# Lipoic acid residues in a take-over mechanism for the pyruvate dehydrogenase multienzyme complex of *Escherichia coli*

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The pyruvate dehydrogenase complex of *Escherichia coli* contains two lipoic acid residues per dihydrolipoamide acetyltransferase chain, and these are known to engage in the part-reactions of the enzyme. The enzyme complex was treated with trypsin at pH 7.0, and a partly proteolysed complex was obtained that had lost almost 60% of its lipoic acid residues although it retained 80% of its pyruvate dehydrogenase-complex activity. When this complex was treated with *N*-ethylmaleimide in the presence of pyruvate and the absence of CoASH, the rate of modification of the remaining *S*-acetyldihydrolipoic acid residues was appoximately equal to the accompanying rate of loss of enzymic activity. This is in contrast with the native pyruvate dehydrogenase complex, where under the same conditions modification proceeds appreciably faster than the loss of enzymic activity. The native pyruvate dehydrogenase complex was also treated with lipoamidase prepared from *Streptococcus faecalis*. The release of lipoic acid from the complex followed zero-order kinetics for most of the reaction, whereas the accompanying loss of pyruvate dehydrogenase-complex activity lagged substantially behind. These results eliminate a model for the enzyme mechanism in which specifically one of the two lipoic acid residues on each dihydrolipoamide acetyltransferase chain is essential for the reaction. They are consistent with a model in which the dihydrolipoamide acetyltransferase component contains more lipoic acid residues than are required to serve the pyruvate decarboxylase subunits under conditions of saturating substrates, enabling the function of an excised or inactivated lipoic acid residue to be taken over by another one. Unusual structural properties of the enzyme complex might permit this novel feature of the enzyme mechanism.

The pyruvate dehydrogenase multienzyme complex of *Escherichia coli* catalyses the following reaction:

$$Pyruvate + NAD^+ + CoASH \rightarrow$$
$$acetyl\text{-}SCoA + NADH + H^+ + CO_2$$
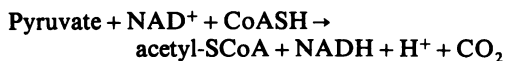
The complex is composed of multiple copies of three different types of polypeptide chain responsible for

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Abbreviations used: E1, pyruvate decarboxylase (EC 1.2.4.1); E2, dihydrolipoamide acetyltransferase (EC 2.3.1.12); E3, lipoamide dehydrogenase (EC 1.6.4.3).

the three constituent enzymic activities: pyruvate decarboxylase (E1) (EC 1.2.4.1), dihydrolipoamide acetyltransferase (E2) (EC 2.3.1.12) and lipoamide dehydrogenase (E3) (EC 1.6.4.3) [for reviews see Reed (1974) and Perham (1975)]. The E2 component forms the structural core of the complex and appears to comprise 24 polypeptide chains arranged with octahedral symmetry (Reed, 1974; Danson *et al.*, 1979). Each E2 chain contains two lipoyl-lysine residues that become reductively acetylated in the presence of pyruvate (Danson & Perham, 1976; Collins & Reed, 1977; Speckhard *et al.*, 1977), and undergo reoxidation by the lipoamide dehydrogenase components and NAD+ (Collins & Reed, 1977; Danson *et al.*, 1981). They act by moving between enzymes as part of the mechanism (Koike *et al.*, 1963; Ambrose & Perham, 1976; Grande *et al.*, 1976). It has been shown further that these lipoyl-lysine 'swinging arms' form an interacting

network in the enzyme, able to pass acetyl groups about within the E2 core by means of intra-molecular transacetylation reactions (Bates *et al.*, 1977; Collins & Reed, 1977; Danson *et al.*, 1978*a*,*b*). This important feature of active site coupling is common to 2-oxo acid dehydrogenase complexes from a wide range of sources (Collins & Reed, 1977; Cate & Roche, 1979; Stanley *et al.*, 1981).

