Cryoelectron Microscopy of Frozen-hydrated α-Ketoacid Dehydrogenase Complexes from Escherichia coli

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The native architectures of the pyruvate and 2-oxoglutarate dehydrogenase complexes have been investigated by cryoelectron microscopy of unstained, frozen-hydrated specimens. In pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex the transacylase (E2) components exist as 24-subunit, cube-shaped assemblies that form the structural cores of the complexes. Multiple copies (12-24) of the α-ketoacid dehydrogenase (E1) and dihydrolipoyl dehydrogenase (E3) components bind to the surface of the cores. Images of the frozen-hydrated enzyme complexes do not appear consistent with a symmetric arrangement of the E1 and E3 subunits about the octahedrally symmetric E2 core. Often the E1 or E3 subunits appear separated from the surface of the E2 core by 3-5 nm, and sometimes thin bridges of density appear in the gap between the E2 core and the bound subunits; studies of subcomplexes consisting of the E2 core from 2-oxoglutarate dehydrogenase complex and E1 or E3 show that both E1 and E3 are bound in this manner. Images of the E2 cores isolated from pyruvate dehydrogenase complex appear surrounded by a faint fuzz that extends ~10 nm from the surface of the core and likely corresponds to the lipoyl domains of the E2.

The α-ketoacid dehydrogenase complexes are among the largest and most structurally complicated enzymes known (for reviews, see 1–3). In Escherichia coli there are two types, one specific for pyruvate and the other for 2-oxoglutarate. In both complexes the transacylase component (E2) exists as an oligomeric, octahedrally symmetric core comprising 24 identical polypeptides and having the overall shape of a cube in which most of the mass is localized at the corners and along the edges (4–6). Bound noncovalently to the E2 core are multiple copies (up to 24) of the other two polypeptide components, pyruvate or 2-oxoglutarate dehydrogenase (E1) and dihydrolipoyl dehydrogenase (E3). The E3 component is identical in pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complexes whereas the E1 and the E2 polypeptides are coded by different genes in the two complexes (7–11).

The E2 polypeptides have a segmented structure (Fig. 1) in which specialized domains are arranged linearly along the amino acid sequence (reviewed in Ref. 3). Domains bearing the lipoic acid moieties (three in E2 from pyruvate dehydrogenase complex (E2p), and one in the E2 from 2-oxoglutarate dehydrogenase complex (E2o)), comprising about 80 residues each, occur beginning at the N terminus. These are followed sequentially by a domain of about 50 residues involved in binding E3 and finally by a large domain bearing the transacylase active site and binding sites for E1 and E2 (hereafter referred to as the “catalytic” domain). Interspersed between these functional domains are segments of polypeptide, typically 20–30 residues in length, which are rich in alanine, proline, and charged amino acids. Based on their distinctive amino acid composition, sensitivity to proteolysis (12–16), NMR spectra (17–20), and appearance in electron micrographs (2, 12) it has been proposed that the (Ala-Pro)-rich sequences are flexible and have an extended structure. They are thought to function as tethers that allow the lipoic acid moieties to access active sites on E1, E2, and E3 and to interact with each other. This model suggests that the reach of the lipoic acid moieties should be several times greater than the 1.4 nm length of a fully extended lipoyl-lysine moiety alone. Large scale dynamic movements of perhaps several nm of the lipoyl and E3 binding domains would also appear to be likely consequences of such a model.

Electron microscopy is a potentially powerful technique for obtaining both qualitative and quantitative information on the nature of movements by domains and subunits in macromolecular complexes such as pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex. A flexible mode of attachment for E1 and E3 to the E2p core has been suggested previously based upon electron microscopy studies of negatively stained pyruvate dehydrogenase complex (2). However, such conclusions must be made with caution because the potential artifacts associated with traditional electron microscopy methods, such as the negative stain technique, can distort native macromolecular structure. In our studies of 2-oxoglutarate dehydrogenase complex (21), for example, it was found that chemical fixation was required to prevent dissociation of the E2 core-bound subunits. Furthermore, scanning transmission electron microscopy of freeze-dried, unstained pyruvate dehydrogenase complex shows a more close packed appearance of the E1 and E3 than in negatively stained specimens (22, 23) and is not suggestive of substantial movements of E1 or E3. Similar uncertainties apply for the 2-oxoglutarate dehydrogenase complex (2, 21, 24-26).
43). Thus, electron microscopy studies of pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex have not led to an unambiguous model for the spatial distribution and mode of attachment of the E1 and E3 subunits about the E2 core.

