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A computer model analysis of the active-site coupling mechanism in the pyruvate dehydrogenase multienzyme complex of *Escherichia coli*

(lipoic acid function/multiple random coupling mechanism/limited proteolysis)

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ABSTRACT **A computer modeling system developed to analyze experimental data for inactivation of the *Escherichia coli* α-ketoglutarate dehydrogenase complex (KGDC) accompanying release of lipoyl moieties by lipoamidase and by trypsin [Hackert, M. L., Oliver, R. M. & Reed, L. J. (1983) *Proc. Natl. Acad. Sci. USA* 80, 2226–2230] was used to analyze analogous data for the *E. coli* pyruvate dehydrogenase complex (PDC). The model studies indicate that the activity of PDC, as found for KGDC, is influenced by redundancies and random processes, which we describe as a multiple random coupling mechanism. In both complexes more than one lipoyl moiety services each pyruvate dehydrogenase (EC 1.2.4.1) or α-ketoglutarate dehydrogenase (EC 1.2.4.2) (E_1) subunit, and an extensive lipoyl–lipoyl interaction network for exchange of electrons and possibly acyl groups must also be present. The best fit between computed and experimental data for PDC was obtained with a model that has four lipoyl domains with four or, more probably, eight lipoyl moieties servicing each E_1 subunit. The lipoyl–lipoyl interaction network for PDC has lipoyl domain interactions similar to those found for KGDC plus the additional possibility of interaction of a lipoyl moiety and its paired mate on each dihydrolipoamide acetyltransferase (EC 2.3.1.12) (E_2) subunit. The two lipoyl moieties on an E_2 subunit in PDC appear to be functionally indistinguishable, each servicing the acetyltransferase site of that E_2 subunit and a dihydrolipoamide dehydrogenase (EC 1.6.4.3) (E_3) subunit if the latter is bound to that particular E_2 subunit. The observed difference between inactivation of PDC by lipoamidase and by trypsin appears to be due to dead-end competitive inhibition by lipoyl domains that have been modified by excision of lipoyl moieties by lipoamidase.**

The pyruvate and α-ketoglutarate dehydrogenase multienzyme complexes (PDC and KGDC, respectively) of *Escherichia coli* each consist of three enzymes: pyruvate dehydrogenase (EC 1.2.4.1) or α-ketoglutarate dehydrogenase (EC 1.2.4.2) (E_1), dihydrolipoamide acetyltransferase (EC 2.3.1.12) or dihydrolipoamide succinyltransferase (EC 2.3.1.61) (E_2), and dihydrolipoamide dehydrogenase (EC 1.6.4.3) (E_3). In both complexes the E_2 component forms a structural core, composed of 24 subunits arranged with octahedral 432 symmetry in a cube-like particle, to which multiple copies of E_1 and E_3 are bound by noncovalent bonds (1, 2). Lipoic acid residues covalently attached to the ε-amino group of lysine residues of E_2 transfer intermediates between the catalytic sites of the component enzymes. There are two lipoyl moieties on each E_2 subunit of the *E. coli* PDC but only one lipoyl moiety per E_2 subunit of the KGDC. The lipoyl moieties are located on protruding regions (lipoyl domains) of the E_2 subunits that are readily excised from the E_2 inner core by proteases (3, 4). Stepp *et al.* (5) used trypsin and lipoamidase to probe the role of lipoyl moieties in PDC and KGDC. The results showed that release of lipoyl domains by trypsin and release of lipoyl moieties by lipoamidase proceeded at rates faster than the accompanying loss of overall activity of the two complexes. Of particular note was the finding that nearly one-half of the lipoyl domains in PDC could be removed before any appreciable loss of activity occurred. The results with PDC were confirmed and extended by Berman *et al.* (6). Hackert *et al.* (7) used computer modeling to quantitatively analyze the inactivation curves obtained for trypsin and lipoamidase treatments of KGDC (5). The model studies indicated that each E_1 subunit is serviced by two lipoyl moieties (i.e., lipoyl domains) and that active site coupling by an extensive interacting network of lipoyl moieties is apparently a characteristic feature of the native complex. This computer modeling system has been extended to analyze the more complicated inactivation curves obtained for trypsin and lipoamidase treatments of PDC (5). These studies are discussed here.

