plexes generates an extra thiol in the dithiolane ring, which is then susceptible to a stable modification with maleimides, resulting in loss of catalytic function (Brown & Perham, 1976).

PDC and OGDC were purified from fresh ox heart (De Marcucci et al., 1985). The activities of both these complexes were not affected by incubation at room temperature with 0.5 mM-N-ethylmaleimide for 1 h. Addition of, respectively, 2 mM-pyruvate and 2 mM-2-oxoglutarate resulted in rapid, irreversible inhibition complete within 10 min. Use of N-ethyl-[2,3-14C]maleimide in this reaction and resolution of the inhibited complexes by SDS/polyacrylamide-gel electrophoresis and fluorography confirmed that the lipoate acetyl-transerase and component X subunits of PDC and the lipoamide succinyltransferase of OGDC were modified by the maleimide. However, when PDC was preincubated at room temperature with pyruvate before addition of N-ethylmaleimide, there was a time-dependent protection against inhibition of overall activity by the maleimide. After 50 min incubation of PDC with pyruvate, addition of N-ethylmaleimide could only produce approx. 10% inhibition of activity. Prior addition of acetyl-CoA to NADH-reduced PDC also prevented the inhibitory effect of N-ethylmaleimide but more rapidly, with 90% protection occurring 10 min after addition of acetyl-CoA. Incubation of PDC with either substrate in the absence of N-ethylmaleimide had no effect on overall activity. There was greatly reduced incorporation of [2,3-14C]maleimide into E2 and component X subunits of PDC and the lipoamide succinyltransferase of OGDC were modified by the maleimide. When PDC was incubated with [2-14C]pyruvate it was found that acquisition of resistance to N-ethylmaleimide inhibition and incorporation did not occur with the ox heart OGDC nor apparently with the bacterial PDC isolated from Escherichia coli (Danson et al., 1981) and may be a unique feature of the mammalian PDC.

Table 1. Incorporation of [2-14C]pyruvate into E2 and X subunits of PDC was incubated with [2-14C]pyruvate in the absence or presence of 0.5 mM-N-ethylmaleimide (NEM). After 30 min incubation, approx. 22 μg of protein was resolved on a 10% (w/v) SDS/polyacrylamide slab gel. Radioactivity in 14C-labelled E2 and X subunits as Coomassie-Blue-stained bands was determined by scintillation counting. Values represent mean ± S.E.M. of three separate determinations.

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Additions</th>
<th>Incorporation of [2-14C]pyruvate (c.p.m./gel slice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>None</td>
<td>1446 ± 247</td>
</tr>
<tr>
<td>Component X</td>
<td>NEM</td>
<td>613 ± 51</td>
</tr>
<tr>
<td>Component X</td>
<td>None</td>
<td>250 ± 20</td>
</tr>
<tr>
<td>Component X</td>
<td>NEM</td>
<td>123 ± 19</td>
</tr>
</tbody>
</table>

formulation of an interlipoyl disulphide bond. This process of substrate-induced protection against N-ethylmaleimide inhibition and incorporation did not occur with the ox heart OGDC nor apparently with the bacterial PDC isolated from Escherichia coli (Danson et al., 1981) and may be a unique feature of the mammalian PDC.


Received 12 April 1988

Biosynthesis of precursor forms of the pyruvate dehydrogenase complex and mitochondrial phosphate transporter in Saccharomyces cerevisiae

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Recently, our group has investigated molecular events in the biosynthesis, targeting and assembly of the eukaryotic pyruvate dehydrogenase multienzyme complex (PDC) and the related 2-oxoacid dehydrogenase complexes, mainly in mammalian cells (Hunter & Lindsay, 1986; De Marcucci et al., 1988). Similar studies are now reported on the precursor states of the E2 and E1β polypeptides of PDC from Saccharomyces cerevisiae and on the identification, purification and import of the phosphate–hydroxyl antiporter (PTP), an integral hydrophobic carrier of the mitochondrial inner membrane.

In the case of PDC, the 2-oxoglutarate dehydrogenase (OGDC) and branched-chain 2-oxoacid dehydrogenase complexes, the distinct substrate-specific dehydrogenases (E1) and the common lipoamide dehydrogenase (E3) polypeptides are synthesized initially as cytosolic precursors with

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In this study, we have utilized the strong cross-reactivity of anti-(ox heart PDC) IgG and anti-(rat liver PTP) IgG to detect the mature forms of the equivalent yeast polypeptides and examine the nature of their precursor states in a rho-sinosome of *S. cerevisiae* (D273-10B-1). This mutant accumulate vast cytoplasmic pools of mitochondrial precursors when grown for two to three generations in the presence of uncouplers of oxidative phosphorylation, e.g. m-chlorocarbonyl cyanide phenylhydrazone (CCCP; Reid & Schatz, 1982).

Initial immune replica analysis of the individual purified enzymes of yeast PDC with anti-(ox heart PDC) IgG demonstrated that a strong cross-reaction was observed only with the E2 and E1β subunits; a weak reaction was also found with E3 while no response was found with E1α. Subsequent immunoblotting of crude yeast extracts, derived from cells grown for 7–8 h in the presence of 1–20 μM-CCCP as in Fig. 1 confirmed the specific detection of the E2 and E1β polypeptides. Moreover, pre-E2 and pre-E1β species were apparent in CCCP-inhibited cultures, exhibiting *M* values 7000–8000 and 3000–4000 greater than their mature states, consistent with earlier observations in mammalian cells. As shown in Fig. 1., densitometric analysis of the immunoblot reveals that appearance of pre-E2 is readily visualized with maximal accumulation occurring in the presence of 5 μM-CCCP; while there is also a corresponding decline in the amount of mature E2 per cell. At high levels of CCCP, protein synthesis (and cell division) is markedly inhibited and the accumulation of precursor forms is less dramatic. Similar results were obtained for the E1β precursor (not shown).

In contrast (Fig. 1), no appearance of a higher *M* form of the PTP protein is observed under these conditions and the level of PTP per cell remains at a constant level during two to three cell divisions, indicative of the continued synthesis of a precursor form of identical *M* value in the presence of CCCP. Thus the yeast PTP may not contain a proteolytically cleavable 'signal' sequence.

The strong cross-reactivity of antibody raised to rat liver PTP, subunit *M*, 34000 with the equivalent yeast protein, subunit *M*, 30000 has facilitated the purification and characterization of the yeast carrier. Yeast PTP has proved amenable to purification using a scheme derived originally for rat liver/beef heart PTP (Gibb et al., 1986), involving pre-extraction of mitochondria in buffer containing 0.5% (v/v) Triton X-100, solubilization in the presence of 8% (v/v) Triton X-100, and absorption chromatography on hydroxylapatite and 'Celite'.


Received 12 April 1988