The relatively new technique of cryoelectron microscopy of frozen-hydrated suspensions of macromolecules is free of most of the artifacts associated with the negative staining and other traditional techniques (24, 25) and is capable of preserving macromolecular structure to or near atomic resolution (26-28). Here we describe our first results in applying this technique to pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex of E coli.

EXPERIMENTAL PROCEDURES

Materials—Pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex were purified and resolved to the component enzymes by established procedures (12, 29-31) with the following modifications. Frozen cells of E. coli; strain B (ATCC 11303, from Grain Processing, Muscatine, IA), were used as the starting material. Some preparations of pyruvate dehydrogenase complex were purified further according to Speckard and Frey (32).

Trypsin treatment of E2 was done as described by Bleile et al. (12) except that the digestion was done in the presence of 0.2 M potassium phosphate (pH 7.0) instead of 0.02 M potassium phosphate to minimize precipitation. The rate of proteolysis, as monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (33), was found to be lower than in 0.02 M potassium phosphate, but the pattern of gel bands generated was identical. The reaction was terminated after 120 min by the addition of pancreatic trypsin inhibitor, at which point it was estimated from the density of the stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis bands that about 75% of the lipoylated lysine residue. Segments connecting the domains are rich in alanine, proline, and charged amino acids, and their length is proportional to the number of residues in the segment. The figure is based largely upon the work of Perham and colleagues (3). A Philips EM420T transmission electron microscope operating at 100 kV was used. The microscope was equipped with a low dose kit and an auxiliary anticontaminator (Gatan model 611N). All microscopy was done at -170 to -180 °C using a Gatan model 626 cryoholder and specimen transfer system. Micrographs were recorded at an instrumental magnification of 48,000 x with a net dose of 1,000-1,500 electrons/nm². The magnification of the microscope was calibrated using tropomyosin paracrystals as a standard (36). Usually, the micrographs were obtained with the objective lens underfocused by 2 μm, but in some cases pairs of micrographs were recorded of the same specimen field at two defocus values, typically 1 and 3 μm (see "Analysis of Images"). Underfocusing is essential for generating phase contrast and is necessary for enzyme complexes to be visible in the micrographs. At 2-μm underfocus the contrast transfer function limits the interpretable resolution of the images to 1/2.9 nm⁻¹. The presence of vitreous ice was verified from electron diffraction patterns recorded for each region of the grid from which micrographs were obtained (37).

Analysis of Images—To improve the contrast of the unstained, frozen-hydrated enzyme complexes, which is inherently low, the micrographs were digitized using a Perkin Elmer PDS microdensitometer (25-μm square scanning aperture corresponding to 0.52 nm on the specimen) and processed using the SPIDER program package (38) implemented on VAX 750 and 6210 computers. Image files containing a single enzyme complex were created by interactive selection from the digitized micrographs, which were displayed on a video graphics monitor. The selected image files were then low pass Fourier-filtered (Gaussian filter function) to a limiting resolution of 1/2.9 nm⁻¹ (39). The montages of images shown in Figs. 2B, 3B, and 6 were processed in this way. To enhance the contrast of the imaged complexes further, most of the images of the E2-E3 subcomplexes shown in Fig. 5 were obtained by combining data from two electron micrographs that were recorded from the same specimen area but with the objective lens underfocused by different amounts, 1.5 (recorded first) and 3.0 μm. The rationale of this procedure was, first, to extend the range of optimally contrasted specimen detail, which is desirable because of the nonuniform nature of the contrast transfer function when the objective lens is underfocused (for example, see Figs. 25 and 40) and second, to improve the signal-to-noise ratio by the averaging of structural information common to both micrographs. Corresponding images from the two micrographs were translationally aligned by cross-correlation (38, 41), low pass filtered, and summed. Only a modest improvement in contrast was achieved by this procedure as compared with the simpler procedure described above in which only a single micrograph was recorded.

Measurements were performed on the processed images, either manually from photographic prints, or by means of an interactively controlled cursor on video displays.

