Biochimica et Biophysica Acta, 430 (1976) 71–82

BBA 47063

THE REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE "OXIDASE" OF *ACHOLEPLASMA LAIDLAWII* MEMBRANES

DAVID C. JINKS and LEONARD L. MATZ

Department of Microbiology, State University of New York at Buffalo, Buffalo, N.Y. 14207 (U.S.A.)

(Received September 1st, 1975)

SUMMARY

An NADH dehydrogenase possessing a specific activity 3–5 times that of membrane-bound enzyme was obtained by extraction of *Acholeplasma laidlawii* membranes with 9.0 % ethanol at 43 °C. This dehydrogenase contained only trace amounts of iron (suggesting an uncoupled respiration), a flavin ratio of 1 : 2 FAD to FMN, and 30–40 % lipid. Its resistance to sedimentation is probably due to the high flotation density of the lipids. It efficiently utilized ferricyanide, menadione and dichlorophenol indophenol as electron acceptors, but not O_2, ubiquinone Q_{10} or cytochrome *c*. Lineweaver-Burk plots of the dehydrogenase were altered to linear functions upon extraction with 9.0 % ethanol. A secondary site of ferricyanide reduction could not be explained by the presence of cytochromes, which these membranes lack. In comparison to other respiratory chain-linked NADH dehydrogenases in cytochrome-containing respiratory chains, this dehydrogenase was characterized by similar K_m's with ferricyanide, dichlorophenol indophenol, menadione as electron acceptors, but considerably smaller V's with ferricyanide, dichlorophenol indophenol, menadione as electron acceptors, and smaller specific activities. It was not stimulated or reactivated by the addition of FAD, FMN, Mg^{2+}, cysteine or membrane lipids, and was less sensitive to respiratory inhibitors than unextracted enzyme. The ineffectiveness of ADP stimulation on O_2 uptake, the insensitivity to oligomycin and the very low iron content of *A. laidlawii* membranes were considered in relation to conservation of energy by these cells. Some kinetic properties of the dehydrogenation, the uniquely high glycolipid content and apparently uncoupled respiration at Site I were noteworthy characteristics of this NADH dehydrogenase from the truncated respiratory chain of *A. laidlawii*.

INTRODUCTION

Acholeplasma laidlawii has recently attracted considerable interest because it is wall-less and its membranes contain high percentages of glycolipids whose fatty acid composition can be easily altered by supplementing the growth medium (1). A facultative anaerobe, it apparently possesses a flavin-terminated respiratory chain, as

no cytochromes have been detected in these cells [2, 3] even when thick suspensions of membrane have been frozen in liquid nitrogen and analyzed spectrophotometrically [35]. Since catalase has not been found in cells of the Mycoplasmatales, the product of this flavin-terminated system is presumably hydrogen peroxide [3].

The flavin-terminated system is membrane-bound in *A. laidlawii*; the membrane-bound reduced β nicotinamide adenine dinucleotide (NADH) oxidase can utilize ferricyanide, dichlorophenol indophenol (DCIP) and menadione as alternate electron acceptors. After cold storage it can be reactivated by FAD [1]. Prolonged sonication [4] or extraction with 1.0 % sodium dodecyl sulfate and 2.0 % sodium deoxycholate [5] can dissociate this NADH oxidase from membrane [36]. Detergent extraction of *A. laidlawii* membranes with sodium dodecyl sulfate and sodium deoxycholate released an oxidase which was excluded from Sephadex-200 [6, 7]. We now report the properties of an ethanol extracted, highly lipoidal NADH "oxidase" which utilizes ferricyanide with much greater efficiency, and therefore has been referred to as an NADH dehydrogenase, and whose easily alterable fatty acid structure could provide dehydrogenases with different fatty acid contents as model systems for study of the fine control of respiratory enzymes by fatty acids [8]. This dehydrogenase is unique in being derived from a truncated electron transport chain containing a relatively stable membrane-bound oxidase [1].

MATERIALS AND METHODS

Cells of *A. laidlawii* (ATCC No. 14192) were routinely grown on 4 % Tryptose media at 37 °C and harvested [9] by centrifugation at $10\,000 \times g$ at 4 °C. Washed cells resuspended three times in β buffer (0.05 M Tris · HCl and 0.01 M mercaptoethanol) [9] plus saline were disrupted by sonic oscillation for 3 min (Bronwill Ultrasonic Disintegrator at maximum power) or by osmotic lysis [10]. Membranes were isolated by centrifugation (Model L2-65B Beckman Ultracentrifuge) of the disrupted cells. Purified membrane suspensions were isolated by density gradient centrifugation of cell lysates on 5–55 % β-buffered sucrose gradients. The gradients were fractionated after centrifugation at $52\,000 \times g$ for 2 h at 4 °C in a Beckman SW-27 rotor with a Buchler Fractomette 200 (10 drops/fraction) and analyzed for A_{280} absorbing material and enzymatic activity.

