Quebec Cooperative Study of
Friedreich's Ataxia

Lipoamide Dehydrogenase in Friedreich's Ataxia Fibroblasts

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SUMMARY: *Lipoamide dehydrogenase was measued in cultivated skin fibroblasts from twelve patients with Friedreich's ataxia and nine normal controls. No difference in specific activity, subcellular distribution and Vmax or Km was observed between patients and controls.*

RÉSUMÉ: *Nous avons mesuré l'activité de la lipoamide déhydrogénase dans les fibroblastes cultivés à partir de biopsies cutanées de douze patients souffrant d'Ataxie de Friedreich et neuf témoins normaux. Nos résultats ne montrent aucune différence entre les deux groupes pour ce qui est de l'activité spécifique de l'enzyme, de sa distribution subcellulaire et des Km et Vmax.*

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INTRODUCTION

Pyruvate dehydrogenase (EC 1.2.4.1) is a major regulatory enzyme located within the inner mitochondrial membrane of the cell, where it catalyses the conversion of pyruvate into acetyl-CoA. This enzymatic reaction proceeds through a series of steps operated by three main components known as (E_1) pyruvate decarboxylase, (E_2) lipoyl reductase transacetylase and (E_3) dihydrolipoyl dehydrogenase (Reed and Cox, 1966). This latter component E_3 is an integral part of the multienzyme α -keto acid dehydrogenase complexes (Linn et al., 1969), where its function is to regenate oxidized lipoic acid. The natural substrate for E_3 is a reduced lipoic acid moiety bound to a lysine residue on the other component enzymes of the dehydrogenase complexes. In vitro, E_3 activity is measured using an artificial substrate, lipoamide, which has been converted to its active dihydrolipoamide form in a slightly alkaline solution of dithiothreithol, thus the name lipoamide dehydrogenase (LAD).

The determination of LAD activity in serum has recently been suggested to reflect liver damage in humans as increased levels of LAD correlated with hyperbilirubinemia (Pelley et al. 1976). A genetic defect in LAD has been postulated to underlie the metabolic alterations observed in some patients with Friedreich's ataxia (Blass et al., 1976). Reduced activity of LAD was documented in blood platelets from one patient (Rodriguez-Budelli and Kark, 1977) and in serum from eighteen other patients with Friedreich's ataxia (Melançon et al., 1977). It was hoped that the present fibroblast studies would confirm these previous investigations, suggesting that LAD deficiency is the major genetic defect underlying Friedreich's ataxia.

SUBJECTS AND METHODS

Cultured skin fibroblasts from 9 normal controls and 12 Friedreich's Ataxia (F.A.) patients were grown according to previously described methods (Melançon et al, 1972) and harvested by scraping after they reached confluency.

Lipoamide dehydrogenase activity was measured by recording the absorbance of NADH at 340 nm as in the serum assay (Pelley et al., 1976), using a Unicam SP1800 ultraviolet spectrophotometer. The complete reaction mixture (one ml) contained 20-60 μg cell protein; NAD, 5mM; DTT, 15mM; lipoamide or lipoic acid, 5mM; glycylglycine buffer, 0.5M, pH 7.8 or Tris HCl buffer, 0.1M; at 30°C. Specific activity of LAD was expressed in μ mole of NAD reduced per minute per mg of cell protein. Differential centrifugation in 0.25N sucrose was performed as previously described (Melançon and Nadler, 1973). Pyruvate dehydrogenase was assayed according to Blass et al. (1972) and citrate synthetase by the method of Schulman and Blass (1971).

RESULTS

The pH range for maximum LAD activity was between 7.4 and 7.8 in both normal and F.A. fibroblasts. The apparent Km and Vmax did not differ significantly between the two groups (fig. 1 a & b). The observed

TABLE I

LIPOAMIDE DEHYDROGENASE* IN FRIEDREICH'S ATAXIA (F.A.) AND NORMAL CULTIVATED SKIN FIBROBLASTS

	Km (mM)		Vmax (μmole/min/mg protein)	
	Normal	F.A.	Normal	F.A.
	0.72	0.96	57.5	128.2
	0.96	0.62	108.7	45.6
	0.44	1.45	96.7	126.6
mean	0.71	0.88	81.3	80.6