RESULTS

Cryoelectron Microscopy of 2-Oxoglutarate Dehydrogenase and Pyruvate Dehydrogenase Complexes A portion of an electron micrograph of 2-oxoglutarate dehydrogenase complex

![Diagram of enzymatic complex](https://example.com/diagram.png)
suspended in a thin film of ice is shown in Fig. 2A. The area shown is from a region of the grid in which a hole in the carbon film was present; the enzyme complexes are not adsorbed to a carbon surface. In most of the images the cube-shaped core formed by the E20 component is visible near the center of the complex. Often the cores appear either as four globular morphological units arranged at the corners of a square, corresponding to views approximately along one of its 4-fold symmetry axes, or as rectangular H-shaped structures, corresponding to views along one of the 2-fold axes (Fig. 2B).

Distributed peripherally about the E20 cores are apparently variable numbers of morphological units corresponding to the bound E1 and E3. The average diameter of the 2-oxoglutarate dehydrogenase complex particles is 32 ± 3 nm.

The number of visibly bound E1 and E3 varies among the images from two to about eight, possibly indicating that 2-oxoglutarate dehydrogenase complex is heterogeneous in subunit stoichiometry. However, we cannot exclude the possibility that some E1 and E3 are lost during the isolation or during preparation for electron microscopy. Biochemical analyses from two independent laboratories indicate a subunit polypeptide stoichiometry of 12:24:12 for E1:E2:E3 (31, 46). Both E1 and E3 exist as dimers (M, 210,000 and 110,000 respectively) in 2-oxoglutarate dehydrogenase complex (42, 43), and so there should be 12 peripherally bound morphological units/molecule of 2-oxoglutarate dehydrogenase complex, not all of which are expected to be visible in any given image because of superposition effects.

Two observations concerning the distribution of E1 and E3 about the E20 core are of particular interest. First, there often appears to be a separation or gap between the bound subunits and the E20 core; and second, the bound E1 and E3 appear not to be distributed in a symmetric manner about the octahedrally symmetric E20 core complex. Although it is difficult to measure precisely the boundaries of the peripheral subunits or of the E20 cores, it appears that the gap between the E20 core and the bound E1 or E3 is maximally in the range of 3-5 nm. Although substructures in the gap regions are not defined clearly in most of the images, in a few images there indeed appears to be a thin bridge of density connecting the bound E1 or E3 subunit to the surface of the E20 core.

The appearance of frozen-hydrated pyruvate dehydrogenase complex (Fig. 3) is similar to that of 2-oxoglutarate dehydrogenase complex except that, not surprisingly, the images are typically somewhat more complex, probably because of the presence of more E1 in pyruvate dehydrogenase complex. The average diameter of pyruvate dehydrogenase complex, 37 ± 3.5 nm, is larger than was found for 2-oxoglutarate dehydrogenase complex and agrees with measurements made previously for negatively stained (2) and unstained, freeze-dried (22) specimens. In many of the images the E2p core complex is discernible, and often the peripherally distributed E1 and E3 subunits appear separated from the surface of the E2p, just as in 2-oxoglutarate dehydrogenase complex.

E2 Core Complexes from 2-Oxoglutarate and Pyruvate Dehydrogenase—To investigate further the ultrastructure of pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex we resolved the complexes into the component enzymes. Electron micrographs of frozen-hydrated E20 and E2p core complexes are shown in Fig. 4, A and B, respectively. Although E20 and E2p have very similar overall structures, they also show some distinct differences.

First, E2p showed a stronger preference to assume orientations in which one of the 4-fold symmetry axes was along the viewing direction (Fig. 4B). In contrast, the E20 complexes displayed more variability in the orientations assumed, and in some experiments views approximately along 2-fold symmetry axes (images appearing as H in Fig. 4A) predominated. Probably these preferences reflect differing interactions of the E20 and E2p at the air-water interfaces present prior to freezing.

A second difference between the E20 and E2p cores was that images of the latter showed varying levels of contrast even in the same area of the grid, where the effect of ice thickness on contrast should have been negligible. The highly contrasted images of E2p (denoted by arrows in Fig. 4B) are likely because of a face-to-face association of E2p complexes such that two or more E2s become stacked up in a direction perpendicular to the plane of the ice film. This interpretation is supported by the frequent occurrence of face-to-face oligomers, mainly dimers, stacked parallel to the plane of the ice film (arrowheads in Fig. 4B).