Ethanol-extracted enzyme was prepared by treatment of membrane in 0.05 M Tris · HCl buffer with 9.0 % ethanol at 43 °C for 30 min [11]. These membrane extracts were chromatographed on Agarose A-50 m (BioRad Laboratories) in a 0.8×30 cm column at 4 °C, and fractions were collected with a Buchler Fractomette with microtube adaptors. Fractions of 12 drops were collected from a 0.8×30 cm column which was developed at a flow rate of 6–8 drops/min. Polyacrylamide gel electrophoresis of mycoplasma membrane proteins [12] and staining procedures for proteins on these gels [13] have been described [13].

The NADH oxidase activity was measured at ΔA_{340} by incubation of enough membrane-bound or ethanol-extracted enzyme to give a 0.1–0.2 decrease in ΔA_{340}/min with 0.576 mM NADH at 37 °C. NADH dehydrogenase or ferricyanide reductase activity was measured at ΔA_{420} by incubating membrane with 0.144 mM NADH or ethanol-extracted enzyme with 0.576 mM NADH and 1.6 mM $K_3Fe(CN)_6$ at 37 °C. Sufficient enzyme was added to give a 0.1–0.2 decrease in ΔA_{420} / min.

Menadione reductase activity was measured at ΔA_{340} with enough enzyme to give a 0.2–0.3 decrease in ΔA_{340}/min with 0.8 mM menadione at 37 C. DCIP was assayed at ΔA_{600} with enough enzyme to give a 0.2–0.3 decrease in ΔA_{600}/min with 0.5 mM DCIP at 37 °C. Coenzyme Q_{10} was assayed at ΔA_{340} with a 0.05–0.1 decrease with 0.35 mM coenzyme Q_{10} at 37 °C. Cytochrome *c* was assayed at ΔA_{550} with enough oxidase to give a decrease in ΔA_{550} of 0.1–0.2/min with 18.75 μM cytochrome *c*. The final reaction mixtures for these assays were made up to 300 μl with 0.05 M phosphate buffer (pH 6.0).

All enzyme activity was calculated from the slope of the linear tracing after 15 or 30s, with adjustments made from proper controls. Assays were made with a Zeiss PMQ Spectrophotometer with attached recorder. An enzyme unit of NADH oxidase, menadione and coenzyme Q_{10} was defined as μmol of NADH oxidized · min^{-1}, and specific activity was defined as μmol of NADH oxidized · min^{-1} · mg^{-1} protein. An enzyme unit of NADH dehydrogenase, DCIP and cytochrome *c* reductase was defined as μmol of electron acceptor reduced · min^{-1}, and specific activity was defined as μmol of electron acceptor reduced · min^{-1} · mg^{-1} protein. Protein was determined by the Lowry modification [14] of the Folin test after addition of 5 μl of 1.0 % sodium dodecyl sulfate (Fisher) and 10 μl of 2 % sodium deoxycholate (Fisher) for each 20–25 μg protein/sample.

Lipids were extracted from lyophilized membrane or ethanol-extracted dehydrogenase with chloroform/methanol (2 : 1) at room temperature, separated on silica gel H chromatoplates with chloroform/methanol/water (60 : 30 : 4.5) or chloroform/methanol (2 : 1), and located with iodine [15]. They were identified by either cochromatography with known compounds or reference R_F values [15]. Lipids bound to silica gel H were quantitated with the dichromate-sulfuric acid assay [16] with glucose as the carbon standard.

FMN and FAD were estimated with the aid of an Aminco-Bowman Spectrophotofluorometer [17], and iron was estimated [18] after release from lyophilized membrane or ethanol-extracted dehydrogenase with 5.0 % mercaptoacetic acid and glacial acetic acid (1 : 2 v/v). O_2 consumption was measured with an O_2 electrode as previously described [19].

ADP, Amobarbital (Amytal), Antimycin A, FAD, FMN, NAD and NADH were obtained from Sigma Chemical Co. 4,4,4-Trifluoro-1-(2-thienyl)-1,3 butanedione (TFTB) was obtained from Wisconsin Alumni Research Foundation.

