Vol. 84, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 29, 1978 Pages 508-514

DIHYDROLIPOAMIDE TRANSACETYLASE FROM ESCHERICHIA COLI: EVIDENCE FOR INTERNAL GENE DUPLICATION

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Received August 21, 1978

SUMMARY

The dihydrolipoamide transacetylase component of the pyruvate dehydrogenase complex from Escherichia coli consists of identical subunits of 89.000 daltons. During the purification procedure it is partially cleaved into active fragments of 82.000 daltons, 37.000 daltons and 35.000 daltons. Fingerprint analysis shows at least 80% similarity of the fragments compared with the native component. This provides evidence for two large homologous domains within same polypeptide chain.

Dihydrolipoamide transacetylase (EC 2.3.1.12) is an enzyme component of the pyruvate dehydrogenase complex and consists of 16 identical polypeptide chains, each with a molecular weight of about 80.000 daltons (80 k) (1-4). It was observed repeatedly that this polypeptide splits into fragments of about half the size of the native protein (3,5). Independently Danson and Perham (6) proved that there are two covalent bound lipoic acid residues per chain and Daigo and Reed (7) showed that there is a distinct amino acid sequence around the bound lipoic acid. This evidence argues for the presence of two catalytic sites with identical functions on one polypeptide chain. Additional

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Abbreviations: PDH, pyruvate dehydrogenase; TA, dihydrolipoamide transacetylase; DLDH, dihydrolipoamide dehydrogenase; BSA, bovine serum albumin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

0006-291X/78/0842-0508$01.00/0

data demonstrate the existence of homologous regions within the transacetylase protein by fingerprint analysis of the fragments.

METHODS AND MATERIALS

The pyruvate dehydrogenase complex from E. coli wild type was purified according to (2). Its enzymic activity ('overall activity') was tested by following spectrophotometrically the reduction of NAD at 340 nm. (8). The partial enzymic reaction of the transacetylase component was measured by acetylation of dihydrolipoamide at 240 nm (9). The enzyme activities were expressed in µmoles per hour and per mg protein, measured at 37°C. For the polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate the discontinuous system of Laemmli (10) was used. The reference proteins were from Boehringer, Mannheim, Myoglobin and ovalbumin from Serva, Heidelberg and bovine serum albumin from Behring, Marburg. Iodination of the proteins with ^{125}I, purchased from Amersham-Buchler, Braunschweig, and tryptic digestion was done according to Elder et al. (11).

RESULTS AND DISCUSSION

Characterization of the fragments

After sodium dodecylsulfate polyacrylamide gel electrophoresis of purified enzyme preparations one observes repeatedly, beside the protein bands for the three different components of the pyruvate dehydrogenase complex, further bands in varying intensities at distinct positions. The occurance of these bands parallels to some extent the disappearance of the transacetylase band (3,5)(Fig. 1). To follow this phenomenon, the pyruvate dehydrogenase complex was incubated at 37°C and aliquots were withdrawn after different times for polyacrylamide gel electrophoresis and enzyme tests. The results are shown in Fig. 2. A decrease in the intensity of the transacetylase band is seen, while different faster migrating bands appear simultaneously. The overall activity of the enzyme complex disappears slowly, while the activity of the transacetylase remains constant, even with complete disappearance of the corresponding native protein band. The fragments seem therefore to have the same enzymic activity as the uncleaved enzyme, though the reactivity of the whole complex was impaired. Three fragments can be clearly identi-

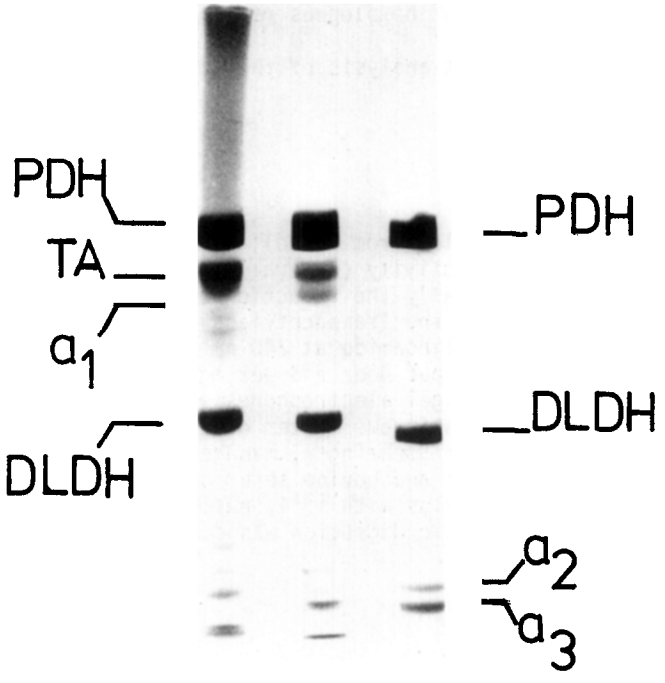

FIGURE 1: Spontaneous decay of the dihydrolipoamide transacetylase component. Three different preparations of the pyruvate dehydrogenase complex were applied to the sodium dodecylsulfate polyacrylamide gel electrophoresis. The left gel shows a nearly native complex preparation, in the sample in the middle the a_1-fragment becomes predominant and in the right one a_2 and a_3 appears while the transacetylase band (TA) is lost. Minor bands seen in the gels were not further characterized here. PDH: pyruvate dehydrogenase, DHLH: dihydrolipoamide dehydrogenase.

