PYRUVATE DEHYDROGENASE COMPLEX OF *ESCHERICHIA COLI.*

Radial Mass Analysis of Subcomplexes by Scanning Transmission Electron Microscopy

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The pyruvate dehydrogenase complex of *Escherichia coli* is composed of three enzymes: pyruvate dehydrogenase (E_1), a dimer of identical subunits with an M_r of 200,000 (1–4); dihydrolipoyl transacetylase (E_2), the 24-subunit core of the complex with an M_r reported to be 1.5×10^6 by equilibrium sedimentation (2) or 2.2×10^6 by scanning transmission electron microscopy (STEM) measurements (4); and dihydrolipoyl dehydrogenase (E_3), a dimer of identical subunits with an M_r of 115,000 (1–4). The subunit composition has been controversial; however, the compositional model advanced by L. J. Reed and associates (1) has been supported by evidence from other laboratories (3, 4). The model postulates that each particle of the complex consists of 24 E_1 chains, 24 E_2 chains, and 12 E_3 chains. Recent particle mass measurements on the complex and its components by STEM have supported this model (4).

The geometrical organization of subunits in the pyruvate dehydrogenase complex has not been established, and little physical evidence regarding this aspect of the structure has been available. The 24-subunit core has been observed as a cubic structure in electron micrographs (2, 5). On the basis of this observation, the postulated subunit composition of 24 E_1:24 E_2:12 E_3, and assuming the cubic symmetry of the core is maintained in the fully constituted complex, Reed has proposed that 24 E_1 subunits are arranged as dimers along the 12 edges of the E_2 core and 6 E_3 dimers are associated with the six faces of the core (6). This model has not previously been verified by direct physical investigations; and results from binding studies have suggested the existence of steric interference between E_1 and E_3 for binding sites on E_2 (7).

We have prepared partially reconstituted subcomplexes of E_2 core containing a few subunits of E_1 or E_3 and subjected them to radial mass analysis by STEM. The results clearly establish that E_1 and E_3 occupy distinct binding sites on E_2. Moreover, the locations of the E_1- and E_3-binding sites were found to be fully consistent with the quaternary structural model described above.

MATERIALS AND METHODS

Pyruvate dehydrogenase complex was purified from *E. coli* cells and resolved into component enzymes as described in the literature (8, 9). Subcomplexes were prepared by mixing E_2 and either E_1 or E_3 in the desired ratios and subjecting them to minimal glutaraldehyde/$NaBH_4$ crosslinking and the samples prepared for mass analysis by STEM as described earlier (4). STEM images were obtained at dose levels of 2–4 $e^-/Å^2$ and the data stored on magnetic tapes. Data on individual particles in each specimen were retrieved from the tapes on the form of masses enclosed within concentric circles centered on the E_2 core. Tobacco mosaic virus included with each sample was the internal standard used in the mass measurements. The radii were increased from 10 Å in 10 Å increments until no further increases in mass were observed. Over 50 similarly oriented particles of each specimen were analyzed and the data averaged.

RESULTS

STEM images of partially reconstituted subcomplexes formed by addition of an average of two E_3 dimers/E_2 core are indistinguishable by visual inspection from the core protein itself. They are easily distinguished, however, by quantitative mass measurements using STEM. Reconstituted subcomplexes similarly prepared from E_2 core and an average of two E_1 dimers/core exhibit distinct morphological differences from the core protein in STEM images. Images of partially reconstituted E_2–E_1 subcomplexes clearly reveal the E_1 subunits protruding from the central core protein.

Shown in Fig. 1 are typical STEM micrographs of the partially reconstituted subcomplexes. Those reconstituted with E_3 and shown in panel *a* appear as essentially cubic structures in various orientations, with the E_3 protein well integrated into the core. Those reconstituted with E_1 and shown in panel *b* appear as core protein with projections extending well outward from the periphery of the core.