In earlier experiments with the pyruvate dehydrogenase complex from *E. coli* we showed that modification of the lipoic acid residues by treatment with *N*-ethylmaleimide in the presence of pyruvate or NADH was accompanied by loss of enzymic activity, but that the rate of chemical modification was appreciably greater than the rate of loss of enzymic activity (Ambrose-Griffin *et al.*, 1980; Danson *et al.*, 1981). This surprising result, which indicated that a full complement of lipoic acid residues was not essential for catalytic activity, was evidently related to the independent finding that about half the lipoic acid residues could be released from the enzyme complex by limited proteolysis with trypsin without major loss of overall catalytic activity (Bleile *et al.*, 1979). Two simple models were advanced to explain the results of the chemical modification experiments: either one lipoic acid residue per E2 chain is essential and the other residue is inessential for the complex reaction as conventionally measured, or the function of a chemically modified lipoic acid residue can be taken over by an unmodified one (Ambrose-Griffin *et al.*, 1980). The experimental results gave a marginally better fit to the first of these two models, which was also more easily reconciled with the report (Frey *et al.*, 1978) that only half the lipoic acid residues in the E2 core are interacting with the E3 (lipoamide dehydrogenase) component, but we pointed out that it was impossible to choose unequivocally between the models. However, it is now clear that all the lipoic acid residues in the E2 core can be coupled with the E3 component (Danson *et al.*, 1981; Collins & Reed, 1977).

The enzyme lipoamidase from *Streptococcus faecalis* (Koike & Suzuki, 1970) can specifically hydrolyse the amide bond of lipoyl-lysine and thus release lipoic acid from the pyruvate dehydrogenase complex (Suzuki & Reed, 1963). In the present paper we describe the kinetics of this release and the accompanying inactivation of the enzyme complex. We have also analysed the chemical modification with *N*-ethylmaleimide of pyruvate dehydrogenase complex partly proteolysed with trypsin. The results extend our earlier findings (Ambrose-Griffin *et al.*, 1980; Danson *et al.*, 1981) and cause us to opt for the second model, a take-over system among an apparent surplus of lipoic acid residues, as the mechanism of the enzyme complex.

Materials and methods

Materials

Protamine sulphate was obtained from ICN Pharmaceuticals, Life Sciences Group, Cleveland, OH, U.S.A. $Na_2{}^{35}SO_4$ (SJS 1) and *N*-ethyl[2,3-^{14}C]maleimide (CFA.293) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were of analytical-reagent grade and are listed by Danson *et al.* (1978*a*).

Enzyme and enzyme assays

Pyruvate dehydrogenase multienzyme complex was purified from a pyruvate dehydrogenase-constitutive mutant of *E. coli* K12 as described by Danson *et al.* (1979). ^{35}S-labelled pyruvate dehydrogenase complex was isolated from bacteria that had been grown in the presence of [^{35}S]-sulphate as described by Hale & Perham (1979*a*), except that the growth medium was supplemented with unlabelled methionine (0.1 g/l) in order to prevent incorporation of radiolabel into that amino acid. Preliminary experiments established that the extent of incorporation of radioactivity into cysteine and lipoic acid was unaffected under these conditions (Hale, 1977). The whole-complex and E3 enzymic activities were assayed spectrophotometrically in the direction of NAD$^+$ reduction (Danson *et al.*, 1978*a*).

Purification of lipoamidase

Streptococcus faecalis strain 10C1, obtained from the American Type Culture Collection (A.T.C.C. 11700), was grown aerobically in 40 litres of lipoic acid-deficient synthetic medium (Gunsalus *et al.*, 1952) to yield 67 g of wet cell paste. The cells were suspended in 200 ml of 20 mM-sodium phosphate buffer, pH 7.0, containing 2 mM-EDTA and 0.02% NaN$_3$, and sonicated in 50 ml batches in a Dawe Soniprobe operated at 90 W for a total of 15–20 min. This and all subsequent steps were performed at 4°C. After removal of the cell debris by centrifugation at 53 700 g for 20 min, lipoamidase was purified by fractionation with protamine sulphate followed by chromatography on DEAE-cellulose as described by Koike & Suzuki (1970). The enzyme eluted from the DEAE-cellulose column was gel-filtered on a Sephadex G-100 column (1.6 m × 7 mm) in 20 mM-sodium phosphate buffer, pH 7.0, containing 2 mM-EDTA and 0.02% NaN$_3$, but this did not give much increase in purity, as most of the protein, together with the lipoamidase activity, was eluted at the void volume of the column. The final specific activity was about 33 units/mg and the total yield was 42 units (1.3 mg of protein). It is unlikely that the enzyme was pure, as several bands were observed after sodium dodecyl sulphate/polyacryl-amide-gel electrophoresis, but it was free of detect-

able proteolytic activity towards the pyruvate dehydrogenase complex under our experimental conditions, as judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. This was not true of the enzyme preparation before the gel-filtration step.