* Lipoamide as substrate

TABLE II

LIPOAMIDE (LAD) AND LIPOIC ACID (LOD) DEHYDROGENASE ACTIVITY IN NORMAL AND FRIEDREICH'S ATAXIA FIBROBLASTS

ENZYME*	SPECIFIC ACTIVITY IN μMOLE/MIN/MG PROTEIN	
	Normal Controls (6) mean ± S.D.	Friedreich's Ataxia (6) mean ± S.D.
LAD (Tris HCl)	26.1 ± 6.8	35.4 ± 11.0
LAD (Glygly)	53.7 ±15.4	75.0 ± 26.9
LOD (Tris HCl)	6.6 ± 2.5	6.7 ± 1.7
LOD (Glygly)	7.0 ± 1.2	8.3 ± 2.2

* Activity measured using both Tris and glycylglycine buffers

TABLE III

LIPOAMIDE (LAD) AND LIPOIC ACID (LOD) DEHYDROGENASE, PYRUVATE DEHYDROGENASE (PDH) AND CITRATE SYNTHETASE (CS) ACTIVITIES IN FRIEDREICH'S ATAXIA FIBROBLASTS

ENZYMES STUDIED	SPECIFIC ACTIVITY IN μMOLE/MIN/MG PROTEIN	
	Normal Controls (3)	Friedreich's Ataxia (6)
LAD (Tris)*	59.9 (52-70.7)	58.5 (45.7-85.3)
LAD (Glygly)	96.6 (85-106.8)	94.8 (71.3-130.8)
LOD (Tris)	4.8 (3.4-5.6)	4.6 (3.7-6.4)
LOD (Glygly)	6.0 (5-6.7)	4.9 (3.6-6.4)
PDH	28.3×10^{-6} (16.3-37.8)	41.3×10^{-6} (21.0-75.6)
CS	1.05×10^{-5} (0.92-1.16)	1.41×10^{-5} (1.10-1.61)

* LAD and LOD activities measured using both Tris and Glycylglycine buffers; values represent mean and range

values for Km and Vmax are given in table 1. It was not possible to study these latter parameters with lipoic acid as substrate because of the low rate of NAD reduction at low substrate concentration. When LAD activity of fibroblasts from 6 F.A. patients previously found to have low serum LAD values was compared with fibroblasts from six low-normal serum-LAD controls (table II), no significant difference could be found.

In a second group of 6 F.A. patients, 3 of whom had low serum LAD activity, we measured the activity of LAD in fibroblasts simultaneously with pyruvate dehydrogenase and citrate synthetase (table III). Once more, our results disclosed comparable values.

The distribution of LAD activity was further studied using subcellular fractions of fibroblast ultrasonicates from two normal controls, one F.A. patient and one Duchenne muscular dystrophy control (table IV). Most of the LAD activity (62-84%) was recovered in the high speed supernatant fraction. However, the specific activity of LAD in the different fractions was equally comparable.

DISCUSSION

Our results of a normal LAD activity in cultured skin fibroblasts from F.A. patients with low serum LAD values suggest that LAD deficiency in F.A. might be (A) restricted to specific tissues, (B) due to the existence of isoenzymatic differences between tissues, (C) appear in serum and platelets as a phenomenon secondary to other metabolic alterations or (D) be restricted to a sub-population of patients with F.A.

The evidence for a defect in LAD being restricted to specific tissues is far from being established. If it were the case, one would expect F.A. patients to demonstrate various clinical symptoms and signs depending on which organ is specifically affected. This is not the case since neither we nor Blass et al. (1976) found different phenotypes associated with serum or platelet LAD deficiency. The existence of differ-

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TABLE IV

DISTRIBUTION OF LAD ACTIVITY IN SUBCELLULAR FRACTIONS OF CULTIVATED SKIN FIBROBLASTS IN NORMAL CONTROLS (2), DUCHENNE MUSCULAR DYSTROPHY (D.M.D.) AND FRIEDREICH'S ATAXIA

FRACTIONS	NORMAL CONTROLS		D.M.D CONTROL	FRIEDREICH
WHOLE CELL LYSATE	85.3* (100)†	51.1 (100)	47.7 (100)	55.0 (100)
LESS NUCLEI	86.0 (95.7)	49.5 (96.8)	47.2 (96.0)	57.0 (99.0)
LESS MITOCHONDRIA	81.5 (91.8)	47.9 (93.7)	45.7 (89.4)	51.2 (95.2)
LESS LYSOSOMES	68.7 (68.2)	48.8 (93.0)	50.6 (88.8)	58.2 (86.7)
LESS MICROSOMES	88.3 (67.7)	43.1 (84.3)	53.5 (70.9)	54.4 (62.2)

