Sequential pathways in this hybrid mechanism can be crucially tipped by mutation of just one amino acid residue, Tyr-177, in the E. coli protein. Further work is in hand to characterize these mutants and to investigate the ability of the mutated residue to protect the flavin from electron loss and to study the environment of the flavin in these mutants. Comparable studies of other mutants can be expected to throw light on other aspects of the structure and mechanism of this important class of dehydrogenase.

We are grateful to the S.E.R.C. for financial support (Grant GR/D 05790) and a Research Studentship (to N.S.S.), and to St John's College, Cambridge, for the award of a Benefactors' Scholarship (to N.S.S.). We thank Dr G. J. Hunter and Mr C. Fuller with help with the synthesis of oligonucleotides and Dr P. J. F. Henderson for useful discussions.


Received 29 September 1987

Dihydrolipoamide dehydrogenase: a ‘new’ function for an old enzyme?

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Introduction

Dihydrolipoamide dehydrogenase (EC 1.8.1.4) catalyses the NAD⁺-dependent oxidation of dihydrolipoamide (Williams, 1976) via a catalytic mechanism which involves the alternate oxidation and reduction of an intrachain disulphide bond and a base (B) on the enzyme (Fig. 1). The enzyme fulfils this function as an integral component of the pyruvate, 2-oxoglutartate and branched-chain 2-oxo acid dehydrogenase complexes (Reed, 1974; Perham, 1975; Petit et al., 1978) and of the glycine enzyme cleavage system (Kikuchi & Hiraga, 1982). These are the established metabolic roles of dihydrolipoamide dehydrogenase but there is accumulating evidence to suggest that the enzyme may serve an as yet undefined additional function and one that is specific to a location in the plasma membrane of the cell. The present communication serves to review the data supporting this possibility.

Dihydrolipoamide dehydrogenase of the archaeabacteria

The archaebacteria are now recognized as a phylogenetically distinct group of organisms, constituting a third evolutionary kingdom in addition to the eubacteria and eukaryotes (reviewed in Fewson, 1986; Woese & Olsen, 1986; Danson, 1988). They comprise three phenotypes: the extreme

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**Fig. 1.** NAD⁺-dependent oxidation of dihydrolipoamide catalysed by dihydrolipoamide dehydrogenase
halophiles, the thermophiles (including thermoacidophilic organisms) and the methanogens. In the present context it is important to note that archaea bacteria have the common feature that none of them possesses the 2-oxo acid dehydrogenase complexes. Rather, they convert pyruvate and 2-oxoglutarate to their corresponding acyl-CoA thio-esters via less complex oxidoreductases, enzymes which have been purified from *Halobacterium halobium* and shown to contain no lipoic acid (Kerscher & Oesterhelt, 1982). It was therefore of considerable interest to discover the presence of dihydrolipoamide dehydrogenase in halophilic (Danson *et al.*, 1984), thermoacidophilic (Smith *et al.*, 1987) and possibly in the methanogenic (Danson, 1988) archaea bacteria. We have purified the enzyme from *H. halobium* and, through the use of dithiol-specific arsenical reagents, have provided evidence that the catalytic mechanism involves the alternate oxidation and reduction of a disulphide bond (Danson *et al.*, 1986). In this and other properties, the enzyme is remarkably similar to dihydrolipoamide dehydrogenases from eubacteria and eukaryotes.

In the case of the thermoacidophilic archaeabacterium *Thermoplasma acidophilum*, the dihydrolipoamide dehydrogenase appears to be associated with the plasma membrane of the cell, a feature which may also be the case in eubacteria and eukaryotes and is discussed below.

The presence of dihydrolipoamide dehydrogenase in the archaea bacteria has at least two important implications. First, it means that the enzyme is found in organisms of all three evolutionary lineages and therefore may be an 'ancient' protein. This contrasts with views on the 2-oxo acid dehydrogenase complexes: these have been found only in non-archaea bacterial respiratory organisms, and Kerscher & Oesterhelt (1982) therefore suggest that these enzyme systems evolved after the development of oxidative phosphorylation. If so, it would appear that dihydrolipoamide dehydrogenase was recruited from another role to serve in such complexes. Secondly, the existence of the enzyme in the absence of the complexes of which it is normally a component (the glycine cleavage system has not been reported in the archaea bacteria) suggests that dihydrolipoamide dehydrogenase may have an additional cellular role, indications to the nature of which come from a study of the enzyme in eukaryotes and eubacteria.