Lipoamidase activity was assayed by mixing samples with $10 \mu g$ of *E. coli* pyruvate dehydrogenase complex in $100 \mu l$ of 20mM-sodium phosphate buffer, pH 7.0, containing 2mM-EDTA and 0.02% NaN_3. After 30min incubation at 30°C, pyruvate dehydrogenase activity was measured and compared with that of a control that had not been treated with lipoamidase. Because the kinetics of loss of pyruvate dehydrogenase activity were markedly non-linear (see the Results section), this assay was only approximately quantitative. One unit of lipoamidase activity was defined as the amount required to diminish the activity of the complex by 50% in this assay.

Measurement of release of [^{35}S]lipoic acid

Samples of ^{35}S-labelled pyruvate dehydrogenase complex ($15-20 \mu l$; 0.6 mg/ml) that had been treated with lipoamidase were added to 0.3 ml of 0.25 M-HCl in a plastic scintillation vial. To this was added 2.5 ml of toluene containing 2,5-diphenyloxazole (5 g/l). The liquids were vigorously mixed for 10s and then centrifuged at 300 g for 1 min. The upper layer (containing any free [^{35}S]lipoic acid) was carefully removed to another vial by means of a Pasteur pipette, and the extraction was repeated. The two organic extracts were combined, and 3.0 ml of scintillant [toluene/Triton X-100 (2:1, v/v) containing 2,5-diphenyloxazole (5 g/l)] was added to the aqueous layer. Radioactivity was counted in an LKB Rackbeta liquid-scintillation counter. Control experiments involving six cycles of extraction showed that 96–97% of the extractable radioactivity was recovered in the first two extracts.

Limited proteolysis of pyruvate dehydrogenase complex

Native pyruvate dehydrogenase complex (24 mg) was mixed with 1 mg of complex in which the lipoic acid residues had previously been radiolabelled by treatment with N-ethyl[2,3-^{14}C]maleimide in the presence of pyruvate (Danson & Perham, 1976), as described below. The mixture (3 ml; 8.3 mg/ml) was incubated with trypsin ($10 \mu g$/ml) at 0°C in 20mM-sodium phosphate buffer, pH 7.0, containing 2mM-EDTA and 0.02% NaN_3. At various times samples were assayed for overall complex activity, and after 27 min, when the overall enzyme activity was 80% of the starting value, proteolysis was halted by adding soya-bean trypsin inhibitor to a final concentration of $30 \mu g$/ml. The mixture was gel-filtered on a

Sepharose 6B column (580 mm × 15 mm) at 4°C in the same buffer.

Modification of pyruvate dehydrogenase complex with N-ethylmaleimide

Native or partly proteolysed pyruvate dehydrogenase complex (1 mg/ml) was incubated at 0°C with 0.25 mM-N-ethyl[2,3-^{14}C]maleimide in 50mM-sodium phosphate buffer, pH 7.0, containing 1.0 mM-sodium pyruvate, 0.5 mM-thiamin pyrophosphate, 5 mM-$MgCl_2$ and 1.0 mM-NAD^+. At measured time intervals samples ($60 \mu l$) were removed and mixed with $1 \mu l$ of 2-mercaptoethanol to halt the reaction. A portion ($40 \mu l$) of the sample was added to 2 ml of ice-cold 10% (w/v) trichloroacetic acid, and the precipitated protein was collected by filtration on micro glass-fibre filters (Whatman GF/C), and washed with 25 ml of 10% trichloroacetic acid, followed by 10 ml of acetone. The filters were dried *in vacuo*, placed in 3 ml of scintillant [2,5-diphenyloxazole (5 g/l) in toluene] and counted for radioactivity. The remainder of each sample was used to measure the catalytic activity of the complex.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Electrophoresis in 7.5% (w/v) polyacrylamide gels containing 0.1% sodium dodecyl sulphate was performed as described by Perham & Thomas (1971). Radioactivity in the protein bands was measured as described by Brown & Perham (1976).

