https://ntrs.nasa.gov/search.jsp?R=19660002909 2020-03-16T22:16:49+00:00Z

ELECTRON MICROSCOPIC AND BIOCHEMICAL CHARACTERIZATION OF FRACTION I PROTEIN

R. Haselkorn
H. Fernandez-Moran
F. J. Kieras
E. F. J. van Bruggen

Department of Biophysics
The University of Chicago

N66-12198
(ACCESSION NUMBER) (THRU)
17 1
(PAGES) (CODE)
CR 68099 04
(NASA CR OR TMX OR AD NUMBER) (CATEGORY)
FACILITY FORM 602

GPO PRICE $ ________
CFSTI PRICE(S) $ ________
Hard copy (HC) 1.00
Microfiche (MF) .50
ff 653 July 65

Electron Microscopic and Biochemical
Characterization of Fraction I Protein

12148

ABSTRACT. High resolution electron micrographs of Fraction I protein from Chinese cabbage leaves have been obtained. The protein, which has ribulose 1, 5-diphosphate carboxylase activity, appears to be a cube with edge of about 120 Å. Substructure can be seen in individual particles, consistent with a model having 24 subunits, the number prescribed by the available physical and chemical data.

When crude extracts of tobacco leaves were examined in the analytical ultracentrifuge over a decade ago, a major soluble protein fraction, termed the Fraction I protein, was found to have a sedimentation coefficient of about 18S (1). Subsequent work showed that the Fraction I protein is located predominantly, if not exclusively, in the chloroplast (2). There, it comprises by weight at least 50 percent of the soluble proteins (2,3). Fraction I protein has been reported to have the following biological activities: (i) protochlorophyll holochrome: the protein to which protochlorophyll is attached, and which catalyzes the light-induced conversion of protochlorophyll to chlorophyll (3,4); (ii) ribose-1-phosphate isomerase (5); (iii) ribulose-1-phosphate kinase (5); and (iv) ribulose 1,5-diphosphate carboxylase (carboxydismutase) (6). Trown has recently shown that the activities under (ii) and (iii) are readily separated from Fraction I protein by gel filtration on Sephadex G-200, whereas carboxydismutase activity (iv) is superimposable on the elution profile of Fraction I protein (7). He concluded that Fraction I protein and carboxydismutase were one and the same.

Trown found the molecular weight of carboxydismutase to be 515,000 by sedimentation equilibrium (7). He, as well as Markham and coworkers, and Wildman and coworkers, found the 18S species prone to form linear aggregates, of which the dimer and trimer have sedimentation coefficients of 25S and 32S respectively (7,8,1). The molecular weight, sedimentation coefficient, and aggregation characteristics of carboxydismutase are very similar to those of the *E. coli* RNA polymerase (9).

A molecular weight of 515,000 is very high for a single polypeptide chain. Simple general considerations of the type that led Watson and Crick to the subunit model for the construction of virus protein shells suggest that carboxydismutase must be made up of subunits (10). If this is so, it becomes germane to inquire whether the rules governing the interaction of subunits are the same as those that apply in the case of small viruses (10). This is a realistic question in the case of carboxydismutase, since large amounts of purified material are readily available.

We therefore undertook a combined electron microscopic and biochemical investigation of Fraction I protein, with the following specific aims:

- (i) Is carboxydismutase composed of subunits?
- (ii) If so, what rules govern their interaction?
- (iii) Do morphological and/or functional relationships exist between carboxydismutase and RNA polymerase?
- (iv) Is Fraction I protein carboxydismutase, and only carboxydismutase?