MODELS AND COMPUTATIONAL PROCEDURE

Computer models used to study the roles of lipoyl moieties in the *E. coli* PDC and KGDC were based on our current understanding of the structure and mechanism of these complexes. The cube-like inner cores of the acetyltransferase and the succinyltransferase are very similar in size and morphology (8–10), whereas the protruding lipoyl domains differ in molecular weight by a factor of about 3, i.e., 31,600 and 11,000, respectively (refs. 3 and 11; unpublished data).

Although there is disagreement concerning the number of E_1 and E_3 subunits that are combined with E_2 in PDC (12, 13), we assume that the stoichiometry of the native complex is $24E_1{:}24E_2{:}12E_3$ (14, 15). Two E_1 subunits are thought to be associated with each of the 12 edges of the E_2 inner core (2). The reaction catalyzed by E_1 is apparently rate limiting in the overall reaction (16–18). E_3 is present in PDC as dimers (19, 20). One E_3 dimer is located on each of the six faces of the E_2 inner core of the complex, randomly bound in one of two equivalent orientations to diagonally opposed morphological units of E_2 (2). Thus a population of particles rather than one unique particle represents the "native" structure in this multienzyme complex.

The indexing scheme developed to uniquely identify each lipoyl moiety and enzyme subunit in KGDC (7) was extended to PDC. The inner core of E_2 is represented by a cube whose faces are assigned numbers from 1 to 6 (Fig. 1). For conve-

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Abbreviations: PDC, pyruvate dehydrogenase complex; KGDC, α-ketoglutarate dehydrogenase complex; E_1, pyruvate dehydrogenase; E_2, dihydrolipoamide acetyltransferase; E_3, dihydrolipoamide dehydrogenase.

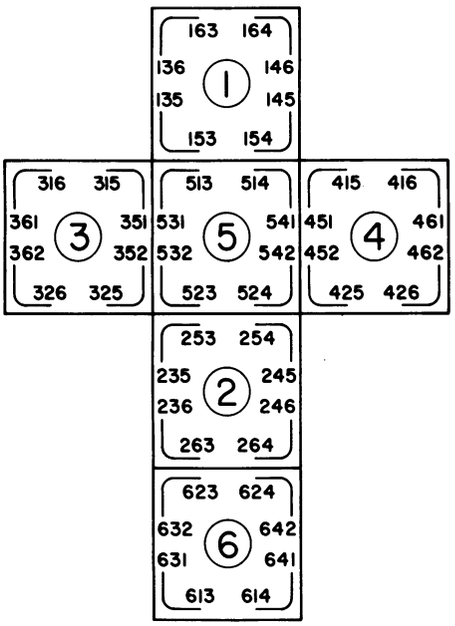

FIG. 1. Indexing scheme used in the model studies. A unique three-digit index is used to identify the relative positions of each of the 48 lipoyl moieties of the pyruvate dehydrogenase complex. The E_2 core is shown as an "unfolded" cube.

nience, each lipoyl moiety is shown as associated with a particular edge and vertex of the E_2 core structure. The first index number indicates the face of the cube on which that lipoyl moiety resides. First and second numbers indicate the particular edge with which that lipoyl moiety is associated. All three numbers associate the lipoyl moieties with a particular vertex. Note that the two lipoyl moieties related on a lipoyl domain can be referenced by exchanging the last two digits, and lipoyl moieties related within a morphological unit by the threefold axis of symmetry can be referenced by a cyclic permutation of the indices. With this scheme the environment of each lipoyl moiety can be specified. Subsets of the 48 indices are used to index the enzyme subunit arrangements so that various models for lipoyl–E_1, lipoyl–lipoyl, lipoyl–E_2, and lipoyl–E_3 interactions can be studied. For convenience, the models tested are also coded (*PQRS*) according to the type of lipoyl interactions employed. Thus, a PDC-4322 model has four lipoyl moieties servicing each E_1 subunit ($P = 4$), each lipoyl moiety capable of interacting with three nearby lipoyl moieties ($Q = 3$), and with both lipoyl moieties on an E_2 subunit capable of servicing both the acetyltransferase site of that E_2 subunit ($R = 2$) and an E_3 subunit ($S = 2$) if the latter is bound to that particular E_2 subunit.