RESULTS

An NADH dehydrogenase which did not sediment at 100 000 × *g* for 1 h was extracted from suspensions of *A. laidlawii* membrane with 9.0 % ethanol at 43 °C. Gel electrophoresis patterns revealed that the extracted and partially purified dehydrogenase contained six proteins or peptides, while membranes contained over 20 (Fig. 1), and indicated that loss of contaminating proteins, rather than non-specific activation by membrane fragmentation, increased the specific activity of the dehydrogenase. The purification scheme for this extracted dehydrogenase disclosed that ethanol-heat treatment of the membrane extracted about 40 % of the original membrane-bound NADH dehydrogenase activity (Table I). During this purification, the loss of activity of the dehydrogenase with O_2 as the acceptor was nearly twice that with ferricyanide

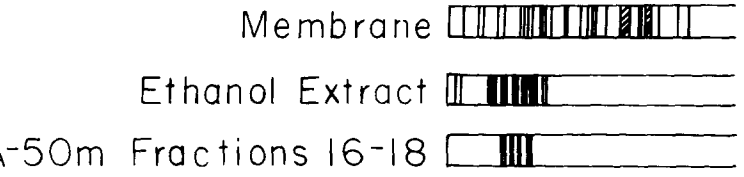

Fig. 1. Polyacrylamide gel electrophoresis of membrane and membrane extracts before and after agarose gel filtration. 100–300 μg protein was layered on the gel while suspended in the milieu of extraction. The gel was then developed for 90 min at 5 mamp/tube and stained with 1.0 % Coomassie Blue. A band of extracted membrane proteins which stained with moderate intensity was exluded from the gels and observed on top of all the gels.

as acceptor, indicating that the NADH dehydrogenase was altered during extraction. In addition, only 60 % of the original membrane-bound dehydrogenase activity was accounted for in the scheme, and the extracted and chromatographed dehydrogenase (Fig. 2) accounted for only 10 % of the original membrane-bound dehydrogenase.

Both the ethanol-extracted dehydrogenase and the enzymes prepared by detergent treatment or sonic oscillation of the membranes migrated as small membrane fragments (which possessed different protein/lipid contents and ratios, but similar specific activities). Further extraction of these membrane fragments with high concentrations of sodium deoxycholate (16 mg/ml), and subsequent gel filtration on Bio-Gel P-200, only slightly increased the specific activity of the enzyme.

Purification schemes were less effective when they were based on extraction with $NaClO_4$, snake venom phospholipase or bee venom phospholipase (kindly supplied by Dr. W. B. Elliott), the detergents (sodium dodecyl sulfate, sodium deoxycholate and Triton X-100), sonication or combinations of these treatments. (Extraction with 1M $NaClO_4$, though it resulted in low yields and suggested the possibility of denaturative fragmentation, did release a soluble enzyme from the membranes, as evidenced by inclusion into BioGel P-200.) Butanol did not release dehydrogenase activity from the membrane, and readdition of the butanol extract to bound or extracted dehydrogenase did not increase the activity of the dehydrogenase. The ethanol-extracted enzyme was slightly more purified, gave very good enzyme $\times$ time

TABLE I

ISOLATION OF AN NADH DEHYDROGENASE EXTRACTED FROM MEMBRANES OF *A. LAIDLAWII* WITH ETHANOL AND HEAT

Purification of an NADH dehydrogenase extracted from membranes of *A. laidlawii* with ethanol and heat.

Sample	Activity units*	Protein (mg)	Spec. act.**	Yield (%)	Purification
Membrane	19.52	8.0	2.44	100	1
9.0 % Ethanolic membrane suspension	18.95	6.6	2.87	97.0	1.17
9.0 % Ethanol-extracted membrane	4.41	6.7	0.93	22.5	0.26
9.0 % Ethanol extract	7.40	2.3	3.36	39.6	1.4
Gel-filtered 9.0 % ethanol extract	1.77	0.19	9.36	9.1	3.8

* units = μmol ferricyanide reduced · min^{-1}

** Spec. act. = μmol ferricyanide reduced per mg protein.

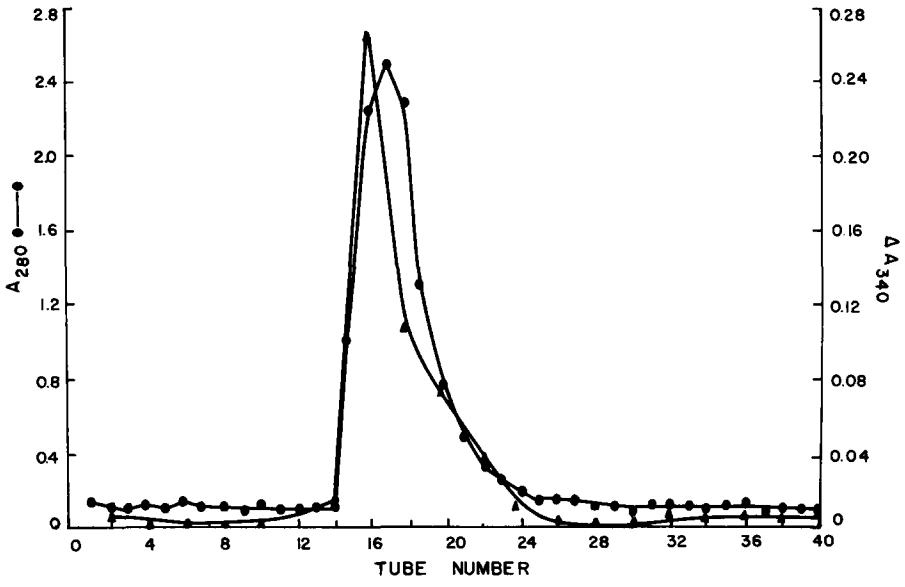

Fig. 2. The effluent pattern of ethanol-extracted oxidase after gel filtration. The oxidase activity (▲—▲) and some A_{280} absorbing protein (●—●) migrated as an intermediate-sized particle (tubes 14–20) on the Bio-Gel A-50m with 10 % ethanolic 1/20 β buffer (pH 7.5) as eluant.