A third difference of the frozen-hydrated E2p from the E20
Cryoelectron Microscopy of α-Ketoacid Dehydrogenase Complexes

FIG. 3. Electron micrographs of frozen-hydrated pyruvate dehydrogenase complex. A, field of enzyme complexes suspended in ice over a hole in the carbon supporting film. The edge of the hole is visible at the far right. Size of scale bar, 50 nm. B, gallery of selected, contrast-enhanced images (see "Experimental Procedures"). In the first and second frames the E2p core appears to be approximately in a 4-fold view; in the third frame it appears in a 2-fold orientation. A few of the subunits (E1 or E3) bound to the E2 core are indicated by the arrowheads. The width of each frame is 69 nm.

cores is that the ice surrounding the E2p complexes usually has a grainy texture whereas the ice surrounding E2o complexes has a very smooth appearance characteristic of vitreous ice. The grainy background observed for E2p complexes appears to be confined to regions near individual complexes or in the vicinity of clusters of complexes. Also, the graininess is sometimes more pronounced surrounding the highly contrasted E2p images mentioned above and gives the impression that small globular particles surround the E2p cores (arrowed images shown in Fig. 4B). We suspect that the grainy texture associated with the E2p images is caused by the lipoyl domains (see "Discussion"). If the lipoyl domains are in their native conformation, these images provide direct evidence that the lipoyl domains of E2p are capable of extending up to 10 nm or more from the surface of the core complex. Further evidence supporting this interpretation is given by Fig. 4C, which shows a micrograph of E2p core complexes that had been treated with trypsin to remove most of the lipoyl domains (12). The ice surrounding trypsin-treated E2p shows a smoother texture, similar to that observed for E2o, but the interpretation of these micrographs is complicated by an increased tendency of trypsin-treated E2p cores to undergo face-to-face aggregation.

E2o-E3, E2o-E1 Subcomplexes—To characterize further the mode of E1 and E3 binding to the E2o core, subcomplexes of E2o and E1 or E3 were prepared by in vitro reconstitution using lower molar ratios, E1:E2o or E3:E2o, than are present in native 2-oxoglutarate dehydrogenase complex. Microscopy of frozen-hydrated subcomplexes yields images that are much less complicated than those of the intact enzyme complexes and also allows the binding modes of the E1 and E3 to be investigated independently of one another (21). Subcomplexes of pyruvate dehydrogenase complex are not treated because the grainy texture of the background surrounding E2p cores and their tendency to aggregate result in subcomplexes that are difficult to interpret.

FIG. 4. Electron micrographs of frozen-hydrated E2 cores. A, E2o. Views of the 4-fold orientation are indicated by arrows; 2-fold views are indicated by arrowheads. B, E2p. Note the fuzzy background associated with the particles, especially for the highly contrasted 4-fold symmetric images (arrows). Side-by-side aggregates are indicated by arrowheads. Inset, enlarged contrast-enhanced image. C, E2p treated with trypsin (see "Experimental Procedures") to remove lipoyl domains partially. Note the smoother texture of the background ice as compared with B. Scale bar, 50 nm.
A gallery of typical images of E20·E3 subcomplexes is shown in Fig. 5. The bound E3 subunits appear as one or more morphological units at the periphery of the E20 core. More than 900 individual images have been examined from two independent experiments employing two different E3:E20 molar ratios (1:2:1 and 4:6:1). Chemical cross-linking, which was necessary to prevent dissociation of E3 and E2 in studies of negatively stained complexes (21), was found to be unnecessary with the frozen-hydrated specimens, underscoring further the superior preservation of macromolecular structure attainable with frozen-hydrated as compared with negatively stained specimens. Although some dissociated E3 appeared in the background, the amount was sufficiently low that bound and free E3 subunits could be distinguished with little uncertainty.

Perhaps the most striking characteristic of the E20·E3 subcomplexes is that in the majority (~85%) of the images the bound E3 does not appear to make extensive contact with the E20 core. In many of the images there is no connection visible at all between the E20 and E3 whereas in some, a thin bridge of connecting density appears to be present (e.g. first and second images in the first row of Fig. 5). The size of the gap separating the E20 and E3 is variable, ranging from 0 to about 5 nm, and is difficult to quantify precisely mainly because of uncertainty in defining the molecular boundaries of E2 and E3. We estimate that maximal size of the gap is 3–5 nm. The position of the bound E3 varies both radially and azimuthally with no clear evidence of preferred locations apparent. These observations are consistent with a structural model of the E20 core in which the E3 molecules are attached to the inner core via extended, flexible portions of polypeptide.