The highlighted images in Fig. 1 are positioned with two faces of the cubic core protein oriented in the field plane and the other four faces perpendicular. Two-dimensional radial mass analysis of such images places the morphological differences between the reconstituted E_2–E_3 and E_2–E_1

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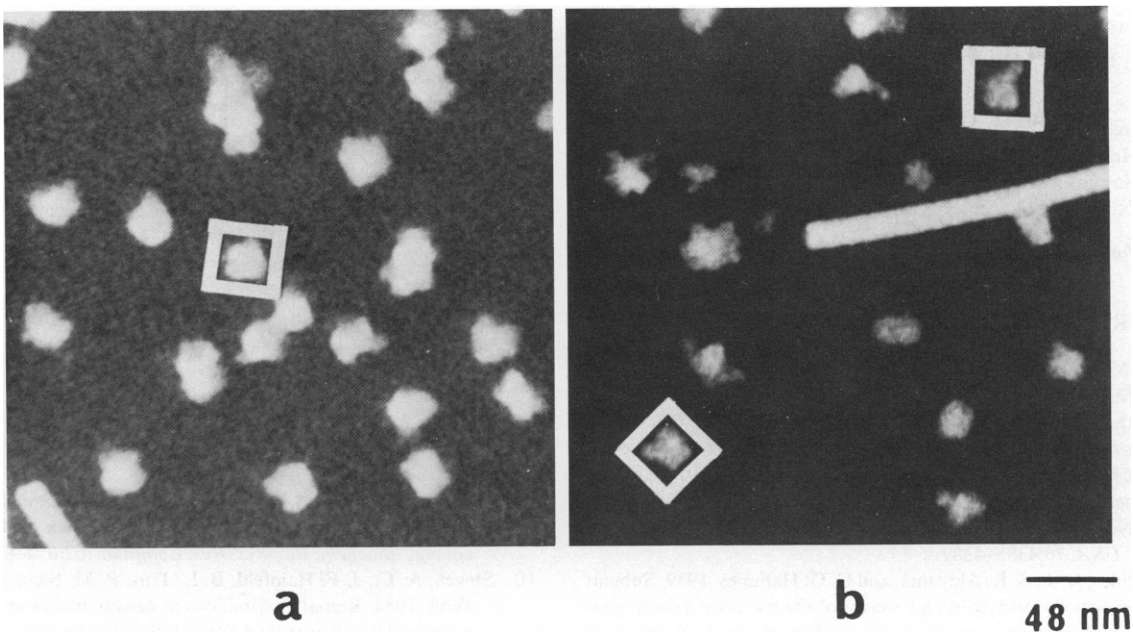

FIGURE 1 Electron micrographs of partially reconstituted subcomplexes. Shown are STEM-images of unstained specimens at a magnification of 2.5×10^5. *a* shows images of E_2 partially reconstituted with an average of 2 E_3 dimers. *b* shows images of E_2 partially reconstituted with an average of 2 E_1 dimers. The rod-like structures are tobacco mosaic virus added as internal mass standard.

subcomplexes on a quantitative basis. This technique is based on the use of STEM to measure masses within concentric rings differing in radius by 10 Å and centered on the E_2 core. The technique is described by Steven et al. (10).

The mass distributions in the subcomplexes are conveniently compared with that in the core protein by computing the ratios of masses enclosed within given radii in a subcomplex to corresponding masses in the core protein. A plot of this ratio vs. the radius reveals the radial locations of mass attributable to E_1 or E_3. Averaged data obtained on over 50 images of each subcomplex and the core are plotted in Fig. 2.

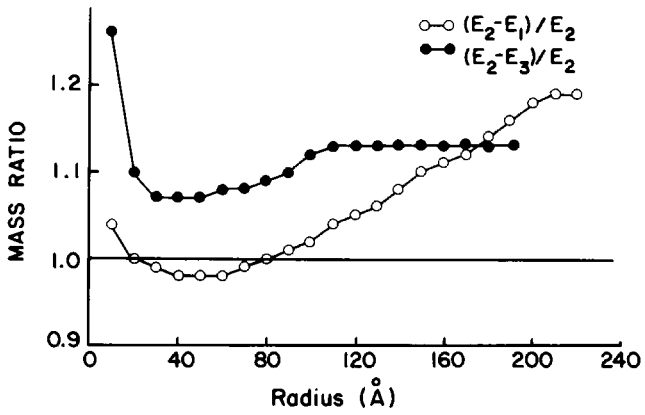

FIGURE 2 Radial mass analysis of partially reconstituted subcomplexes. Radial mass distributions on subcomplexes shown in Fig. 1 were obtained on over 50 images of each type. The results are here compared with similar data on E_2-core protein by computing mass ratios as a function of radius from the core center, as described in the text. Ratios >1.0 reflect the presence of mass due to E_1 or E_3.