reproducibility, and contained 1/3 to 1/4 the protein of the membrane, and was therefore routinely utilized in this study.

The extracted dehydrogenase contained only 1.3 nmol of heme and non-heme iron per mg protein (Table II). In order to ascertain whether the low iron content could be traced to the extraction of the iron-sulfur proteins by ethanol-heat, membranes were grown on tryptose media (which contains ample iron) and analyzed. The results confirmed the unusually low iron content of the membrane and the dehydrogenase. Estimation of molecular weight by analytical ultracentrifugation was not possible, as the dehydrogenase contained sufficient lipid to float. The lipid content of the dehydrogenase, 0.65 mg lipid/mg protein (Table II), was quantitatively similar to the lipid content of the membranes. The lipids isolated from membrane-bound or extracted dehydrogenase were also qualitatively similar, although the carotenoid and cardiolipin content of the soluble dehydrogenase was slightly enriched. This extracted dehydrogenase was unique in having 28 % glycolipids, the same proportion of glycolipids as the membranes from which it originates. About twice as much FMN as FAD was found in the extracted dehydrogenase.

The dehydrogenase was more active in 0.05 M phosphate than in 0.05 M Tris · HCl, and was most active at pH 6.0 and 37 °C; the pH and temperature optima fell within a very narrow range. It oxidized NADH in direct proportion to the protein present between 0.04–1.15 mg protein with ferricyanide as an electron acceptor with greater efficiency than O_2. The extracted dehydrogenase also reacted efficiently with menadione and DCIP, but only weakly with cytochrome *c*, coenzyme Q_{10}, and O_2 (Table III). The NADH oxidase activity of the extracted dehydrogenase was stimulated 3–5 times by catalase. (Catalase stimulates some oxidases by forming O_2 from

TABLE II

CHEMICAL COMPONENTS OF THE EXTRACTED DEHYDROGENASE

Chemical components of the extracted dehydrogenase. ND, no data.

Component	Composition (mg protein)	
	Membrane*	Ethanol-extracted dehydrogenase**
	μg	μg
Lipid	669	642
Carotenoids	74	118
Cardiolipin	136	157
Monoglucosyl diglyceride	113	72
Diglucosyl diglyceride	60	63
Cholesterol	135	76
Phosphatidyl glycerol	98	61
Glycerophosphoryl-diglucosyl diglyceride	23	47
Unknown	30	48
	nmol	nmol
Flavin		
FAD	ND	0.19
FMN	ND	0.30
Metal		
Iron	5.0	1.3

* Compilation of two determinations
** Compilation of four determinations

TABLE III

ACTIVITY OF EXTRACTED NADH DEHYDROGENASE WITH DIFFERENT ELECTRON ACCEPTORS

Activity of extracted NADH dehydrogenase with different electron acceptors. NA, not applicable; ND, not determined.

Electron Acceptor	Concentration (mM)	Spec. Act. (μmol)	K_m acceptor (mM)	V acceptor ($\mu mol \cdot min^{-1}$)
Ferricyanide	1.6	5.61	0.570	0.167
Menadione	0.8	1.97	0.556	0.025
DCIP	0.5	0.894	0.180	0.006
O_2	NA	0.0558	ND	ND
Coenzyme Q_{10}	0.35	0.0474	ND	ND
Cytochrome c	0.0187	0.0198	ND	ND