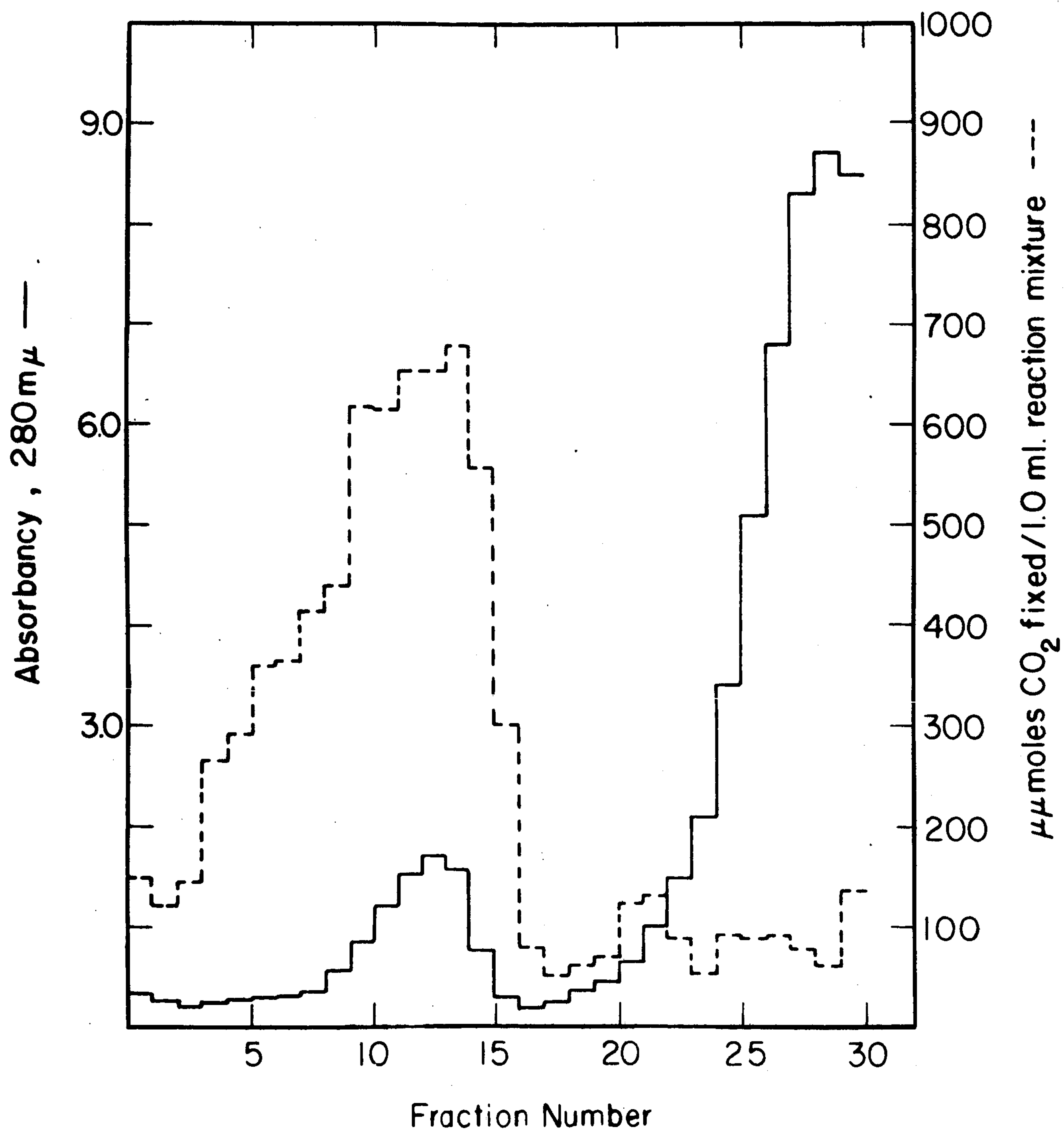
In this communication we provide an affirmative answer to (i), a tentative answer to (ii), a negative answer to (iii), and no answer to (iv).

Fresh, re-ribbed Chinese cabbage leaves were ground in the cold and filtered through several layers of cheese cloth. This extract was then centrifuged for ten minutes at 12,000 rpm in a Servall centrifuge, and the supernatant made 50 percent saturated with ammonium sulfate. The precipitate was collected by centrifugation, dissolved in a small volume of 0.01 M tris, pH 7.5, and dialysed in the cold against the same buffer. One ml of the dialysed preparation was then subjected to zone centrifugation on a glycerol gradient. Carboxydismutase activity is seen (Figure 1) to be associated solely with the peak of ultra-violet absorbing material at 18S. This result is consistent with two interpretations. The one favored by Trown is that the 18S protein is in fact carboxydismutase. Alternatively, it might be argued that carboxydismutase is a small molecule active only when associated with the 18S protein.

Each Fraction in Figure 1 was assayed for RNA polymerase activity using calf thymus DNA as template under conditions found to be optimal for the E. coli enzyme. (9). No significant activity was found.

In some preparations, material having an absorption spectrum characteristic of reduced pyridine nucleotide was observed specifically associated with the 18S peak. These fractions were tested for triose phosphate dehydrogenase activity, but none was found. The significance of the bound pyridine nucleotide remains unknown.