Performic acid oxidation, acid hydrolysis and paper electrophoresis

Samples of protein were oxidized with performic acid by the method of Hirs (1956). Hydrolysis of proteins with 6 M-HCl, amino acid analysis and paper electrophoresis were performed as described by Perham (1978).

Results

Reaction of the partly proteolysed complex with N-ethylmaleimide

Pyruvate dehydrogenase complex mixed with a small amount (4%) of N-ethyl[2,3-^{14}C]maleimide-labelled complex as a marker was treated with trypsin as described in the Materials and methods section. When the complex activity had begun to fall (80% remaining), soya-bean trypsin inhibitor was added and the mixture was gel-filtered on a column of Sepharose 6B (Fig. 1). Most of the protein was eluted in the same position as native complex would have been. Analysis of this protein by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed that the E3 polypeptide chain was essentially unaffected, but that the E1 chain was
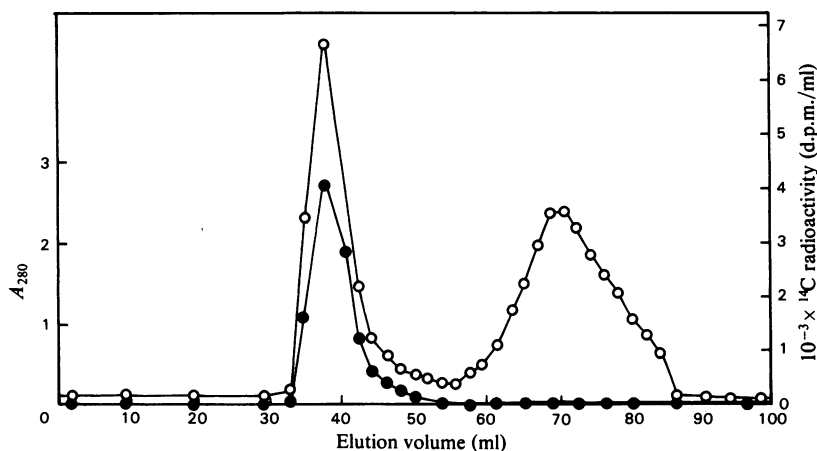
Fig. 1. *Gel filtration of trypsin-treated pyruvate dehydrogenase complex*
Pyruvate dehydrogenase complex, a small proportion (4%) of which had been labelled with N-ethyl[2,3-^{14}C]maleimide, was incubated with trypsin under very mild conditions as described in the text. The mixture was gel-filtered on Sepharose 6B and the eluate was monitored for radioactivity (O) and absorbance at 280 nm (●).

partly degraded to a fragment of apparent M_r 65 000 and that the E2 polypeptide chain was almost completely degraded into fragments of apparent M_r 48 000, 46 000, 39 000 and 36 000, as described previously (Hale & Perham, 1979b,c; Bleile *et al.*, 1979). However, only 42% of the radioactivity associated with the N-ethyl[2,3-^{14}C]maleimide-labelled lipoic acid residues was eluted with the high-M_r enzyme complex, the rest of the radioactivity appearing in lower-M_r polypeptides (Fig. 1). On the reasonable assumption that the pattern of proteolysis is the same for the native and radio-labelled enzyme complexes, these results are consistent with the report (Bleile *et al.*, 1979) that a substantial proportion of the lipoyl groups can be excised by treatment with trypsin under these conditions before there is a correspondingly large loss of enzymic activity.

Samples of native and trypsin-treated enzyme complex were inhibited by treatment with N-ethyl-[2,3-^{14}C]maleimide in the presence of pyruvate, as described in the Materials and methods section. The rate of loss of overall enzyme activity was compared with the rate of modification in the lipoyl groups, as determined by incorporation of radioactivity into the protein (Fig. 2). For the partly proteolysed complex, in which a trace amount (4%) already contained radiolabelled lipoic acid residues, the results were corrected by subtraction of the radioactivity present before the second treatment with N-ethyl[2,3-^{14}C]-maleimide.