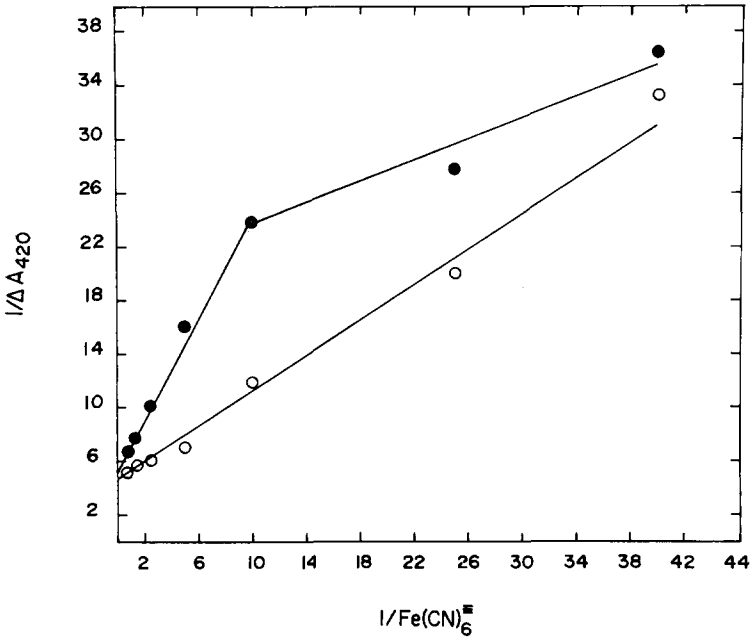

Fig. 3. A Lineweaver-Burke plot of the membrane-bound and ethanol-extracted NADH dehydrogenase. The incubation mixture was similar to that described in the legend of Fig. 5, except that the mixture was made up to 0.144 mM NADH. The kinetics of both membrane-bound (●–●) and ethanol-extracted (○–○) NADH dehydrogenase are plotted on this graph.

H_2O_2, the apparent product of this dehydrogenase, and by increasing the O_2 concentration of the incubation mixtures.) Lineweaver-Burke plots of the membrane-bound or extracted ferricyanide reductase activities changed from a biphasic function of the membrane-bound enzyme to a linear function for the extracted enzyme (Fig. 3). These altered kinetics confirmed the increased homogeneity of the dehydrogenase suggested by the polyacrylamide gel patterns, and reflect the removal of the dehydrogenase from the microenvironment of the membrane and its other possible electron acceptors. This inverse proportionality showed also that the dehydrogenase possessed an increased reactivity after extraction, an indication that the dehydrogenase was not detrimentally altered by extraction. Indeed, detailed inverse plots at the higher concentrations of ferricyanide revealed that the higher concentrations of NADH no longer inhibited the extracted dehydrogenase as they did the membrane-bound dehydrogenase (Fig. 4). The regulatory controls on the membrane-bound dehydrogenase were therefore ineffective after extraction. Determination of the K_m and V for the extracted dehydrogenase with different electron acceptors (Fig. 5) confirmed an ordered affinity of the dehydrogenase for different electron acceptors. This reactivity of the dehydrogenase with several electron acceptors may reflect the presence of several respiratory components, even though some purified dehydrogenases do react with several electron acceptors. That reactivity of this dehydrogenase with O_2 is very limited is confirmed by these kinetic properties.

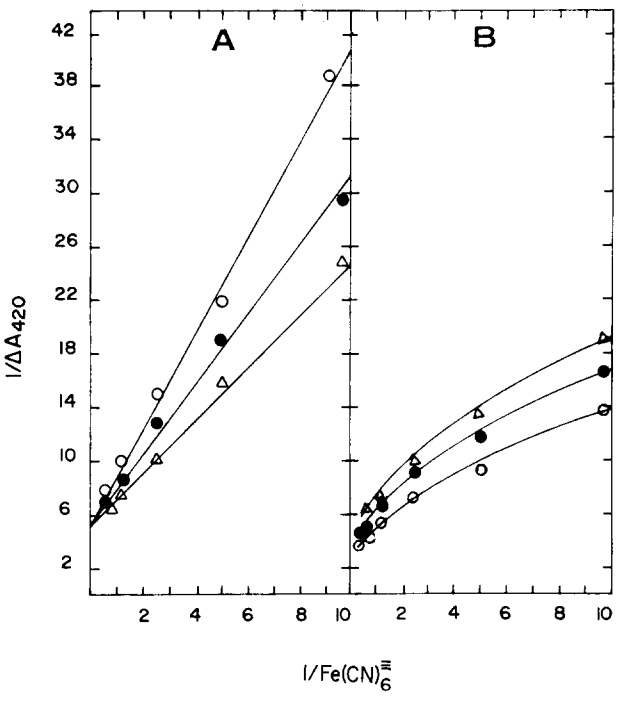

Fig. 4. Effect of NADH on the (a) membrane-bound, and (b) extracted NADH dehydrogenase. The NADH concentration was varied from 0.144 to 0.576 in the incubation mixture as described in Fig 5. These patterns show an inhibition with membrane-bound dehydrogenase and stimulation with extracted NADH dehydrogenase. 0.576 mM NADH (○–○), 0.288 mM NADH (●–●) and 0.144 mM NADH (△–△) were the final concentrations of NADH in the incubation mixtures.

TABLE IV

INHIBITION OF MEMBRANE-BOUND NADH OXIDASE AND EXTRACTED NADH DEHYDROGENASE OF *A. LAIDLAWII*

Inhibition of membrane-bound NADH oxidase and extracted NADH dehydrogenase of *A. laidlawii*. ND, no data; NI, no inhibition.