Material from the peak of absorbancy in a gradient like that of Figure 1 was then examined in the electron microscope.



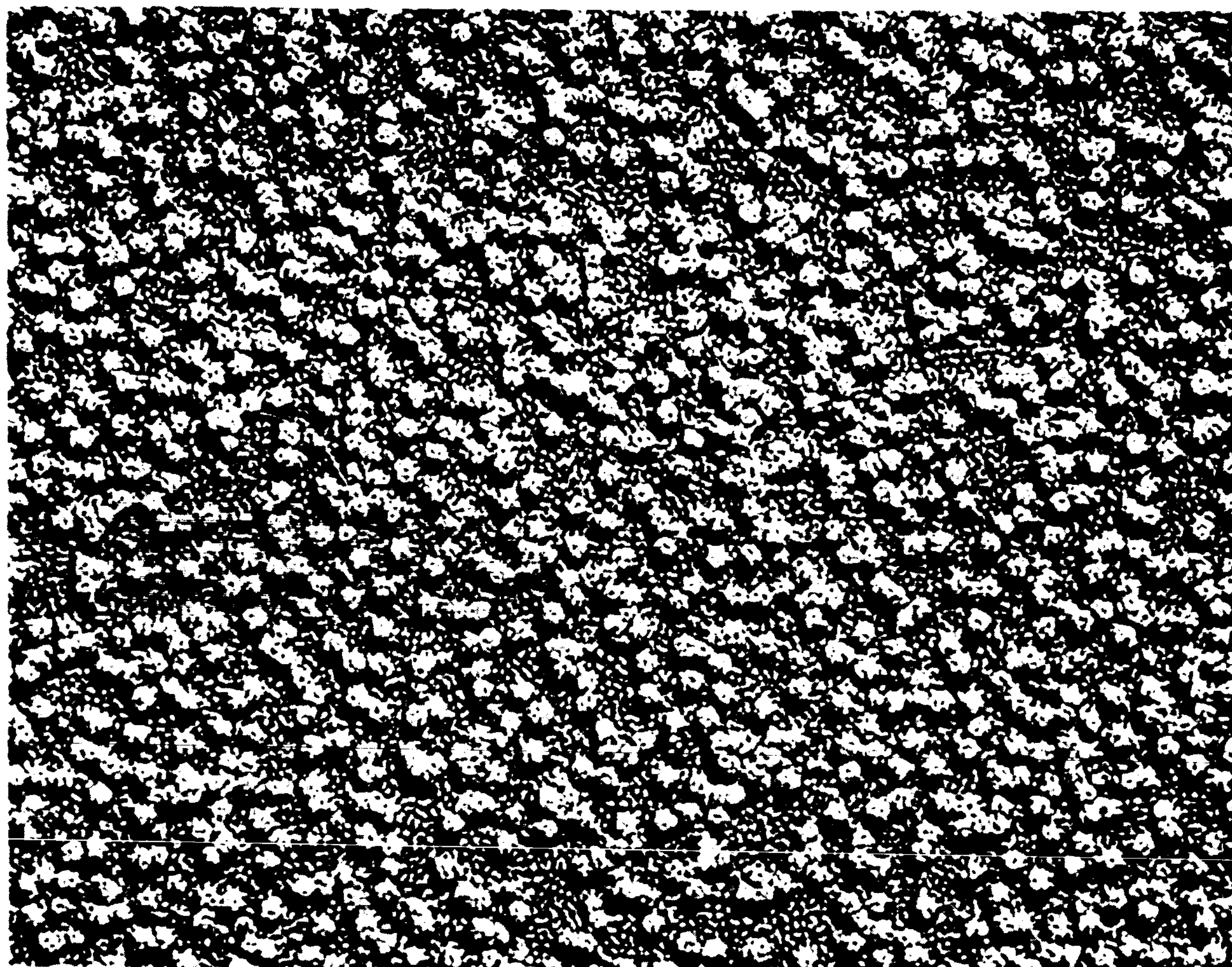
Absorbancy , 280 mμ —
9.0
6.0
3.0
μμmoles CO_2 fixed/1.0 ml. reaction mixture ---
1000
900
800
700
600
500
400
300
200
100
5
10
15
20
25
30
Fraction Number

The specimens were prepared for electron microscopy using shadow casting and negative staining techniques.