As for KGDC (7), a computer program was written that initially constructs a model of the "native" complex consisting of the E_2 core with its full complement of 48 lipoic acid residues, 24 E_1 subunits, and 12 E_3 subunits which were allowed to bind randomly as six dimers. Lipoic acid residues were then removed from this "native" complex on a random basis, individually to mimic the lipoamidase treatment and pairwise to simulate the trypsin treatment. The "activity" of the modified complex was calculated by determining the fraction of E_1 subunits that remain coupled to E_2 and E_3, with different results being obtained depending on the lipoyl interactions allowed for a particular model. Each calculated inactivation curve was fitted to 25 points, each point representing the average value for 1,000 such randomly treated molecules of the complex. In this manner inactivation curves could be computed for selected patterns of lipoyl interactions of the types discussed above.

RESULTS

For PDC, in contrast to KGDC, the experimental inactivation curves for lipoamidase and trypsin treatments are markedly different (5). Limited tryptic digestion does not affect overall activity of PDC until about 50% of the lipoyl moieties (i.e., lipoyl domains) are removed (Fig. 2). Because trypsin excises lipoyl domains, each containing two lipoyl moieties, the computed trypsin inactivation curves cannot distinguish between models in which either one or two lipoyl moieties per domain service an E_1 subunit. Thus models of the type PDC-*P* for the series $P = 1, 2, 3$, or 4, in which one lipoyl moiety per lipoyl domain services an E_1 subunit, cannot be distinguished from models of the type PDC-*P* for the series $P = 2, 4, 6$, or 8, in which two lipoyl moieties per lipoyl domain service an E_1 subunit (Fig. 2). For these calculations each lipoyl moiety was assumed to have access to E_2 and E_3; therefore these curves represent a maximal possible activity for each *P*-type model. The results demonstrate that at least three or, more probably, four lipoyl domains can service each E_1 subunit. However, the predicted activities for the PDC-4 model (Fig. 2, curve 4) are too high when most of the lipoyl moieties have been removed. Therefore, the effects of introducing constraints on the access of lipoyl moieties to E_2 and E_3 were studied. Because there are two lipoyl moieties per E_2 subunit, models of the general class PDC-*PQRS* were explored, in which *Q* could have values ranging from 1 to 11 and *R* and *S* could have values of either 1 or 2. All models involving $P = 3$ (or 6) and any of the limited lipoyl–lipoyl interaction networks produced calculated trypsin/PDC inactivation curves too low to fit the experimental data (not shown). Therefore, we conclude that four lipoyl domains service each E_1 subunit.

The effect of different lipoyl–lipoyl interaction patterns on the shape of the calculated trypsin/PDC inactivation curves is illustrated by selected results from models of the type PDC-4*Q*22 shown in Fig. 3. Lipoyl–lipoyl interaction networks that restrict the potential for electron pair exchange to the edge of the E_2 inner core yield results similar to curve 1 (i.e., $Q = 3B$, in which each lipoyl moiety can exchange only with the three