Inhibitor	Concentration (mM)	% Inhibition	
		Membrane	Ethanol-extracted enzyme
Amytal	0.01	10.5	ND
	10.0	34.0	ND
Arsenate	0.01	52.3	NI
Azide	10.0	46.2	NI
Rotenone	0.01	15.3	NI
	0.02	35.7	NI
	0.04	60.2	NI
	0.08	90.0	NI
TFTB	0.018	60.0	3 × stimulation
	0.035	100.0	3 × stimulation
	0.070	100.0	77.0
Oligomycin	0.0001	NI	ND
	0.001	NI	ND

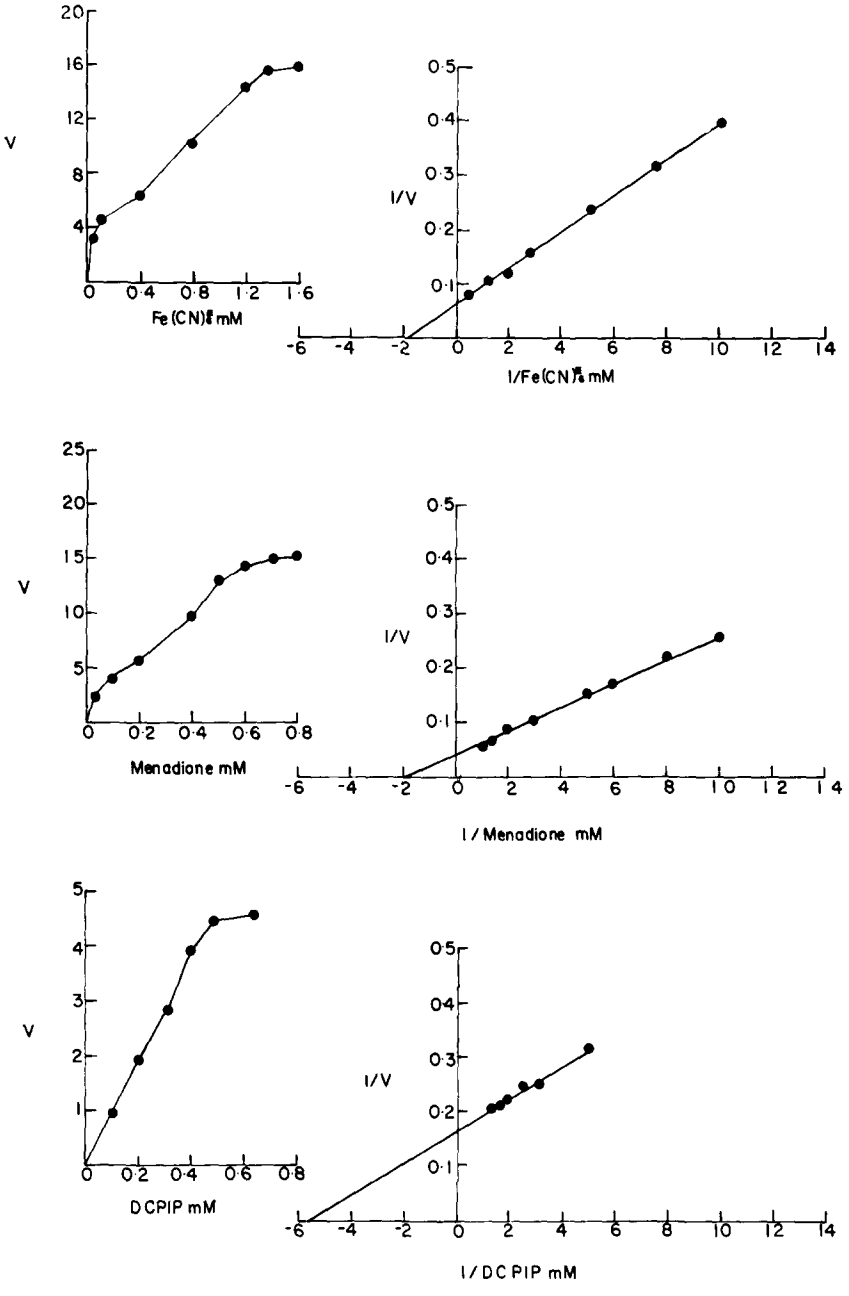

Fig. 5. Determination of the K_m acceptor and V acceptor of the ethanol-extracted NADH dehydrogenase. The incubation mixture for assay of the NADH dehydrogenase was composed of 5 μl NADH to make the mixture 0.576 mM, 10 μl acceptor, 100 μl enzyme (0.2–0.4 mg/ml) made up to 300 μl with 0.05 M phosphate buffer (pH 6.0). The ferricyanide reductase was assayed at A_{420}, the DCIP reductase at A_{600} and the menadione reductase at A_{340} after incubation at 37 °C for 30 s. The ordinates are expressed as mM of electron acceptor in all plots. The abscissa for ferricyanide is expressed as mmol $\cdot 10^{-5} \cdot \text{min}^{-1}$, and those for menadione and DCIP are expressed as mmol $\cdot 10^{-6} \cdot \text{min}^{-1}$.