fied (Fig. 1). Their molecular weights were determined as shown in Fig. 3. For the native transacetylase chain, a value of 89 k was found which is about 10 k higher than the molecular weights reported by other groups (1,3,4). In fact the largest fragment found (a_1, Fig. 1) had a size of about 82 k. Since a rapid purification procedure for the pyruvate dehydrogenase complex was employed to circumvent proteolytic cleavage (2), it may be that our band a_1 is identical with that formerly assigned to the native transacetylase. The previously determined molecular weights for the other two components, which are less sensitive to proteolytic cleavage were confirmed (cf, Fig. 3). The size of the two

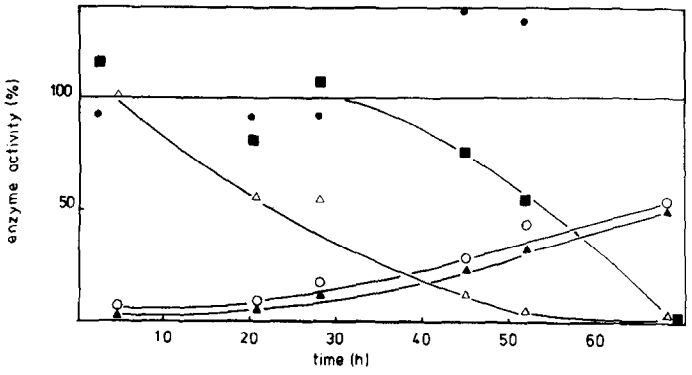

FIGURE 2: Incubation of the native pyruvate dehydrogenase complex at 37°C in 0.1 M tricine buffer, pH 8.0. Aliquots were taken after the times indicated, tested for the overall activity (■), for the partial activity of the transacetylase component (●), and applied to the sodium dodecylsulfate polyacrylamide gel electrophoresis. Relative peak intensities of the protein bands (indicated as percentage of the native transacetylase): Δ , transacetylase; ▲ , a_2; O , a_3. The fragment a_1 was not clearly resolved in this experiment.

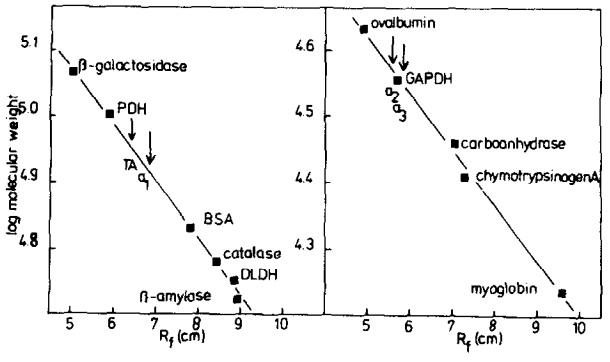

FIGURE 3: Molecular weight determination of the dihydrolipoamide transacetylase (TA) and of the fragments a_1, a_2 and a_3. Molecular weights of the reference proteins; β-galactosidase (E. coli), 116 k; pyruvate dehydrogenase (PDH, E. coli), 100 k; bovine serum albumin (BSA), 68 k; catalase (beef liver), 60 k; dihydrolipoamide dehydrogenase (DLDH, E. coli), 56 k; β-amylase (Ipomoea patatas), 54 k; ovalbumin, 43 k; glyceraldehyde-3-phosphate dehydrogenase (GAPDH, yeast), 36 k; carboanhydrase (bovine erythrocytes), 29 k; chymotrypsinogen A (bovine pankreas) 25.7 k; myoglobin (whale) 17.2 k. The gels were 7% (left) and 13% (w/v) (right) in the acrylamide concentration.

fragments a_2 and a_3 was calculated to be 37 k and 35 k respectively.