* Specific activity expressed in μmoles/min/mg protein

† per cent residual activity as compared to whole cell homogenate

ent enzymatic forms of LAD in different tissues is quite obvious. Serum LAD activity as a function of different concentrations of lipoamide was shown by Pelley et al. (1976) to follow a curve quite similar to the one we observed using skin fibroblasts lysates (fig. 1a). The Km for lipoamide calculated from serum LAD values would fall between 0.6-1.0 mM. We found Km values of 0.71 and 0.88 mM respectively in normal and F.A. fibroblasts. Platelets as studied by other investigators (Rodriguez-Budelli and Kark, 1977) showed two apparent Km values of 85.1 ± 14.3 μM and 26.0 ± 5.0 μM in 6 normal controls and 3 to 5 times larger figures in one F.A. patient.

The elevated serum LAD levels in acute liver damage and hyperbilirubinemia (Pelley et al., 1976) would suggest that serum LAD originates from liver mitochondria and that liver, serum and fibroblasts LAD is a unique enzyme. Platelet LAD could then be a different form of the enzyme found in liver, serum and fibroblasts. We have no data as to the nature of LAD in other body tissues.

Recent experiments by one of us (Barbeau A., personal communication) indicate that taurine, calcium and pyridoxal phosphate have a regulatory effect on LAD in brain. Since we have previously found a renal conservation defect of taurine in F.A. patients (Lemieux et al., 1976), the occurrence of reduced serum and platelets levels of LAD

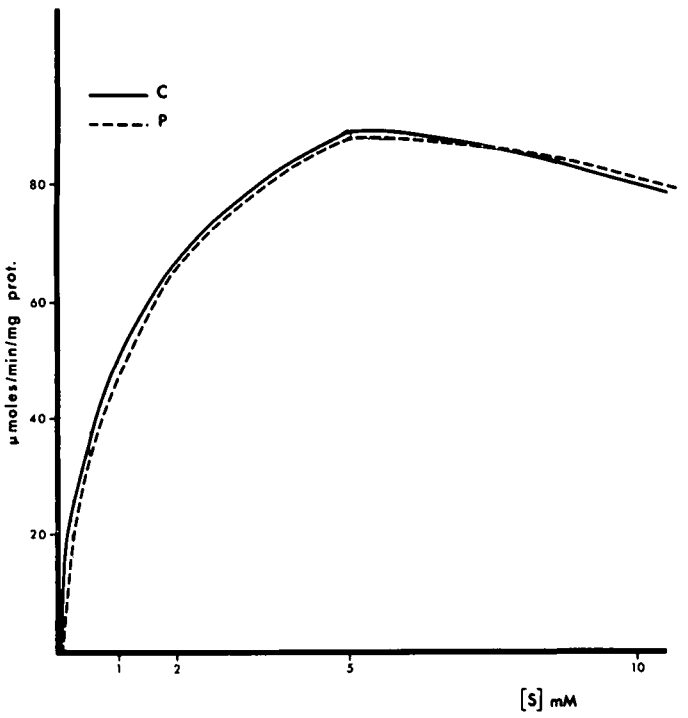

Figure 1a—Lipoamide dehydrogenase (LAD) activities as a function of lipoamide (S), in 3 fibroblast lines from patients with Friedreich's ataxia (P) and normal controls (C).