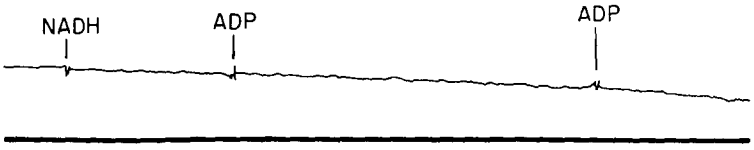

Fig. 6. O_2 incorporation of a thick suspension of membranes of *A. laidlawii* upon addition of NADH and ADP. Membranes from a culture of *A. laidlawii* in tryptose media were suspended in 0.02 M phosphate buffer and checked for endogenous respiration. After determining that the endogenous respiration was nearly zero, 20 μl of 0.1 M NADH was added to the reaction mixture at 37 °C, and the rate of O_2 uptake recorded. Then two samples of 20 μl of 0.1 M ADP were added and the rate of O_2 uptake again recorded after each addition. These membranes incorporated 6.0 μmol O_2/min upon addition of NADH. This rate of uptake of O_2 was affected very little by the first addition of ADP but went up to 8.0 μmol O_2/min with the second addition of ADP.

A flavoprotein dehydrogenase was suggested by inhibition of membrane-bound oxidase activity with rotenone and amytal, even though 0.01 M rotenone was ineffective against the ferricyanide reductases of both membrane-bound and extracted dehydrogenases. TFTB inhibited the ethanol extracted oxidase activity but not ferricyanide reductase activity. Oligomycin did not inhibit the membrane-bound oxidase activity (Table IV), and the rate of oxygen uptake was not altered by addition of ADP (Fig VI), implying no coupling between electron transport and oxidative phosphorylation.

DISCUSSION

This extracted NADH "oxidase" of *A. laidlawii* is reminiscent of NADH oxidase complexes found in several other bacterial membranes [2, 20]. Because this "oxidase" preferred ferricyanide as an electron acceptor, was competitively inhibited by higher concentrations of NADH, and contained flavins, it is apparent that at least one operative respiratory enzyme of this complex is a respiratory chain-linked NADH dehydrogenase [21]. The Lineweaver-Burk plots of the membrane-bound and extracted dehydrogenases, however, disclose the possibility that two sites of ferricyanide reduction are found in the intact respiratory chain of the membrane. Reduction of ferricyanide with a large multifunctional protein [22] or a second flavoprotein [23] or cuproprotein [24] could account for these results. Other possible explanations include nonspecific effects of permeability or specific denaturation of the native dehydrogenase and use of an alternate site of action which exhibits linear kinetics [23]. This denaturative mechanism, which may involve fragmentation of the dehydrogenase, has been suggested for beef heart mitochondrial dehydrogenase. Utilization of an alternate site of action resulted in lowered activities, loss of competitive inhibition with NADH and reactivity with a wide range of electron acceptors [25]. That our extracted dehydrogenase was involved in this type of denaturation was suggested by the loss of competitive inhibition with NADH. But evidence that the reaction rate of the extracted dehydrogenase was greater than that of membrane-bound dehydrogenases, and the dehydrogenase did not fragment when extracted with ethanol-heat, seems strongly to reject the explanations using this denaturative mechanism. Whatever the reason for the biphasicity of ferricyanide reduction is thought to be, the product of the respiratory chain is generally accepted as H_2O_2 [3], even though this dehydroge-

nase reacts only marginally with O_2. Therefore, this respiratory chain from a facultative anaerobe appears to involve more labile respiratory chain-linked components which increase its efficiency in these membranes, or minor utilization of this aerobic respiration and major utilization of substrate-level dehydrogenations.

The sensitivity of the membrane-bound and extracted dehydrogenase to amytal and rotenone was evidence of a flavin-dehydrogenase [26–28]. Finding both flavins suggests the presence of more than one flavoprotein. The insensitivity of our dehydrogenase to stimulation with FAD or FMN is apparently due to tightly bound flavin not released by our manipulation. Efforts at removal of essential flavin cofactors have not been undertaken.