The computed mass ratios can have values ≥ 1.0 and will reach constant values at radii beyond the outermost limits of the images. The mass ratios for the E_2–E_3 subcomplex exceed 1.0 at all radii and reach a constant value at 110 Å, which is the effective radius of the core protein. This result demonstrates that E_3 is well integrated with the core protein. The mass ratios for the E_2–E_1 subcomplex are near 1.0 at radii from 10 to 80 Å; they increase beyond 100 Å and reach a constant maximum value at 220 Å, the effective radius of the pyruvate dehydrogenase complex itself. Mass attributable to E_1 is, therefore, not integrated within the core structure, but appears at the edge and beyond the periphery of the core.

DISCUSSION

The present results demonstrate that the high affinity sites for E_1 and E_3 binding to the E_2 core are distinct, overlapping only slightly at the core edges. Mass attributable to E_3 is readily detected on the faces of oriented core protein particles and does not extend beyond the core images, whereas mass attributable to E_1 is found at the core edges extending out to 220 Å from the core centers. These results are fully consistent with, and even predicted by, the structural model of Reed, in which six E_3 dimers are postulated to be associated with the faces and 12 E_1 dimers with the edges of the cubic core (7). If cubic symmetry is maintained in the fully constituted complex and the com-

position is 24 E_1:24 E_2:12 E_3; where E_1 and E_3 are dimeric proteins, no other subunit arrangement is possible.

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APPLICATION OF RESTRAINED LEAST-SQUARES REFINEMENT TO FIBER DIFFRACTION FROM MACROMOLECULAR ASSEMBLIES

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The principal concern in the refinement of the structure of a macromolecular assembly against fiber diffraction data is the relatively small number of independent data available compared with the number of adjustable molecular parameters. At the resolution limit of the best fiber diffraction patterns from helical viruses (~3 Å) there are too few measurable intensities per model parameter for stable refinement of the structure using diffraction data alone. This is because the intensities observed in a fiber diffraction pattern are the cylindrical average of the intensity distribution on layer planes in reciprocal space. Thus, far fewer data are available as restraints on the values of the molecular parameters. For example, cylindrical averaging reduces the effective number of observable diffraction data for tobacco mosaic virus (TMV) at 3 Å resolution by a factor of ~2.5 and for the bacteriophage Pf1 at the same resolution by a factor of 1.7 (Makowski, 1982). Consequently, stereochemical information must be incorporated into the refinement process to increase the ratio of observations to parameters. This can be done in two ways. Stereochemical information can be used in the form of constraints that fix the values of selected bond lengths and angles, reducing the number of parameters to be refined. This is the approach taken in the linked-atom least-squares (LALS) method of Arnott and his collaborators (Arnott and Wonacott, 1966; Smith and Arnott, 1978), in which dihedral bond angles are refined. This has been particularly effective in refining the structure of helical polymers with relatively small repeating units such as nucleic acids and polysaccharides using diffraction data from crystalline fibers. An alternative approach is one in which stereochemical information is used in the form of restraints. This method as developed by Hendrickson and Konnert (1980) has been widely used in the refinement of protein structures against crystallographic data. In this approach, the ideal values of bond lengths and angles are treated as additional observational equations and the refinement of atomic coordinates attempts to minimize deviations from these values while simultaneously minimizing the difference between calculated and observed diffraction data. Additional restraints can readily be incorporated into the refinement. Because restrained least-squares methods offer some advantages in speed and in final agreement with the observed diffraction data (Dod-

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