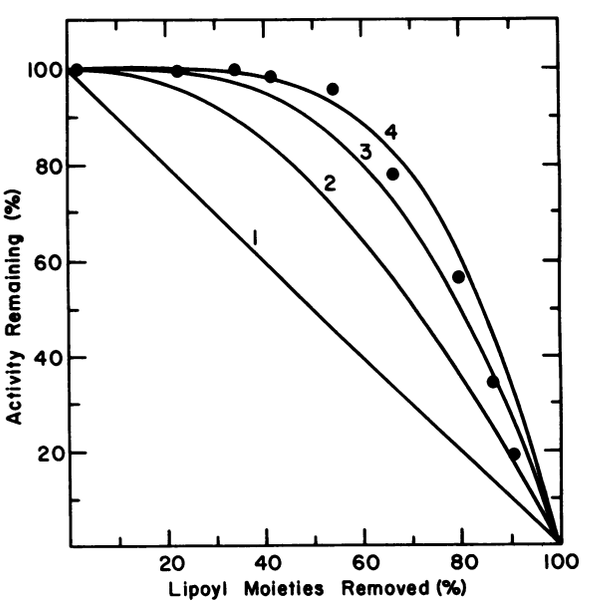

FIG. 2. Computed curves for inactivation of PDC by trypsin for models of the type PDC-*P*, illustrating the effect of varying the number of lipoyl domains that service an E_1 subunit. Curves 1–4 were computed from models in which $P = 1, 2, 3$, and 4, respectively. Experimental data points are represented by ●.

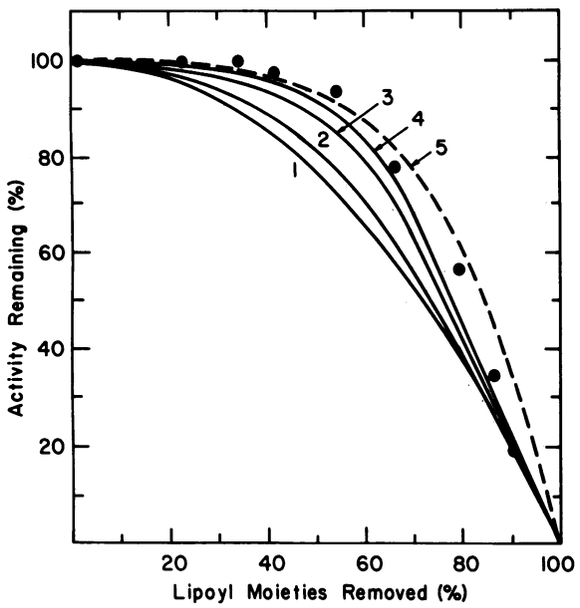

FIG. 3. Computed curves for inactivation of PDC by trypsin for models of the type PDC-4Q22. Curves 1 and 2 were computed from models with limited lipoyl–lipoyl interaction networks (Q = 3B and 2, respectively). Curves 3 and 4 were computed from models with extensive networks (Q = 3A and 4, respectively). Curve 5 is the P = 4 limiting case from Fig. 2. Experimental data points are represented by ●.

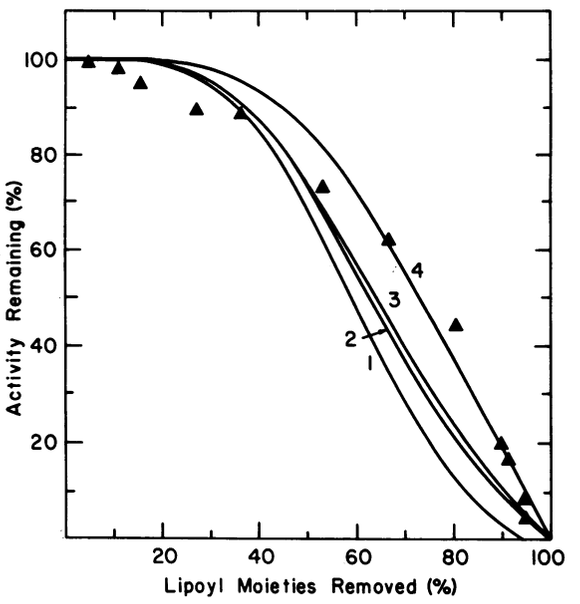

FIG. 4. Computed curves for inactivation of PDC by lipoamidase for models of the type PDC-44RS, illustrating the effect of limiting access to active sites on E_2 and E_3 to only one of the pair of lipoyl moieties on a lipoyl domain. Curves 1–4 were computed from models PDC-4411, -4421, -4412, and -4422, respectively. Experimental data points are represented by ▲.