Although the locations of the bound E3 relative to the E20 core vary, analysis of those images in which a connecting bridge of density between the E20 and E3 is visible should reveal from where on the surface of the E20 core the connecting bridges emanate. We emphasize, however, that caution should be exercised in interpreting those images in which the density of the connecting bridge is comparable to background noise. Images in which the E20 is in the 2-fold orientation (third and fourth rows of Fig. 5) and in which the bound E3 is located near one of the two short edges are especially informative because these edges correspond to the actual edges of the cube-shaped E20 core complex (unlike the situation for the 4-fold views in which each edge arises from the superposition to two edges of the E20 complex). The connecting bridges appear to emanate from positions on the edges and near the corners of the E20 core (e.g., see second image in third row of Fig. 5).

A gallery of selected E20·E1 subcomplexes is shown in Fig. 6. In the micrographs from which the images were extracted, the E20 cores had a much stronger preference for the 4-fold orientation as compared with that obtained in the experiments of Figs. 4A and 5. Consequently, there were fewer images of 2-fold views available for analysis (third row of Fig. 6). Otherwise, the results obtained were similar to those obtained for the E20·E3 subcomplexes described above. The bound E1 subunits, which are dimeric (43), appear as globular masses 6–8 nm in diameter. In most of the images analyzed (n = 203) there is an apparent gap of 2–4 nm between the bound E1 and the E20 core. Frequently it appears that the bound E1 is connected to the E20 by a thin bridge of density that appears to contact the surface of the E20 core along one of its edges and offset toward a corner, at or near the same location as was found for E3 attachment. The bridging density was visible more frequently than was the case for the E20·E3 subcomplexes, suggesting that perhaps it represents more mass than that of the E20·E3 images (cf. Figs. 5 and 6).

DISCUSSION

One of the main results of this study is that in electron micrographs of frozen-hydrated 2-oxoglutarate dehydrogenase complex and subcomplexes thereof, the E1 and E3 subunits often appear separated by a gap of up to several nm from the surface of the E20 core complex to which they are bound. The same behavior has also been observed for pyruvate dehydrogenase complex, but it has not been investigated as thoroughly as for 2-oxoglutarate dehydrogenase complex. Our interpretation of these observations is that the bound E1 and E3 are connected by thin bridges of polypeptide which are usually not visible because of the limited resolution (~3 nm) and contrast of the micrographs. These results may be relevant to understanding the mode of active site coupling that is mediated by the lipoic acid cofactors of 2-oxoglutarate dehydrogenase complex and pyruvate dehydrogenase complex.

Similar results have been described previously for pyruvate dehydrogenase complex in which the negative staining tech-
Cryoelectron Microscopy of α-Ketoacid Dehydrogenase Complexes

nique was used (2, 47). However, in those studies it was not possible to rule out the alternative interpretation that the observed separation between the bound E1 or E3 and the E2p core resulted from dissociation of the bound subunits caused by the air-drying or interactions with the negative stain. It is extremely unlikely that this interpretation applies to our

results, in which the complexes were suspended in a thin film of buffer and rapidly (~100,000 °C/s) frozen for cryoelectron microscopy.

One potential source of damage for frozen-hydrated specimens is the interaction of the specimen with the air-buffer interfaces present prior to freezing (26). It is, however, difficult to envision how surface effects could account for the apparent separation of the bound E1/E3 subunits from the E2 cores observed for the frozen-hydrated specimens. Because the specimens were not adsorbed to a carbon film, as was the case in the previous negative stain studies, diffusion of E1 or E3 following its dissociation from the E2 core would occur, and so the probability of observing a dissociated E1 or E3 subunit still within a few nm of the E2 core to which it had been bound in a frozen-hydrated specimen would be very low. We conclude that the gap observed between the E1 and E3 subunits and the E2 core is a characteristic of native 2-oxoglutarate dehydrogenase complex and pyruvate dehydrogenase complex.

