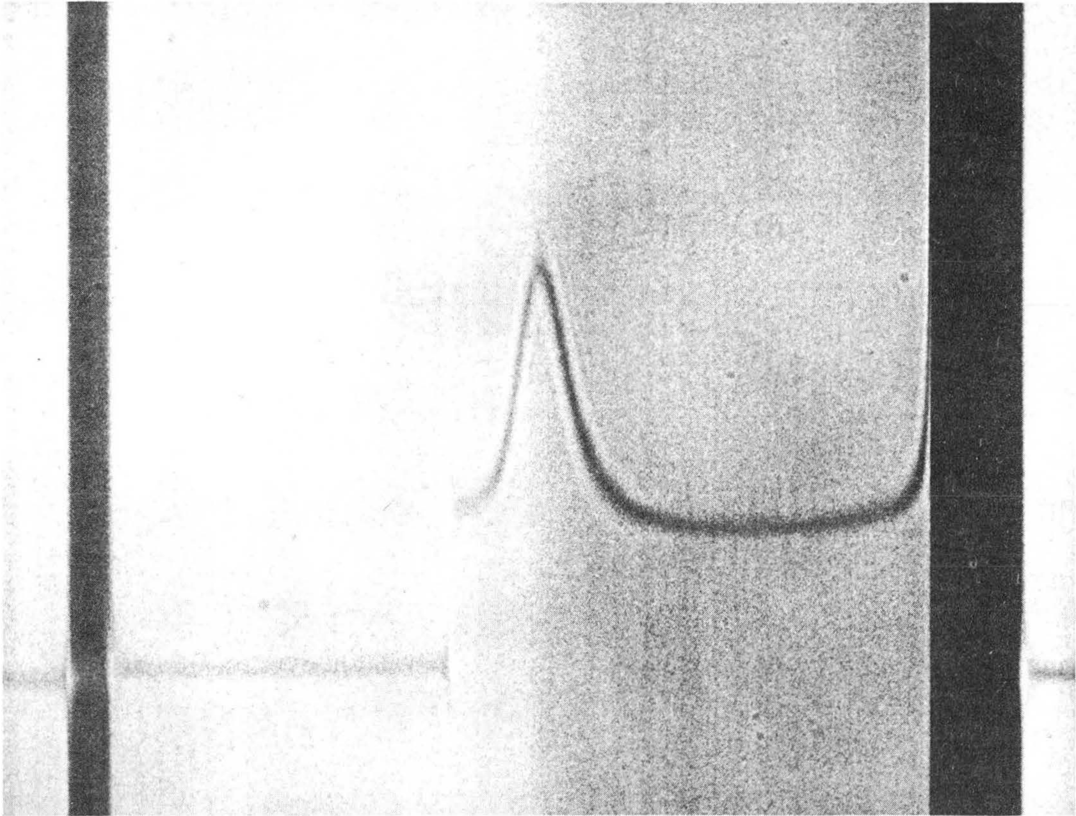


Fig 5



ZN-4609

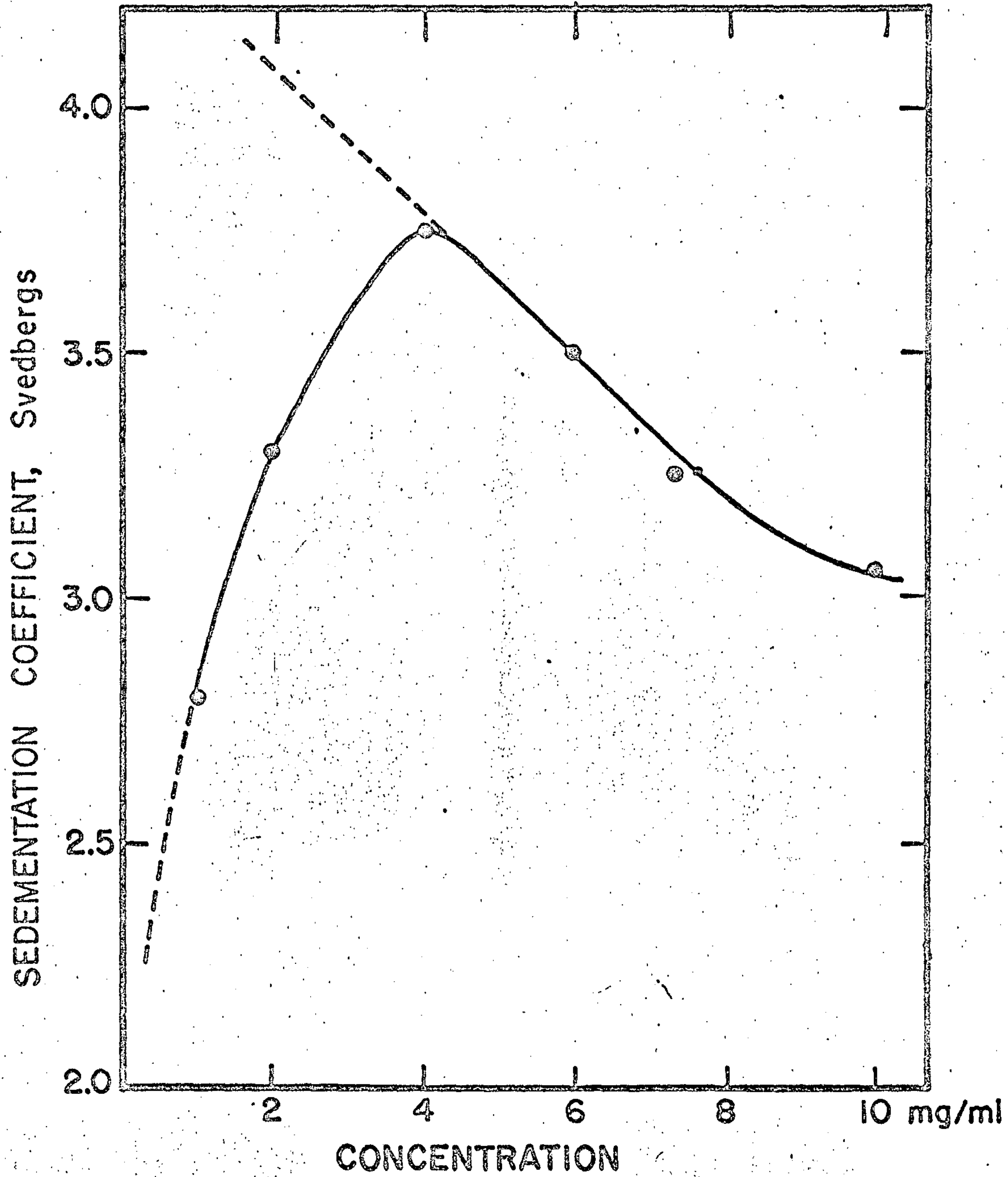


Fig 7

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.