other lipoyl moieties related to an edge). Curve 2 results from a model in which the lipoyl interaction network is confined to a morphological unit of the E_2 inner core (i.e., Q = 2, in which each lipoyl moiety can interact with the lipoyl moiety with which it is paired on the same lipoyl domain and one lipoyl moiety on the adjacent domain that is part of the same morphological unit). The model giving rise to curve 3 permits each lipoyl moiety to interact with its paired mate on its lipoyl domain and with an additional lipoyl moiety on each of two other lipoyl domains (Q = 3A). Curve 4 results from a Q = 4 pattern in which each lipoyl moiety interacts with its paired mate and with an additional lipoyl moiety on each of the three other domains that define an edge of the E_2 core. Models for Q = 3A and Q = 4 yield very similar results because they both incorporate lipoyl–lipoyl interactions that form a closed network over the whole of the E_2 core. Caution should be exercised not to over-interpret the importance of small differences in the computed curves. However, it is clear that only those models that generate a complete lipoyl–lipoyl interaction network give calculated results similar to those observed experimentally, with Q = 4 providing slightly better agreement than does Q = 3A.

Models of the type PDC-$PQRS$ with P = 4 (or 8), Q = 4, and R = S = 2 provide reasonable fits between computed and experimental curves for the trypsin/PDC inactivation data. However, even the PDC-4422 model predicts too high an activity for the initial phase of lipoamidase inactivation of PDC as shown in curve 4 of Fig. 4. Because trypsin releases lipoyl moieties in pairs, the computed trypsin/PDC inactivation curves (Fig. 3) are independent of whether R and S values are 1 or 2. However, because lipoamidase removes lipoyl moieties one at a time, it is possible to determine the effect of limiting access to E_2 and E_3 catalytic sites to only one of the pair of lipoyl moieties on a given lipoyl domain—i.e., imposing functional nonequivalence on the two lipoyl moieties. They are, of course, structurally nonequivalent. The results are shown in Fig. 4. This limitation does bring about a decrease in the predicted residual PDC activity but does not improve the agreement between the computed and experimental curves. All four curves still reflect too high an initial retention of activity followed by too sharp a fall-off during the later stages of lipoamidase inactivation of PDC.

Because lipoamidase treatment removes the lipoyl moieties without removing the lipoyl domain, the possibility of the modified lipoyl domains, lacking one or both lipoyl moieties, serving as dead-end competitive inhibitors was examined. Fig. 5 presents the inhibition results for the PDC-4422 model (solid curves). Curve 3 corresponds to no inhibition by modified lipoyl domains, and curve 1 corresponds to the results obtained on the assumption that modified lipoyl domains are fully com-

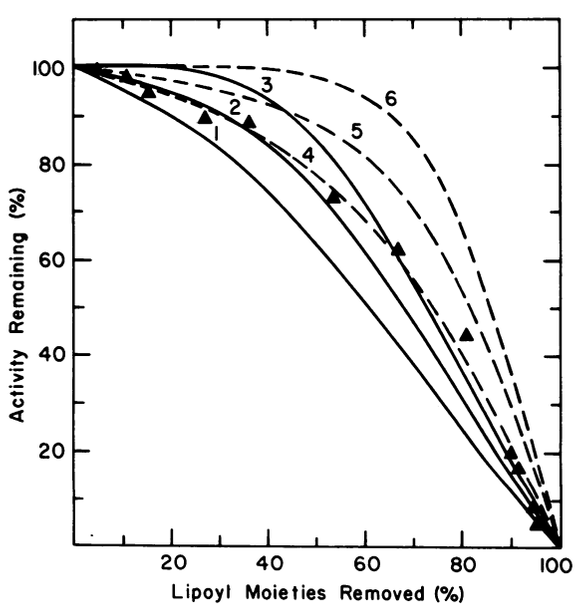

FIG. 5. Computed curves for inactivation of PDC by lipoamidase illustrating the effects of competitive dead-end inhibition by the modified lipoyl domains. Curves 1–3 refer to model PDC-4422 with full, one-half, and no inhibition, respectively. Curves 4–6 refer to model PDC-8422 with full, one-half, and no inhibition, respectively. Experimental data points are represented by ▲.