With the native complex, chemical modification was found to occur more rapidly than loss of enzymic activity, as described previously (Ambrose-

Griffin *et al.*, 1980). The rate constant for loss of activity was 0.099 (±0.005) min^{-1}, whereas a single-exponential fit to the incorporation of radio-activity gave a rate constant of 0.48 (±0.08) min^{-1}. With the partly proteolysed complex, however, a different result was obtained. The rate of incorporation of radioactivity was comparable with that observed for the native complex; a single-exponential fit gave a rate constant of 0.31 (±0.04) min^{-1}. On the other hand, the loss of enzyme activity was now more rapid, with a rate constant of 0.26 (±0.01) min^{-1}, which is close to that for the chemical modification. It appears that, after about 50% of the lipoyl groups have been removed by limited proteolysis with trypsin, the enzyme activity becomes more nearly proportional to the number of lipoic acid residues that remain unmodified.

Treatment of pyruvate dehydrogenase complex with lipoamidase

In preliminary experiments, native pyruvate dehydrogenase complex was incubated at 30°C with lipoamidase and the overall activity of the complex was measured at various times. It was found that there was a pronounced lag in the loss of enzyme activity, followed by a more rapid fall, suggesting that some lipoic acid residues could be removed without affecting the enzyme activity. The rate of loss of overall activity of the complex in the rapid phase did not depend on the concentration of pyruvate dehydrogenase complex in the range 0.1–2.5 mg/ml (about 1–25 μM in lipoic acid residues), which implies that the lipoamidase was fully saturated with substrate in this range.
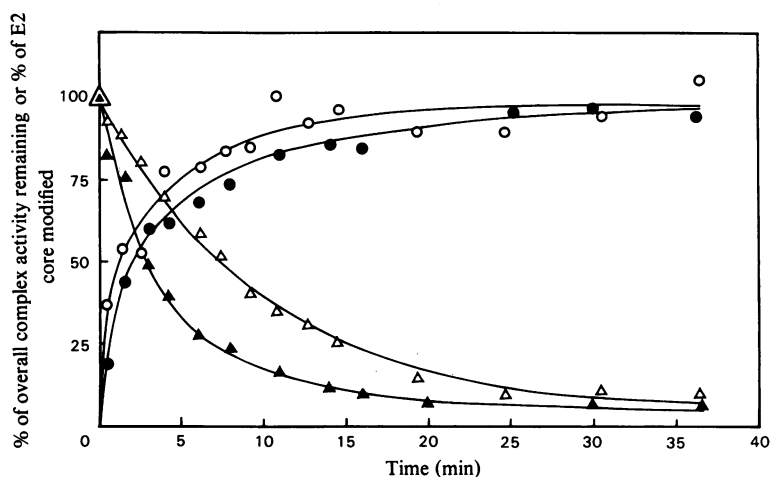
Fig. 2. *Modification of native or trypsin-treated pyruvate dehydrogenase complex with N-ethyl[2,3-^{14}C]maleimide* Treatment with 0.25 mM-*N*-ethyl[2,3-^{14}C]maleimide in the presence of pyruvate was performed as described in the text. △, Overall complex activity of native complex; ▲, overall complex activity of trypsin-treated complex; ○, percentage of the E2 core modified by maleimide in native complex; ●, percentage of the E2 core modified by maleimide in trypsin-treated complex. The lines represent the best fit of the data to single-exponential curves as described in the text.

In order to correlate the loss of enzymic activity with the presumed release of lipoic acid residues, we repeated the experiment but used ^{35}S-labelled pyruvate dehydrogenase complex (Hale & Perham, 1979a), in which the cysteine and lipoic acid residues were radiolabelled by growing the bacteria in the presence of [^{35}S]sulphate and unlabelled methionine. The release of [^{35}S]lipoic acid could easily be followed by extracting acidified samples with a toluene-based scintillant, into which only free lipoic acid would be extracted (Suzuki & Reed, 1963; Hale & Perham, 1979a).