**Dihydrolipoamide dehydrogenase of eukaryotes**

Most eukaryotic organisms possess the mitochondrial 2-oxo acid dehydrogenase complexes and glycine cleavage system, although the latter is a minor activity. However, some eukaryotes lack functional mitochondria and also, therefore, the associated enzymes normally found in these organelles. *Trypanosoma brucei*, the causative agent of African sleeping sickness, is one such organism, the bloodstream form of which is totally dependent on glycolysis for ATP production. Thus, it does not have the 2-oxo acid dehydrogenase complexes and, under aerobic conditions, glucose is metabolized almost completely to pyruvate which is then excreted (Farlamb, 1982). We have recently discovered the presence of dihydrolipoamide dehydrogenase in the bloodstream form of *T. brucei* (Danson *et al.*, 1987). Kinetic analysis and chemical modification studies again indicate it to be a true dihydrolipoamide dehydrogenase, but in addition to these expected properties, we have found that the enzyme is specifically located within the plasma membrane of the organism. Plasma membrane sheets were prepared by the method of Voorheis *et al.* (1979); the dehydrogenase co-purified with these membranes through a number of purification procedures and washing steps, and after extraction with Triton X-100, it could be reconstituted into soyabean l-α-phosphatidylcholine liposomes to a specific activity the same as that in the purified enzyme.

In an attempt to determine if this membrane association is also found in eukaryotic cells that do possess functional mitochondria, plasma membranes have been prepared from isolated rat adipocyte cells (A. R. Karim, G. D. Holman & M. J. Danson, unpublished work). Using 5'-nucleotidase as a plasma-membrane marker, and succinate dehydrogenase to assess contamination with mitochondrial membranes, we have evidence that the adipocyte plasma membrane has associated dihydrolipoamide dehydrogenase activity. The data from the adipocyte cells are preliminary and must be viewed with some caution, but they do suggest that there are two locations for the enzyme, one inside the mitochondrion (presumably in association with the 2-oxo acid dehydrogenases and the glycine cleavage complex) and the other in an extramitochondrial position. It will be interesting to see if the association of the enzyme with the eukaryotic plasma membrane is dependent on cell type or whether it is a general phenomenon.

**Dihydrolipoamide dehydrogenase of eubacteria**

As in eukaryotes, dihydrolipoamide dehydrogenase is present in respiratory eubacteria as a component of various multi-enzyme systems. Again, however, the enzyme is not confined to these locations. Using crossed immunoelectrophoresis for the analysis of antigens present in transporting membrane vesicles of *Escherichia coli*, dihydrolipoamide dehydrogenase has been identified as an enzyme fractionating to similar extents between membrane and cytoplasm (Owen *et al.*, 1980). Furthermore, evidence was presented to suggest that the dihydrolipoamide dehydrogenase was responsible for ubiquinone-mediated NADH-dependent transport of amino acids in the vesicle system. Indeed, the authors point out that the enzyme can use certain quinones as electron acceptors instead of NADH and that even purified dihydrolipoamide dehydrogenase has ubiquinone reductase activity.

To my knowledge this is the only known example of a eubacterium with a membrane-associated dihydrolipoamide dehydrogenase. It is obviously important to look at further species, but this one report does serve to establish the phenomenon as existing within this evolutionary kingdom in addition to the archaea bacteria and the eukaryotes.

**A membrane-associated function for dihydrolipoamide dehydrogenase?**

The occurrence of a plasma-membrane-associated dihydrolipoamide dehydrogenase in such an evolutionarily diverse range of species suggests that the enzyme may play a fundamental role in the membrane, such as in the transport of solutes into and out of the cell. If one is looking for a common mechanism of participation in such processes, then an oxidation-reduction reaction might be ideally suited to dihydrolipoamide dehydrogenase and to its lipid-soluble substrate, lipoamide. The evidence supporting a role in transport phenomena is outlined below.

