Component X of mammalian pyruvate dehydrogenase complex: properties of lipoyl groups

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Pyruvate dehydrogenase complex (PDC) from mammalian sources comprises a structural core formed by 60 lipoate acetyltransferase (E2) subunits assembled as a pentagonal dodecahedron, to which pyruvate dehydrogenase (E1 αβ M, 154,000) and lipoamide dehydrogenase (E3), a homodimer of M, 110,000, subunits are bound to give a large array of approx. M, 8.5 × 10⁶ (Koike & Koike, 1976). Pyruvate dehydrogenase complex is located in the mitochondrial matrix and catalyses the overall reaction:

pyruvate + NAD⁺ + CoASH → acetyl-CoA + NADH + H⁺ + CO₂

A recently recognized subunit of M, 50,000 forms a close physical and functional union with the E2 assembly (De Marcucci & Lindsay, 1986; Jilka et al., 1986). Similarly to E2, component X incorporates acetyl groups in the presence of pyruvate and absence of CoASH (De Marcucci et al., 1986). Acetylation of component X requires active E1 subunits and rapid deacetylation of both E2 and X occurs on addition of CoASH. The site of acetyl group incorporation on the E2 polypeptides is lipoic acid which is covalently attached by means of the Nα-amino group of a lysine residue. Most evidence suggests that only a single lipoyl moiety is present on each E2 polypeptide (Hamada et al., 1975; White et al., 1980), although there have been some conflicting observations (Cate & Roche, 1979). Studies employing proton n.m.r. or electron microscopy (Bleile et al., 1979; Perham et al., 1981) have shown that lipoyl groups on the E2 subunits are situated on extended regions of polypeptide which are highly mobile relative to other parts of the multienzyme complex. S-Acetylation in the presence of pyruvate generates a thiol on the adjacent sulphur atom in the diithiole ring of lipoic acid, which may thus be specifically modified with N-ethylmaleimide (Brown & Perham, 1976).

PDC was purified from fresh ox heart by a modification (De Marcucci et al., 1985) of the procedure of Stanley & Perham (1980). N-Ethyl[2,3-¹⁴C]maleimide treatment of the complex resulted in major incorporation of ¹⁴C label into the E1 subunits whether or not pyruvate was added. This pyruvate-independent modification was eliminated by pretreatment of the complex with non-radioactive N-ethylmaleimide or phenylenemaleimide (Brown & Perham, 1979). S-Acetylation may not be reductive acetylation of a second lipoyl group and is therefore E2 component X can interact spatially and that therefore E2 and component X are present on the same core assemblies. The rapid generation in the presence of pyruvate of two cross-linkable thiol domains on the lipoyl domain on the E2 subunit and may have catalytic significance.


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