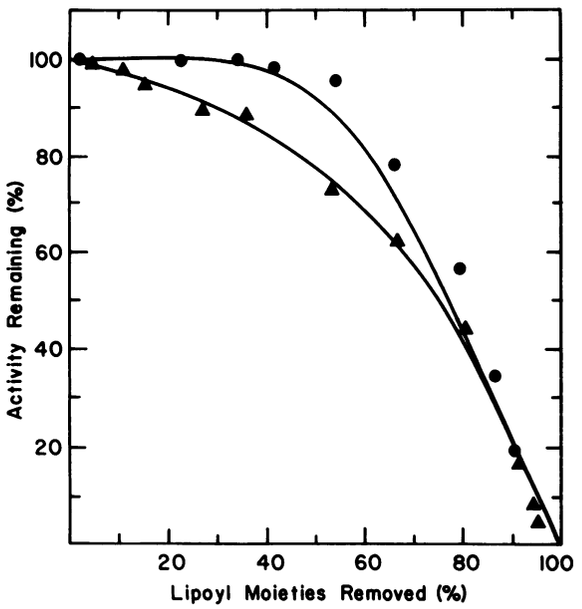

FIG. 6. Computed (solid lines) and experimental curves for inactivation of PDC by trypsin (●) and by lipoamidase (▲) for model PDC-8922.

petitive with unmodified lipoyl domains in servicing E_1 subunits. Curve 2 represents the results obtained if each modified domain is assumed to be only half effective in competing with the unmodified domains for E_1 service. The latter results give the best fit obtained thus far for the lipoamidase data.

The use of dead-end competitive inhibition produces the correct trend in the computed lipoamidase/PDC inactivation curve, but it predicts too low an activity profile. Because the computed trypsin/PDC inactivation results were indistinguishable for $P = 4$ and 8, the dead-end competitive inhibition analysis was carried out for the PDC-8422 model (Fig. 5, broken lines). The improved fit suggests the rather surprising result that the preferred model has $P = 8$, in which both lipoyl moieties on each of four lipoyl domains can service an E_1 subunit, and the modified domains act as full dead-end competitive inhibitors. Because the $P = 8$ model implies that the two lipoyl moieties on a lipoyl domain act independently in servicing an E_1 subunit, a more interactive lipoyl network might also be expected. Therefore, an analysis was carried out for a PDC-8922 model, which generates trypsin/PDC and lipoamidase/PDC inactivation curves similar to those computed for the PDC-8422 model. The new model also has a complete lipoyl network, but with the advantage that the new model employs a lipoyl domain interaction network similar to that found for the KGDC (7). The agreement of the computed results for a PDC-8922 model, invoking full dead-end competitive inhibition, and the experimental curves for trypsin and lipoamidase inactivation of PDC is shown in Fig. 6. A reaction diagram representing the interactions implicit in a model of the PDC-8922 type is shown in Fig. 7. In either case, $P = 4$ or $P = 8$, the appearance of the curves in Figs. 5 and 6 is certainly highly suggestive that dead-end inhibition by modified lipoyl domains is probably responsible for the differences in the lipoamidase/PDC and trypsin/PDC results.