The specimens were examined in the native state shortly after preparation. The final concentration of the specimens was generally 0.1 mg. of protein per ml. in 0.01 tris or TMA (10^{-2} M tris, 10^{-2} M magnesium acetate and 2.2 x 10^{-2} M ammonium chloride) at pH 7.15. Ammonium acetate buffer was used for the shadowing experiments. The specimens were mounted on extremely thin carbon films (10 Å to 50 Å thick), prepared by evaporation on freshly cleaved mica in a Varian Vac-ion pump 921 unit at a vacuum of 10^{-8} to 10^{-9} mm Hg. These ultrathin, practically structureless carbon films are supported on special fenestrated substrates (11).

The microdroplet cross-spraying technique (11,12) and a droplet technique (13) were used for negative staining (Figure 3). By means of a special multiple-spraying device microdroplets of the specimen and of 1 to 2 percent potassium phosphotungstate at pH 7.2 to 7.4 collide, and interact very rapidly shortly before impinging on the specimen grid. Positive staining with 1 or 2 percent uranyl acetate was also obtained with this technique.

A droplet of the specimen diluted with tris or TMA buffer was placed on the carbon coated fenestrated films. After 1 to 2 minutes excess enxyme solution was removed by floating the grid upside down on TMA buffer for about 15 seconds. Then the specimen was stained by floating on 0.5 percent to 1 percent neutralized potassium phosphotungstate (PTA) or on 1 percent to 2 percent unbuffered uranyl acetate (UA). After 1 to 2 minutes the specimen was dried by blotting the edge of the grid on filter paper.



2

The electron microscopic studies were carried out in a facility designed to provide optimum conditions for consistent attainment of high resolution. Electron micrographs were recorded with Siemens Elmiskops I and Ia, at electron optical magnifications of 40,000 and 46,000 at 80 kV. These microscopes were operated with a highly regulated power supply, and used pointed filaments of single-crystal tungsten (14,15) to provide microbeam illumination of high coherence yielding enhanced contrast. For critical high resolution analysis through-focus series were taken in steps of 80 Å to 200 Å, and the measured astigmatism of the objective lens was 0.05 μ or less. A liquid nitrogen anti-contamination device was used while recording most of the micrographs. Calibrations were carried out using a diffraction grating replica of 54,864 lines per inch, and checked by using combined electron microscopy and electron diffraction of selected planes from copper phthalocyanine and chrysotile crystals.