E2–E3 Interaction—The mode of E3 binding to the E2o core revealed by this study can be interpreted in terms of the segmented structural model for the E2o and E2p polypeptides described by Perham and co-workers (3) and discussed above (Fig. 1). Recall that in this model the lipoyl, E3 binding, and catalytic functional domains are separated from one another by sequences, typically 20–30 amino acid residues long, which are unusually rich in alanine, proline, and charged amino acids. Since the E3 binding domain is linked to the catalytic domain by one of the (Ala-Pro)-rich sequences (Fig. 1), the E3 subunit in E2o E3 subcomplexes (Fig. 5) can be thought of as a label of the E3 binding domain. Note that the E3 binding domain is itself too small (M, 6000) to be visualized directly. The apparent gap of up to 3–5 nm which is frequently observed between the surfaces of the E3 and the E2 core complex is consistent with the extended conformation predicted for the (Ala-Pro)-rich connector sequence. A portion of the gap could be because of the E3 binding domain, which is in a folded conformation (45). The faint bridges of material which are occasionally visible in the gap regions might correspond to the (Ala-Pro)-rich sequences. According to the segmental structural model the (Ala-Pro)-rich sequences are also flexible. This flexibility probably accounts for the wide distribution of locations observed for the positions of the bound E3 subunits relative to the E2o core.

E2o and E2p Cores—Electron micrographs of frozen-hydrated E2p cores are also consistent with an extended, flexible structure for the (Ala-Pro) rich sequences. Images of E2p cores (Fig. 4B) often appear surrounded by a halo of granary or fuzzy material which extends out to about 10 nm from the surface of the cores. We believe that this fuzzy material corresponds to the N-terminal half of the E2p polypeptide, which contains the three lipoyl-bearing domains and (Ala-Pro)-rich sequences.

Fuzzy material surrounding E2p cores has been described previously in electron micrographs of negatively stained specimens (2, 12). In those studies the fuzziness was also attributed to the lipoyl domains, but it did not appear to extend as far from the surface of the core as we have observed for the frozen-hydrated specimens.

In micrographs of nonstained, freeze-dried E2p cores obfained by scanning transmission electron microscopy, a fuzzy background was not detected, but the edge length of the core (in the 4-fold orientation) was reported to be 20 nm (22), significantly larger than the length determined from negatively stained and freeze-hydrated specimens, about 12 nm. An explanation for the larger edges observed in the freeze-dried specimens is that during the preparation for microscopy, which included extensive cross-linking with glutaraldehyde, the lipoyl domains collapsed from their native extended conformation onto the surface of the cube-shaped mass formed by the catalytic domains. In contrast to these results, a study by scanning transmission electron microscopy of freeze-dried E2 cores derived from a mammalian branched chain α-ketoacid dehydrogenase indicated that the lipoyl domains extend from the surface of the core by up to 5 nm (44). This E2 complex, like the E2o core studied here, contains just 1 lipid acid residue/E2 polypeptide.

E2o–E1 Subcomplexes—Cryoelectron microscopy shows that the mode of E1 binding to the E2 complex appears essentially identical to that of E3 (Fig. 6). A gap of 2–4 nm is frequently present between the bound E1 and the E2o, and sometimes a thin strand of density appears in the gap region. The bridging density is more easily visualized in the E2o–E1 subcomplexes than in the E2p–E3 subcomplexes, possibly indicating that it represents more mass than the E2o–E3 bridges. The locations at which the bridging densities appear to emanate from the E2o complex are along the edges and displaced from their midpoints. A specialized domain for binding the E1 subunit has not yet been identified in the E2o or E2p amino acid sequences, and it is also clear from the sequences that no further (Ala-Pro)-rich segments are present to link such a domain to the catalytic domain (8, 11). From our results we cannot yet ascertain whether the bridging density corresponds to polypeptide derived from E2o, E1, or both. Finally, the distribution of positions of the bound E1 relative to the E2o core complex is suggestive of a flexible coupling between the two.

Evidence is mounting (48–50) for the hypothesis that the (Ala-Pro)-rich sequences confer mobility and an extended reach to the lipoyl domains thereby enabling the lipoy acid moieties to visit active sites that are widely separated in the complexes, much greater than the 2.5-nm separation that would be permitted by the lipoyl-lysine moiety alone. The results presented here for the E2o–E3 subcomplexes support this type of structural model for the (Ala-Pro)-rich regions. Additionally, evidence that both E1 and E3 may be linked to the E2o cores by extended flexible regions of polypeptide raises the possibility that active site coupling in 2-oxoglutarate dehydrogenase complex and pyruvate dehydrogenase complex also involves movements of the E1 and E3 subunits.

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Cryoelectron Microscopy of α-Ketoacid Dehydrogenase Complexes

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