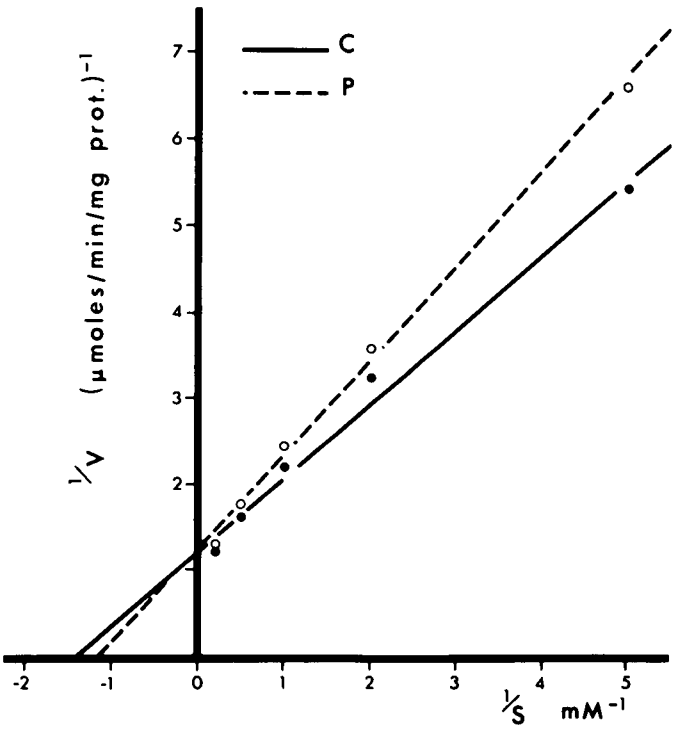

Figure 1b—Lineweaver-Burk plot of LAD activity in the same fibroblast lines.

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may well be a secondary phenomenon.

Finally, by analogy with the observation of Blass et al. (1977), who within a group of 16 patients with spinocerebellar degeneration found reduced pyruvate dehydrogenase activity in only 8, we recognize that LAD deficiency may be restricted to a sub-population of patients with Friedreich's ataxia.

In conclusion, we have been unable to confirm the presence of a defect in lipoamide dehydrogenase activity using skin fibroblasts from 12 patients with Friedreich's ataxia, 9 of whom had low serum LAD levels.

We believe that the low serum LAD activity observed in our patients reflects the effect of other primary and genetic derangements within the cell or about the cell membranes.

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REFERENCES

BLASS, J. P., SCHULMAN, J. D. and YOUNG, D. S. (1972). An inherited defect affecting the tricarboxylic acid cycle in a patient with congenital lactic acidosis. J. Clin. Invest., 51, 1845-1851.

BLASS, J. P., KARK, R. A. P. and MENON, N. K. (1976). Low activities of the pyruvate and oxoglutarate dehydrogenase complexes in five patients with Friedreich's ataxia. New Engl. J. Med., 195, 62-67.

BLASS, J. P., KARK, R. A. P. and RODRIGUEZ-BUDELLI, M. (1977). Pyruvate dehydrogenase in spinocerebellar degeneration. Clin. Res., 25, 321A.

LEMIEUX, B., BARBEAU, A., BERONIADE, V., SHAPCOTT, D., BRETON, G., GEOFFROY, G. and MELANCON, S. B. (1976). Amino acid metabolism in Friedreich's ataxia. Can. J. Neurol Sci., 3, 373-378.

LINN, T. L., PETTIT, F. H., HUCHO, F. and REED, L. J. (1969). α-Keto acid dehydrogenase complexes. XI Comparative studies of regulatory properties of the pyruvate dehydrogenase complexes from kidney, heart and liver mitochondria. Proc. Natl. Acad. Sci. U.S.A., 64, 227-234.

MELANCON, S. B., TAYCO, J. and NADLER, H. L. (1972). The free amino acid pool of cultivated human skin fibroblasts. Proc. Soc. Exp. Biol. and Med., 141, 391-395.

MELANCON, S. B., and NADLER, H. L. (1973). Prenatal diagnosis of genetic disorders leading to mental retardation. In Methods of Neurochemistry. Ed. R. Fried, Publ. Marcel Dekker, New York, 1-57.

MELANCON, S. B., POTIER, M., DALLAIRE, L., GEOFFROY, G., LEMIEUX, B. and BARBEAU, A. (1977). Serum lipoamide dehydrogenase in Friedreich's ataxia. Pediat. Res., 11, 460.

PELLEY, J. W., LITTLE, G. H., LINN, T. C. and HALL, F. F. (1976). Lipoamide dehydrogenase in serum: A preliminary report. Clin. Chem., 22, 275-277.

REED, L. J. and COX, D. J. (1966). Macromolecular organization of enzyme systems. Annu. Rev. Biochem., 35, 57-84.

RODRIGUEZ-BUDELLI, M. and KARK, R. A. P. (1977). Analysis of a defect in lipoamide dehydrogenase in Friedreich's ataxia. Trans. Am. Soc. Neurochem., 8, 116.

SCHULMAN, J. D. and BLASS, J. P. (1971). Measurement of citrate synthetase activity in human fibroblasts. Clin. Chim. Acta, 33, 467-468.