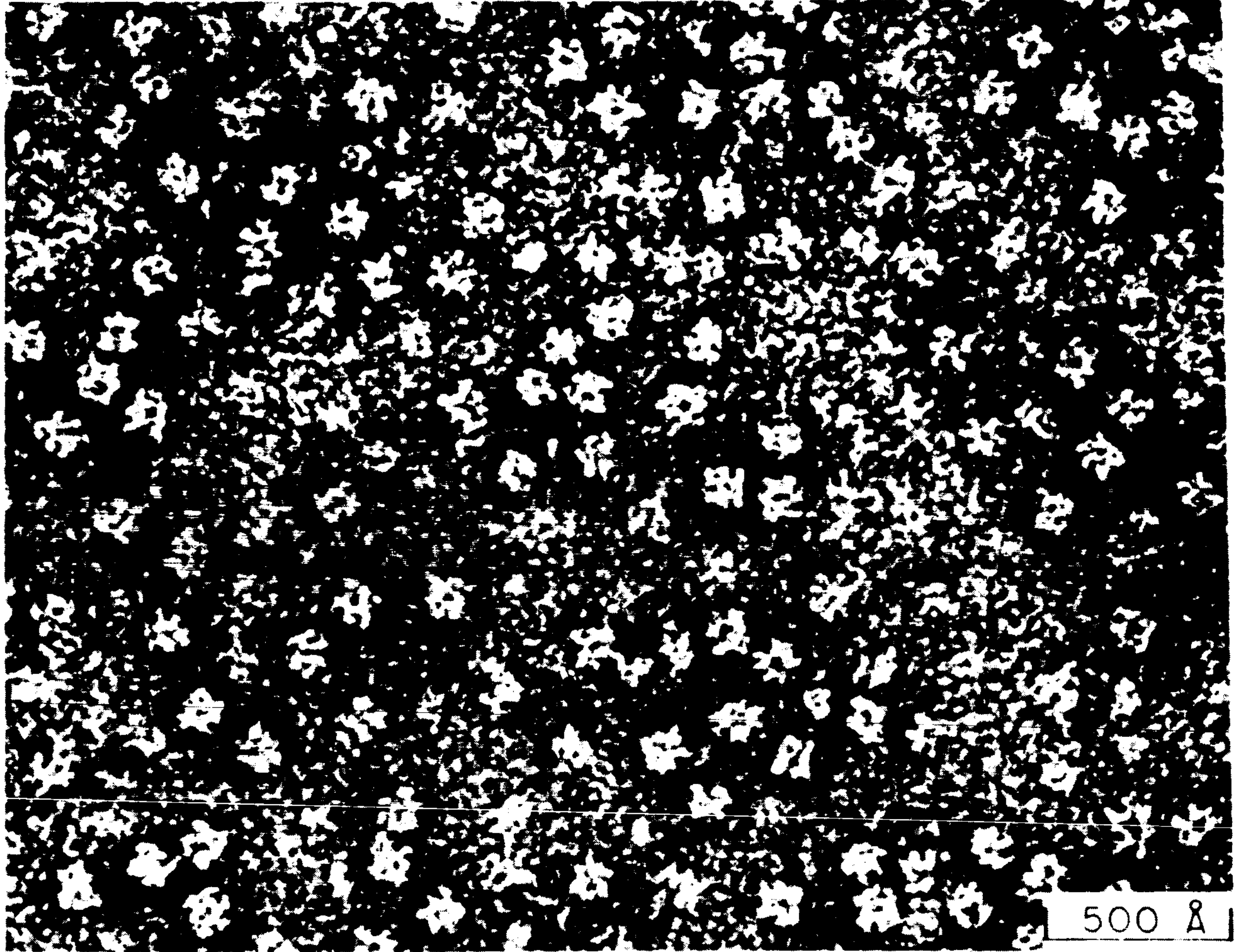
The analytical data obtained by Rees (17) established that the bulk of Fraction I protein consists of 24 identical subunits, each of molecular weight 22,500. Twenty-four subunits can be fitted into equivalent positions by placing them in groups of three on the vertices of a cube. Such a model for the enzyme can be ruled out on several grounds. First, the electron micrographs of concentrated droplets show no tendency toward aggregation in regular arrays. Secondly, attempts at crystallization from ammonium sulfate solutions have produced fibrous precipitates instead. Thirdly, when aggregates do occur, they are seen to be linear. Hence, the correct molecular model must be one which contains a unique two-fold axis.

The shadowed preparations (Figure 2) show uniform cubical particles often with rounded edges and a central depression which may be partly due to preparative (dehydration) effects. A model consistent with the views