Radiolabelled pyruvate dehydrogenase complex (0.6 mg/ml; approx. 2.7 × 10^6 d.p.m./mg) was incubated at 30°C with lipoamidase (5 units/ml) in 50 mM-sodium phosphate buffer, pH 7.0, containing 2 mM-EDTA and 0.02% NaN$_3$. Samples were assayed at various times for activity of the complex and for radioactivity extracted into toluene, as described in the Materials and methods section. After 24 h incubation with lipoamidase, 24% of the total radioactivity could be extracted, whereas in a control experiment, in which lipoamidase was not present, less than 0.2% of the total radioactivity was extractable. The kinetics of release of lipoic acid and of loss of overall activity of the complex are shown in Fig. 3. Although the activity of the complex initially declined relatively slowly, lipoic acid residues were released more rapidly, in an apparently zero-order process. No loss of activity was observed in the absence of lipoamidase.

At the end of the experiment samples of the control and lipoamidase-treated complex were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The stained gels showed only three protein bands, corresponding to the three component enzymes of the complex (Perham & Thomas, 1971), indicating that there was no detectable proteolysis during the incubation with lipoamidase.

In a further experiment, the control and lipoamidase-treated complex were analysed by performic acid oxidation, acid hydrolysis and paper electrophoresis (Hale & Perham, 1979a) to assess whether or not all of the lipoic acid residues could be removed by lipoamidase. Radiolabelled pyruvate dehydrogenase (1.1 mg; 2.7 × 10^6 d.p.m./mg) was given an extended incubation (46 h) with lipoamidase (2.5 units) at 37°C in a final volume of 0.75 ml of 20 mM-sodium phosphate buffer, pH 7.0, containing 2 mM-EDTA and 0.02% NaN$_3$. Another sample of complex was incubated under identical conditions in the absence of lipoamidase. At the end of the incubation, the pyruvate dehdyrogenase-complex activity in the treated sample was less than 1% of the control, and 23% of the total radioactivity could be extracted into toluene, whereas less than 0.1% of the total radioactivity was extractable from the untreated sample. These values agree rather well with those found for the lipoamidase treatment shown in Fig. 3. The remainder of each incubation mixture was then acidified by adding 0.24 ml of
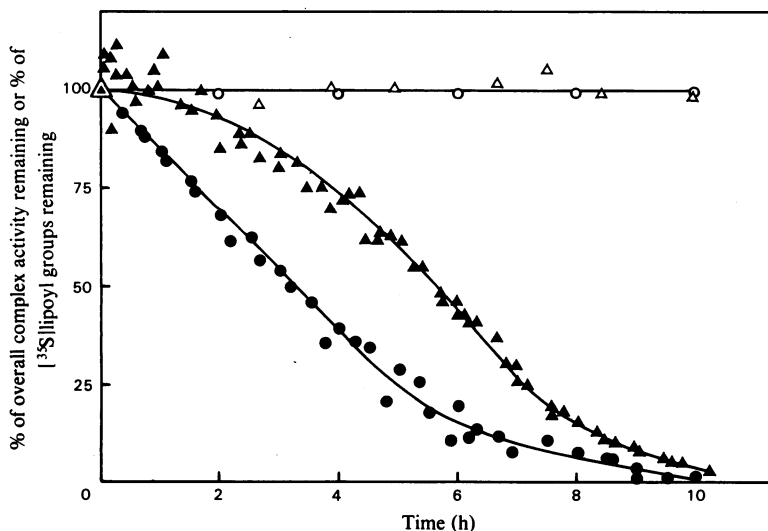
Fig. 3. *Release of [³⁵S]lipoic acid and loss of enzyme activity during incubation of ³⁵S-labelled pyruvate dehydrogenase complex with lipoamidase*