*E. coli*, deficient in the synthesis of lipoic acid, can grow in the absence of the cofactor in minimal medium supplemented with acetate and succinate. While lactose permease and phosphoenolpyruvate (PEP)-glucose phosphotransferase are unaffected by this lipoic acid deprivation, the binding-protein-dependent transport of ribose, galactose and glucose is severely reduced (Richarme, 1985). Moreover, when lipoic acid is supplied in the growth media, these lipoic-acid-dependent transports are completely inhibited by the diethyl-specific arsenite. Richarme & Heine (1986) have
further shown that a dihydrolipoamide dehydrogenase activity is also detected in a strain of E. coli deleted for the lpd gene coding for the dihydrolipoamide dehydrogenase component common to the 2-oxo acid dehydrogenase complexes. The activity detected in this mutant accounted for approximately 15% of the activity in the parental strain and was stimulated by galactose and maltose. Conversely, no stimulation was observed by these sugars in mutants deficient in the corresponding binding-protein-dependent transport system. The data implicate both lipoic acid and dihydrolipoamide dehydrogenase in these sugar transport systems; however, the authors offer no mechanism for the involvement except to note that a membrane potential may be required for the transport processes.

While this is the most direct evidence for a plasma-membrane-linked transport function for the enzyme, Frost & Lane [15] have observed that the tervalent arsenical, phenylarsine oxide, inactivates insulin-stimulated hexe transport by 3T3-L1 adipocytes. The precise site of modification has not been defined although the specificity of the reagent and the reversal of its effects by 2,3-dimercaptopropane sulphonate are both consistent with the involvement in transport of a pair of vicinal thiols. Recent evidence (Bernier et al., 1987) suggests that this dithiol functions in the signal transduction pathway downstream from the protein whose phosphorylation is stimulated by insulin. It has also been suggested that insulin may cause an interconversion of its receptor from one hydrodynamic form to another by a process involving a disulphide-thiol interchange and that this receptor change may relate to the biological action of insulin (Maturo et al., 1983). Furthermore, if an oxidation-reduction reaction involving cysteine residues is involved in the effect of insulin or its regulation, it is important to note that dihydrolipoamide can reduce the disulphides of insulin through the catalytic reaction of thioredoxin (Holmgren, 1979). Our finding that dihydrolipoamide dehydrogenase is present in adipocyte membranes thus takes on further significance even if the relationship and significance of these effects are still unclear.

In the context of these speculations, one should keep in mind the evidence that dithiol-disulphide interchanges may play a general role in membrane-related processes such as solute transport and energy transduction (reviewed by Robillard & Konings, 1982) and cell surface receptor activations (reviewed by Malbon et al., 1987). Whether or not these interactions are mediated through a cofactor such as lipoamide is unknown, but with our finding that dihydrolipoamide dehydrogenase is commonly associated with cell membranes it is imperative to determine if the cofactor is also present in these locations. To this end, we have developed a method using gas liquid chromatography (g.l.c.)-mass spectrometry to detect lipoic acid as its oxidized lipoprotein methyl ester (Pratt et al., 1986). The sample is first acid hydrolysed to release any protein-bound lipoic acid and then extracted into benzene and dried. This hydrolysate is then reduced with NaBH₄ to convert lipoic acid to the dihydro form and filtered through an agarose column containing an immobilized arsenical ligand to bind covalently any molecules with vicinal thiol groups. Material retained on the column is eluted with 2,3-dimercaptopropane sulphonate acid and allowed to re-oxidise before extraction and then methylation with diazomethane. Subsequent g.l.c.-mass spectrometry provides an absolute identification of methylated lipoic acid. The method has been successfully tested both with pure proteins known to contain lipoic acid and with hydrolysed cell extracts of E. coli which possess these proteins. It is now our intention to apply the methodology to the membrane fractions where we have found the dihydrolipoamide dehydrogenase. The presence or absence of lipoic acid will determine the nature of experiments designed to elucidate the function of the membrane-associated enzyme.

Concluding remarks

There is increasing evidence that a dihydrolipoamide dehydrogenase is associated with the cellular plasma membrane of a diverse range of organisms. While the enzyme has been implicated in sugar transport in E. coli, its possible functions in other membranes remain entirely speculative. However, the view that many processes at the plasma membrane may involve disulphide-thiol interchanges serves to heighten the interest in the biological function of this enzyme with its catalytic redox-active disulphide bond.

I gratefully acknowledge financial support in the form of grants (GR/C/02969 and GR/D/17793) from the S.E.R.C., travel grants from The Royal Society and the British Council and a N.A.T.O. grant for International Collaboration in Research with Professor K. 1. Stevenson, University of Calgary, Canada. I thank Professor K. J. Stevenson, Dr G. Holman, Dr R. Eisenthal and Dr D. Hough for their considerable help and advice.


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Received 29 September 1987