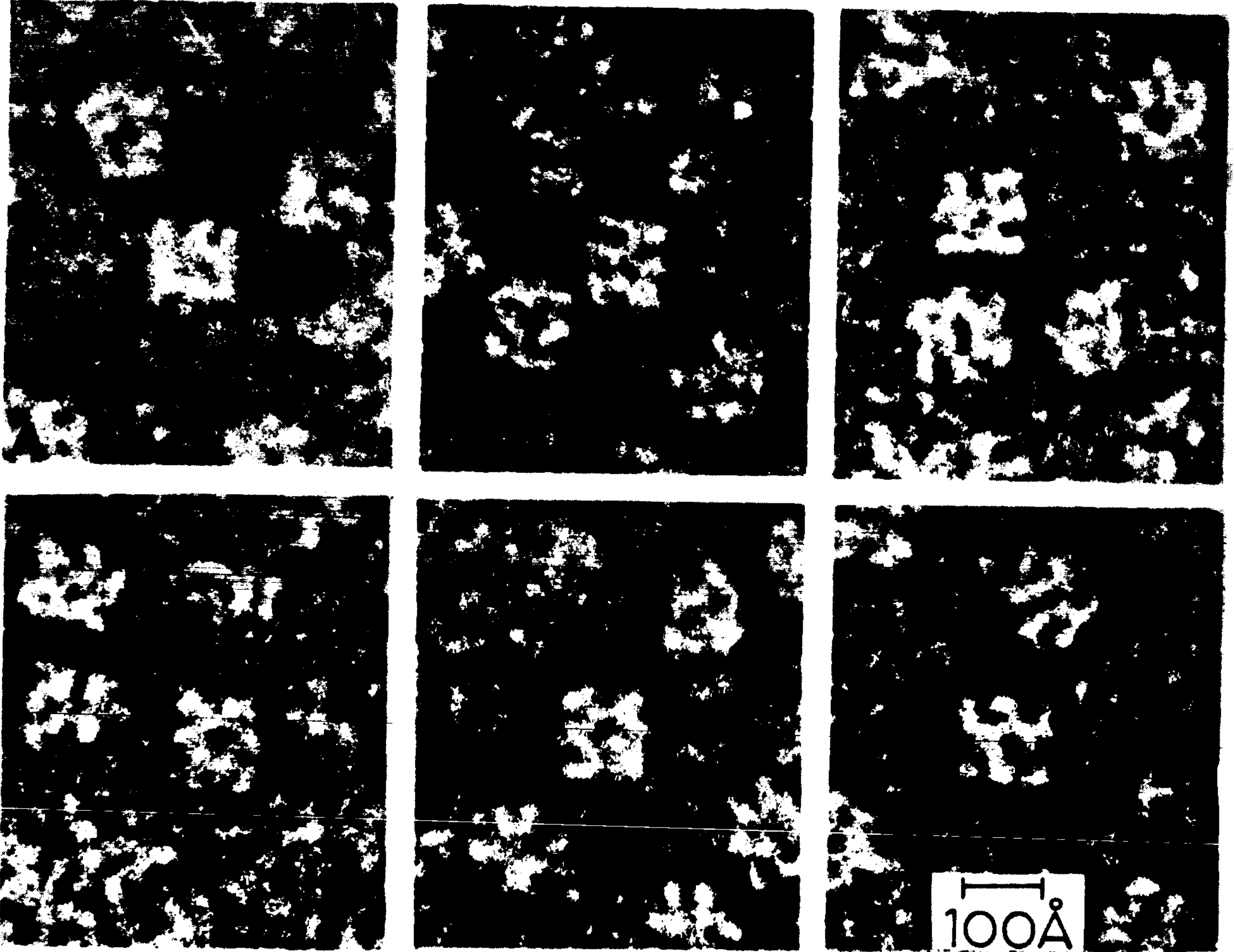
shown in electron micrographs (Figures 3 and 4), as well as with macroscopic properties of the protein, is that of a cube with three subunits along each edge, and one in the center of each of four faces. Particles resting on a side face will show three rows of three subunits (Figure 4a). Particles resting on a top or bottom face will show a ring of eight subunits (Figure 4b) corresponding to the side view with the central subunit missing. The subunits have a diameter of approximately 20 Å to 30 Å. Particles resting on an edge should show three rows, with the particle length in the direction of the rows up to 40 percent greater than its width perpendicular to the rows. Particles resting on a vertex should have a hexagonal appearance, with a central subunit and twelve subunits around the perimeter. Although several particles have been observed corresponding to the latter two views, these are rare, and could be due to distortion or association of particles on the substrate. Particles with hexagonal outline are seen occasionally, but these usually have one right-angle corner, and probably arise from partial embedding of the square face.

The diameter of a Stokes sphere of molecular weight 500,000 and sedimentation coefficient 18S (7) is calculated to be 120 Å. This value is in excellent agreement with the measured dimensions of the cubical particles (120 Å edge) as shown by electron microscopy using several preparation techniques.