³⁵S-labelled pyruvate dehydrogenase complex was incubated at 30°C with lipoamidase as described in the text. At various times samples were assayed for overall enzyme activity (▲) and for toluene-extractable radioactivity. The extractable radioactivity at any time was expressed as a percentage of the total extractable radioactivity after 24 h treatment and subtracted from 100% to give the percentage of lipoic acid remaining enzyme-bound at that time (●). In a control experiment the enzyme activity (△) and lipoic acid content (○) were measured for an identical sample of radiolabelled complex incubated in the absence of lipoamidase.
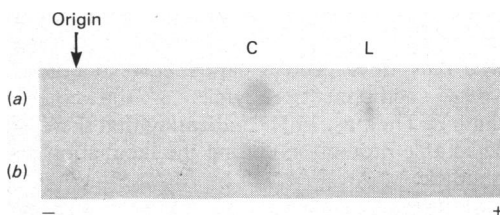

Fig. 4. *Electrophoresis of ³⁵S-labelled compounds in a 24 h hydrolysate of pyruvate dehydrogenase complex after performic acid oxidation*

High-voltage electrophoresis was performed on Whatman no. 1 paper at pH 6.5, and a radio-autograph was prepared. Track (*a*), ³⁵S-labelled pyruvate dehydrogenase complex; track (*b*), ³⁵S-labelled pyruvate dehydrogenase complex after treatment with lipoamidase. Abbreviations used: C, cysteic acid; L, 6,8-disulpho-octanoic acid (presumed, the oxidation product of lipoic acid). For other details see the text.

1 M-HCl and extracted four times with 3 ml of toluene to ensure complete removal of free lipoic acid. The aqueous layers, containing precipitated protein, were freeze-dried, oxidized with performic acid and then hydrolysed with 6 M-HCl for 24 h at 105°C. Samples of the hydrolysate were submitted

to paper electrophoresis at pH 6.5 (Fig. 4). The two large radioactive spots in the control sample were readily identified as 6,8-disulpho-octanoic acid (L) and cysteic acid (C), the oxidation products of lipoic acid and cysteine respectively (Hale & Perham, 1979*a*). No radioactive methionine was detected. The spots were excised and their radioactivity was counted: the ratio (L/C) was found to be 30%, implying that a maximum of 23% of the radioactivity in the enzyme complex can be attributed to lipoic acid residues. This value agrees well with the earlier estimates of the amount of radioactivity extractable into toluene after treatment of the enzyme complex with lipoamidase (see above). It appears that lipoamidase is able eventually to remove all the lipoic acid residues from the pyruvate dehydrogenase complex, a view sustained by the virtual absence of 6,8-disulpho-octanoic acid from the sample of lipoamidase-treated enzyme shown in Fig. 4.

Discussion

Treatment of the pyruvate dehydrogenase complex with trypsin enabled us to isolate an enzyme that retained most of its pyruvate dehydrogenase complex activity despite having lost at least half of its lipoic acid residues (Fig. 1), as reported by Bleile *et al.* (1979). When the native enzyme is inhibited by

treatment with N-ethylmaleimide in the presence of pyruvate, chemical modification of the lipoic acid residues proceeds appreciably faster than the accompanying loss of enzymic activity (Ambrose-Griffin et al., 1980; and Fig. 2). On the other hand, with the trypsin-treated complex the rate of loss of enzyme activity and the rate of modification of lipoic acid residues were approximately equal, the apparent first-order rate constants for both processes being significantly larger than that for inactivation of the native enzyme under identical conditions (Fig. 2). Assuming that the modification with trypsin has no effect on the chemical reactivity of the lipoic acid residues remaining in the complex, this experiment narrows the choice of model for the enzymic mechanism. If the essential/inessential lipoic acid model (Ambrose-Griffin et al., 1980) is applied, it might be argued that trypsin is selectively excising peptides carrying inessential lipoic acid residues. However, the increased rate constant for the inactivation of the trypsin-treated complex shown in Fig. 2 would then be hard to explain. On the other hand, if we take the alternative model, which postulates that one lipoic acid residue is able to take over the catalytic role of another inactivated (or excised) lipoyl group (Ambrose-Griffin et al., 1980), our present results can be explained by supposing that trypsin randomly excises lipoic acid-containing peptides from the enzyme complex until 'take-over' is no longer possible. The rate of inactivation of the trypsin-treated enzyme by N-ethylmaleimide in the presence of pyruvate might then be expected to rise and to coincide more nearly with the rate of modification of lipoic acid residues, as observed (Fig. 2).