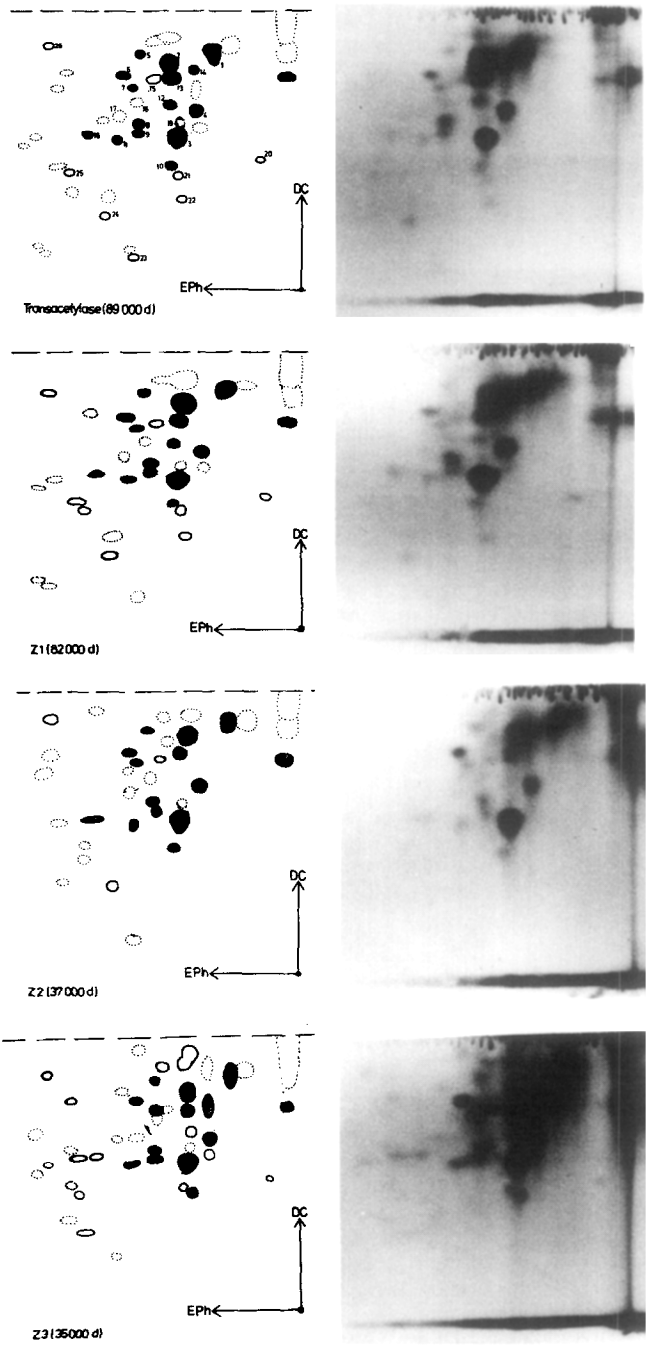

FIGURE 4: Fingerprint analysis of the native transacetylase chain and of the fragments a_1, a_2 and a_3 (from top to bottom). The ^{125}I-labelled tryptic peptides (0.5 µCi per plate) were separated by electrophoresis in the horizontal dimension (EPh), using the solvent formic acid-acetic

Peptide maps of the fragments

To explore the origin of the different fragments a_1, a_2 and a_3, the three protein bands and the bands of the three native enzyme components of the pyruvate dehydrogenase complex in the polyacrylamide gel electrophoresis were labelled with ^{125}I according to the technique of Elder et al. (11). After tryptic digestion we separated the peptides by electrophoresis and thin layer chromatography and identified the spots by autoradiography. Fig. 4 shows the fingerprint pattern of the native transacetylase as well as that of the three fragments. In all cases a high degree of homology can be seen. The high molecular fragment a_1 differs from the two small fragments a_2 and a_3 in only 5 of the 22 intense spots. No similarity was found within the peptide pattern of the other two enzyme components, the pyruvate dehydrogenase and the dihydrolipoamide dehydrogenase (data not shown). It thereby appears that the dihydrolipoamide transacetylase component decays via a 82 k fragment into enzymatically active fragments of 37 k and 35 k. Though they possess only 40% of the length of the native polypeptide chain, they exhibit about 80% homology in the tryptic peptide pattern. From this it is concluded that the functional unit of the transacetylase is a domain of about 35 k in size and that the native polypeptide chain consists of two of these homologous domains. Such structures can be evolved by gene duplication and gene fusion (12). Serum albumin, phosphofructokinase and various tRNA-synthetases are examples of proteins with such homologous regions where the original enzymatic function has been conserved in one domain, while the other region showed divergence (12). In the case postulated here both domains seem to function equally well (6).

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acid-water (1 : 4 : 16, v/v); and by thin layer chromatography in the vertical dimension (DC) with the solvent n-butanol-pyridine-water-acetic acid (39.4 : 30.3 : 24.2 : 6.0, v/v). This solvent contained PPO in a final concentration of 7%. The plates (20 x 20 cm) were exposed for 3-9 days with an AGFA-Curix X-ray film. Right site: photographs of the autoradiogramms; left site: tracings of the respective fingerprints.

ACKNOWLEDGEMENT

We thank Mrs. E. Schmincke-Ott for her valuable suggestion and J.A. Koshul for reviewing the manuscript. This work was supported by the 'Deutsche Forschungsgemeinschaft'.

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