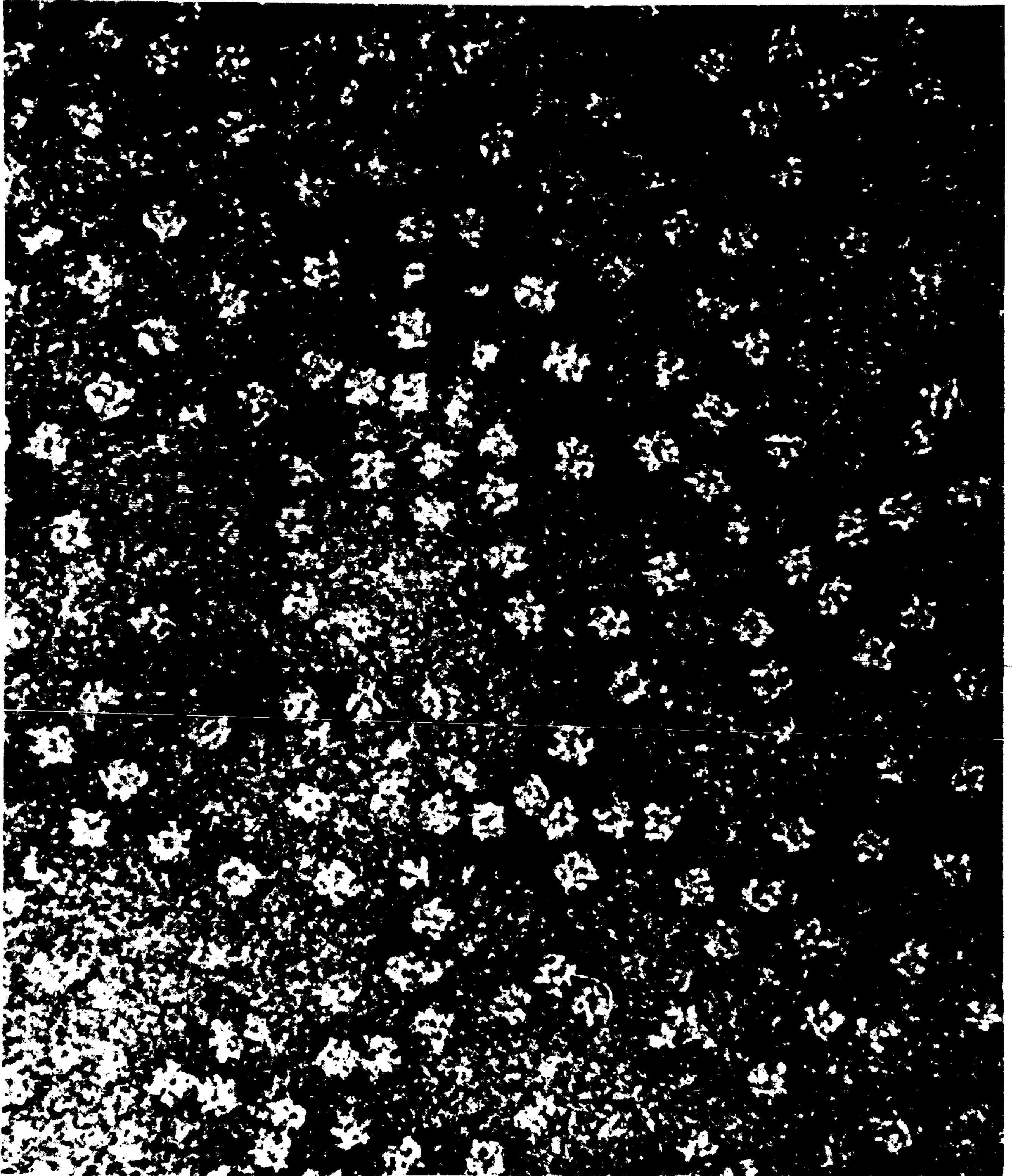
Subunits in the model proposed are not related by the principle of quasi-equivalence that governs the structure of many small viruses (10). In the model proposed, subunits are found in three positions: on faces, edges, or vertices. We assume that the plane net, from which the structure may formally originate, consists of a square lattice. Then the distortion required to place a subunit



3



4



on a vertex, maintaining the same bonding rule, becomes unacceptable (10). We are therefore obliged to propose that either subunits are capable of making several kinds of bond, or that subunits undergo considerable conformational changes as a result of their statistical location in the completed particle.

These findings may be summarized: Fraction I protein consists of a cube, about 120 Å along each edge, containing 24 subunits visualized in high resolution electron micrographs. The protein contains all the carboxydismutase activity recoverable from glycerol gradients. It has the same sedimentation coefficient and molecular weight as bacterial RNA polymerase, but lacks the latter activity. The particle structure does not appear to be governed by the same rules that apply in the case of small virus protein shells. We believe this to be the first occasion upon which the substructure of a protein enzyme of about 120 Å diameter has been resolved in electron micrographs.

R. Haselkorn
H. Fernández-Morán
F.J. Kieras
E.F.J. van Bruggen (20)

Department of Biophysics
The University of Chicago

LEGENDS TO FIGURES

Figure 1. Glycerol gradient centrifugation of Fraction I protein. One ml of a dialysed 0 percent to 50 percent saturated ammonium sulfate fraction was layered on a 5 percent to 30 percent glycerol gradient containing 0.01 M tris sulfate, 0.001 M glutathione, pH 7.7, and centrifuged for 24 hours at $4^{o}C$ at 23,000 rpm in the SW25 rotor of the Model L Spinco. After centrifugation, one ml fractions were collected in the following system: 0.07 ml glycerol fraction, 1.5 μ mole $NaHC^{14}O_3$ (5.1 mC/m mole), 2 μ moles $MgCl_2$, 15 μ moles tris, pH 7.7, and 0.06 μ mole ribulose 1,5-diphosphate in a final volume of 0.20 ml were incubated at $25^{o}C$ for 15 minutes. Glacial acetic acid (0.05 ml) was then added, and 0.05 ml aliquots were dried on planchets and counted (16).