The experiments with lipoamidase are similarly informative. The only structural effect of lipoamidase on the pyruvate dehydrogenase complex appeared to be release of the lipoic acid residues; in particular, no proteolysis was detectable. In principle this makes the results easier to interpret than those with trypsin. The loss of lipoic acid residues from the complex was a zero-order process until it neared completion (Fig. 3). This is consistent with an enzyme-catalysed reaction in which the enzyme lipoamidase was initially saturated with substrate and showed no particular preference for either of the two lipoyl-lysine residues in each E2 polypeptide chain. However, the accompanying loss of pyruvate dehydrogenase complex activity was not a similar zero-order process. There was a pronounced lag behind the release of lipoic acid residues over most of the course of the reaction (Fig. 3). This result again favours the 'take-over' model, since the loss of enzymic activity would otherwise be expected to be zero-order, coinciding with the release of the 'essential' lipoic acid residues (Ambrose-Griffin et al., 1980).

The 'take-over' model can be satisfyingly fitted into an emerging picture of the mechanism and structure of the 2-oxo acid dehydrogenase complexes. The lipoic acid residues in these enzymes are able to participate in an extensive network of intramolecular transacylation reactions within the E2 core (Bates et al., 1977; Collins & Reed, 1977; Cate & Roche, 1979; Stanley et al., 1981). With the E. coli pyruvate dehydrogenase complex, it has been shown that these reactions are not rate-determining, implying that the rate-determining step precedes them and involves the E1 component (Danson et al., 1978b). It was suggested that this step is the generation of the intermediate hydroxyethylthiamin pyrophosphate (Collins & Reed, 1977; Akiyama & Hammes, 1980), whereas for the ox kidney enzyme it has been identified as the subsequent reductive acetylation of lipoyl groups (Cate et al., 1980). In either case, if the same E1 active site can be served by more than one lipoic acid residue, chemical modification or excision of lipoic acid residues could occur without loss of enzymic activity (Ambrose-Griffin et al., 1980; Cate et al., 1980).

The existence of structural properties of the enzyme that might permit this curious feature of the enzyme mechanism lends further plausibility to it. Thus the lipoic acid residues in the pyruvate dehydrogenase complexes from E. coli (Hale & Perham, 1979b,c; Bleile et al., 1979), from Bacillus stearothermophilus (Perham & Wilkie, 1980) and from ox kidney (Kresze & Ronft, 1980) and ox heart (Bleile et al., 1981) are located in protruding regions of the E2 polypeptide chains that are readily excised from the E2 core by proteinases. These regions are difficult to detect in electron micrographs of the enzyme (Bleile et al., 1979; Perham & Wilkie, 1980; Bleile et al., 1981), and it has been conjectured that they might be flexible or extended, interdigitating between E1 and E3 subunits and adding considerably to the distance (2.8 nm) that can be spanned by a single lipoyl-lysine swinging arm. Direct evidence for unusually high internal mobility of these regions of the E2 chains has come from ^{1}H n.m.r. studies of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes (Perham et al., 1981; Wawrzynczak et al., 1981). It is now easy to envisage, therefore, that the high mobility of these parts of the E2 core could allow a given E1 active site in the pyruvate dehydrogenase complex of E. coli to be served by more than one lipoic acid residue, perhaps even by lipoic acid residues bound to different E2 chains in the core (Bates et al., 1977; Ambrose-Griffin et al., 1980; Cate et al., 1980).

The E2 core of the pyruvate dehydrogenase complex of E. coli evidently has some properties not yet found in simpler enzymes. These peculiarities of structure and mechanism, however, all contribute to ensuring the most effective coupling of the rate-

determining E1 reaction with the faster acetyl-transferase and lipoamide dehydrogenase reactions that follow. They can overcome the problems for a multi-step reaction that might arise from low substrate concentrations (Danson *et al.*, 1978*b*), from the unequivalent numbers of the three types of active sites in the enzyme particle (Reed, 1974; Bates *et al.*, 1975) and, if it turns out to be a true reflection of affairs *in vivo*, from the physical heterogeneity of the enzyme particles inferred from their hydrodynamic properties *in vitro* (Schmitt & Cohen, 1980; Gilbert & Gilbert, 1980).

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