DISCUSSION

PDC and KGDC are very similar in their structural organization. The obvious differences are that PDC contains twice as many E_1 subunits and lipoyl moieties as does KGDC and that the mass of the lipoyl domain in the acetyltransferase subunit,

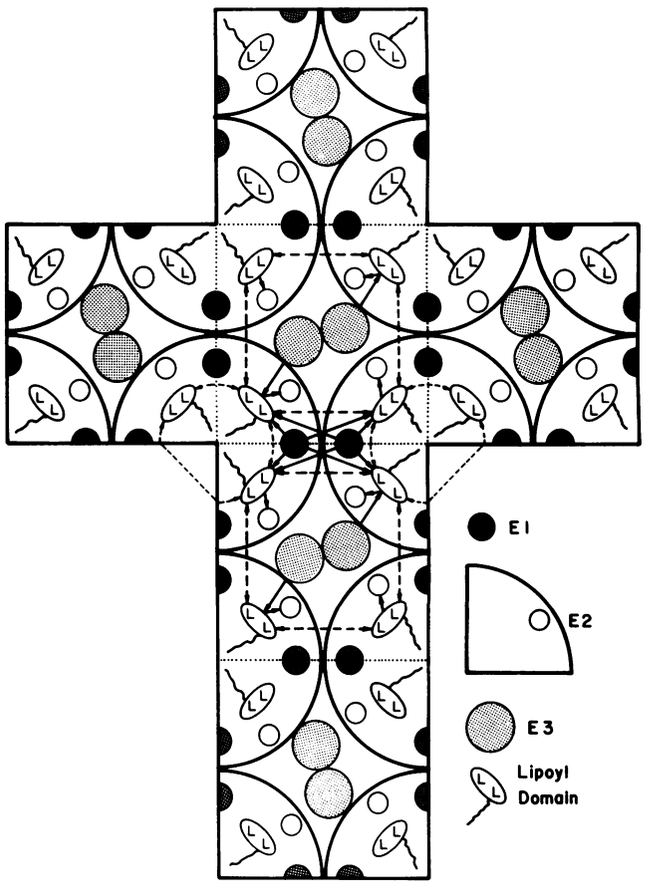

FIG. 7. Reaction diagram illustrating the coupling of active centers in model PDC-8922. For simplicity only representative interactions involving the central edge are shown.

with its two lipoyl moieties, is about three times the mass of the succinyltransferase lipoyl domain. The experimental inactivation curve for lipoamidase treatment of PDC is similar to that observed with KGDC—i.e., a slight decline in activity at the beginning, followed by a sharp decline (5). Apparently E_1 subunits in both complexes are served by more than one lipoyl moiety. In contrast, the trypsin inactivation curves are markedly different. The overall activity of PDC is not affected until about 50% of the lipoyl domains have been removed. Because trypsin releases lipoyl moieties in pairs by virtue of removal of an entire lipoyl domain, this result suggests that E_1 subunits are serviced by more than one lipoyl domain. A computer model analysis of the lipoamidase/KGDC and trypsin/KGDC inactivation curves, utilizing differing interaction patterns for lipoyl–E_1, lipoyl–lipoyl, lipoyl–E_2, and lipoyl–E_3, showed the best fit between computed and experimental data was obtained with a model that had two lipoyl moieties (on two lipoyl domains) servicing each E_1 subunit and an extensive interacting network of lipoyl moieties (7). Computer simulation of trypsin inactivation of PDC indicates that four lipoyl domains can probably service each E_1 subunit. Although this result is independent of particular structural models, the structure of the acetyltransferase (E_2) core and the postulated distribution of E_1 subunits on the E_2 core implies that each lipoyl domain probably interacts with each of the dyad-related E_1 subunits on an edge and also must be able to service two such edges of the E_2 core. Such service involves four or possibly eight lipoyl moieties per E_1 subunit, implying that the relative orientation of these components must not be very critical.

The extent and geometry of the relay network in PDC was also investigated by observing the effect of different lipoyl–li-

poyl interaction networks on the computed inactivation curves. The results shown in Fig. 3 indicate that each lipoyl moiety has the capability to interact with at least three other lipoyl moieties. As with KGDC, it appears that a closed network that couples all lipoyl moieties is a characteristic of the native complex. A reaction diagram illustrating the best model for PDC is shown in Fig. 7.