Figure 2. Fraction I protein from Chinese cabbage leaves shadowed with platinum on thin carbon substrate(X 160,000).

Figure 3. Fraction I protein from Chinese cabbage leaves negatively stained with 2 percent phosphotungstate (pH 7.4) by microdroplet cross-spraying technique (X 360,000).

Figure 4 (a,b). Particles of Fraction I protein negatively stained with 2 percent phosphotungstate (pH 7.4) in different orientations (X 800,000).

Text of (possible) Cover Picture: Electron micrograph of Fraction I protein, the major soluble protein component in green leaf chloroplasts, showing subunit organization of these enzyme protein particles of about 120 Å (X 360,000 - micrograph A; or X 640,000-micrograph B).

REFERENCES AND NOTES

1. S.J. Singer, L. Eggman, J.M. Campbell, and S.G. Wildman, J. Biol. Chem. 197, 233 (1952).
L. Eggman, S.J. Singer, and S.G. Wildman, J. Biol. Chem. 205, 969 (1953).

2. J.W. Lyttleton and P.O.P. T'so, Arch. Biochem. Biophys. 73, 120 (1958).

3. D.W. Kupke, J. Biol. Chem. 237, 3287 (1962).

4. N.K. Boardman, Biochem. Biophys. Acta 62, 63 (1962).
_____________, Biochem. Biophys. Acta 64, 279 (1962).

5. G. van Noort, W. Hudson and S.G. Wildman, Plant Physiol. 36, Suppl XIX (1961).
L. Mendiola and T. Akazawa, Biochemistry 3, 174 (1964).

6. A. Weissbach, B.L. Horecker and J. Hurwitz, J. Biol. Chem. 218, 795 (1956).
R.W. Dorner, A. Kahn and S.G. Wildman, J. Biol. Chem. 229, 945 (1957).
R.B. Park and N.G. Pon, J. Mol. Biol. 3, 1 (1961).

7. P.W. Trown, Biochemistry 4, 908 (1965).

8. R. Markham, Adv. in Virus Research 9 , 241 (1962).

9. E. Fuchs, W. Zillig, P.H. Hofschneider and A. Preuss, J. Mol. Biol. 10, 546 (1964).
A.J.E. Colvill, E.F.J. van Bruggen and H. Fernandez-Moran, to be submitted to Science.

REFERENCES AND NOTES CONT.

10. F.H.C. Crick and J.D. Watson, Nature 177, 473 (1956). D.L.D. Caspar and A. Klug, Cold Spring. Harbor Symp. Quant Biol. 27, 1 (1962).

11. H. Fernández-Morán, Circulation 26, 1039 (1962).

12. ________________, L.J. Reed, M. Koike, and C.R.Willms, Science 145, 3635 (1964).

13. H.E. Huxley, J. Mol. Biol. 1, 281 (1963).

14. H. Fernández-Morán, J. Appl. Phys. 31, 1840 (1960).

15. ________________, T. Oda, P.V. Blair, and D.E. Green, J. Cell Biol. 22, 63 (1964).

16. B.R. Rabin and P.W. Trown, Proc. Natl. Acad. Sci. U.S. 51, 497 (1964).

17. M.W. Rees, Personal Communication.

18. We are indebted to Misses C.M. Runner and D.A. Meddoff for their technical assistance in specimen preparation and to Mrs. L. Deloria for her technical assistance, and to Mr. C. L. Hough for expert assistance with photographic reproductions.

19. This work was supported by the following grants: AI-14448 and AI-06279, NB-04267 and GM-13243 of the United States Public Health Service; by NsG 441-63 of the National Aeronautics and Space Administration; by U.S. Atomic Energy Commission Contract AT-(11-1)-1344; by the L. Block Fund; and by the Otho Sprague Memorial Fund of The University of Chicago.

REFERENCES AND NOTES CONT.

20. RH is a recipient of a Research Career Development Award from the United States Public Health Service; FJK is a predoctoral trainee of the United States Public Health Service; EFJvB was an International Postdoctoral Research Fellow of the United States Public Health Service, present address Laboratorium voor Structuurchemie, Bloemsingel 10, Groningen, The Netherlands.