Insight into the possibility of different functions for the two lipoyl moieties residing on an E_2 subunit can be gained from the lipoamidase/PDC inactivation results. The shapes of the lipoamidase/PDC and the trypsin/PDC inactivation curves differ significantly (Fig. 6). Our studies indicate that this result is best explained by dead-end inhibition by modified lipoyl domains. If one lipoyl moiety, or both, is removed by lipoamidase, the modified lipoyl domain could still interact with the catalytic site of an E_1 subunit and thereby decrease its activity. Because trypsin releases an entire lipoyl domain, dead-end inhibition during trypsin treatment is precluded. Although the native PDC has two structurally different sets of 24 lipoyl moieties, the two lipoyl moieties on each E_2 subunit appear, in the computer simulation studies, to be functionally equivalent. This finding is consistent with previous reports that all 48 lipoyl moieties in PDC are reductively acetylated in the presence of pyruvate and the absence of CoA, that all acetyl groups on the dihydrolipoyl moieties can be transferred to CoA (16, 17, 21, 22), and that all lipoyl moieties are functionally connected to E_3 (22, 23). These findings argue against proposals that there are two functional classes of lipoyl moieties in the acetyltransferase, one class participating in the normal catalytic mechanism and the other class being nonessential (17) or having a different but as yet undetermined catalytic function (24).

The lipoamidase/PDC inactivation results are similar to those obtained by pyruvate-dependent inactivation of PDC by *N*-ethylmaleimide in that chemical modification of lipoic acid residues proceeds faster than the accompanying loss of enzymatic activity (25). The *N*-ethylmaleimide/PDC inactivation results were interpreted qualitatively to imply that one lipoic acid residue can take over the function of another. Our finding that dead-end competitive inhibition by modified lipoyl domains must be invoked to account for the marked difference in the lipoamidase/PDC and trypsin/PDC inactivation data suggests that this inhibition may also be involved in the *N*-ethylmaleimide/PDC inactivation results. Inactivation of a lipoyl moiety by covalent modification with *N*-ethylmaleimide is functionally equivalent to release of a lipoyl moiety by lipoamidase. In either case the modified lipoyl domain could act as a dead-end inhibitor, competing with an unmodified lipoyl domain for access to catalytic sites on PDC. It is interesting to note in this connection that for KGDC, in contrast to PDC, the experimental inactivation curves for lipoamidase and trypsin treatments are essentially identical (5). Apparently, a lipoic acid-less (i.e., modified) lipoyl domain in KGDC does not act as an effective dead-end inhibitor. This may be due, at least in part, to the fact that the mass of the lipoyl domain in a succinyltransferase subunit is only one-third that of the lipoyl domain in an acetyltransferase subunit.

It should be emphasized that although the high symmetry of the E_2 core in PDC and KGDC is important in establishing the framework for activity relationships, the particular pathways through which the overall activity is manifested appear to be influenced by a number of random processes, which we have described as a multiple random coupling mechanism (7). Thus the unique nature of the oligomeric E_2 core, with its extended lipoyl domains, apparently obviates the necessity for a particular juxtapositioning of the three catalytic components while still ensuring an efficient coupling of their activities.

In summary, the computer simulation studies of inactivation of PDC by release of lipoyl moieties or lipoyl domains suggest: (*i*) that each E_1 subunit is serviced by four lipoyl domains; (*ii*) that active site coupling by an extensive interacting network of lipoyl moieties is apparently a characteristic feature of the native complex; (*iii*) that, although two structural classes of lipoyl moieties must exist in PDC, there is no evidence from these studies that there are any functional differences between the two sets of lipoyl moieties; and (*iv*) that dead-end competitive inhibition by lipoic acid-less lipoyl domains accounts for the observed differences between lipoamidase/PDC and trypsin/PDC inactivation curves.

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