Our results are indicative of uncoupled respiration and oxidative phosphorylation in *A. laidlawii*. First, ADP, which usually stimulates incorporation of O_2 in coupled systems, had little effect on O_2 uptake when added to thick suspensions of *A. laidlawii* membranes. In fact, spectrophotometric data show that ADP actually inhibits the oxidation of NADH oxidase with O_2 as the acceptor in sonicated membrane from *A laidlawii* [4]. This control of the NADH oxidase activity might be traceable to the similarities in structure of ADP and NADH [3]; our data substantiate the product inhibition of NAD on the NADH dehydrogenase. Second, oligomycin, which inhibits coupled systems by interfering with the coupling factor ATPase [29], was ineffective in this system. Oligomycin is very effective in the pigeon heart mitochondrial system, requiring only 2.8 nmol/mg protein, and inhibits to some degree in most systems [30]. Last, only traces of iron are found in the membranes and extracted NADH dehydrogenases of *A. laidlawii*. This is noteworthy because iron-sulfur complexes have recently been associated with the conservation of energy as intermediates of coupling in some yeasts [31, 32] and mammalian submitochondrial particles [32]. Their association with NADH dehydrogenase molecules [33] supercedes previous work which indicated that they were distinct components of the NADH dehydrogenase region of the respiratory chain [34]. Not all yeast submitochondrial particles which contain NADH dehydrogenase flavoproteins exhibit the electron paramagnetic resonance signals of the iron-sulfur centers [32]; the yeast dehydrogenases from *cerevisiae* and *Streptomyces carlbergensis* are from uncoupled systems [32]. The extracted or membrane-bound NADH dehydrogenase of *A. laidlawii*, which does not contain appreciable iron and is apparently not coupled, seems comparable to the *S. cerevisiae* and *S. carlbergensis* dehydrogenases. Iron-sulfur proteins and conservation of energy of the dehydrogenase region are closely related to rotenone sensitivity [31]. The sensitivity of the *A. laidlawii* NADH dehydrogenase to rotenone with O_2 as electron acceptor contradicts the general conclusion that the *A. laidlawii* system is uncoupled. Under some conditions, though, rotenone insensitivity has been observed in coupled systems [31]; upon further investigation, other uncoupled bacterial systems may prove to be rotenone sensitive. In general, however, the data at this point support the view that this is an uncoupled or loosely coupled system, or that coupling occurs by a mechanism far different from those observed in other microorganisms.

ACKNOWLEDGEMENTS

This investigation was supported by General Research Support Grant No. 5440 from the General Research Support Branch, Division of Research Resources,

National Institutes of Health. The authors wish to thank Dr. W. B. Elliott and Dr. J. M. Merrick for the loan of some instrumentation and for their helpful suggestions upon reading this manuscript.

REFERENCES

1 Razin, S. (1973) Adv. in Microbiol. Phys. 10, 1–80
2 Smith, S. L., Van Demark, P. J. and Fabricant, J. (1963) J. Bacteriol. 86, 893–897
3 Smith, P. F. (1971) The Biology of the Mycoplasmas, pp. 171–176, Academic Press, New York
4 Stopkie, R. J. and Weber, M. W. (1967) Biochem. Biophys. Res. Commun. 28, 1034–1039
5 Rodwell, W. W., Razin, S. Rottem, S. and Aragmann, M. (1967) Arch. Biochem. Biophys. 122, 621–628
6 Ne'eman, B., Kahane, I., Kovartsky, J. and Razin, S. (1972) Biochim. Biophys. Acta 266, 255–268
7 Razin, S., Morowitz, J. J. and Terry, T. M. (1965) P.N.A.S. 54, 219–225
8 DeKruyff, B., DeGrief, W. J., Van Eyk, R. V. W., Demel, R. A. and Van Deenen, L. L. M. (1973) Biochim, Biophys. Acta 298, 479–499
9 Pollack, J. D., Razin, S. and Cleverdon, R. C. (1965) J. Bacteriol. 90, 617–622
10 Razin, S. (1963) J. Gen. Microbiol. 33, 471–475
11 Mahler, J. R., Sarkar, N. K., Vernon, L. P. and Alberty, A. R. (1952) J. Biol. Chem. 199, 585–597
12 Rottem, S. and Razin, S. (1967) J. Bacteriol. 94, 359–364
13 Chrambach, A., Reisfeld, R. A., Wychoff, M., and Zaccari, J. (1967) Anal. Biochem. 20, 150–154
14 Lowry, O. H., Rosenbough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–278
15 Shaw, N., Smith, P. F. and Koostra, W. L. (1968) Biochem. J. 107, 329–333
16 Amenta, J. S. (1964) J. Lipid Res. 5, 270–272
17 Faeder, E. J. and Siegel, L. M. (1973) Anal. Biochem. 53, 332–336
18 Doeg, K. A., and Ziegler, D. M. (1962) Arch. Biochem. Biophys. 97, 37–40
19 Malviya, A. N., Nichols, P. and Elliott, W. B. (1968) Biochim. Biophys. Acta 153, 920
20 Yu, L. and Wolin, M. J. (1972) J. Bacteriol. 109, 51–58
21 Minikami, S., Ringler, R. L. and Singer, T. P. (1962) J. Biol. Chem. 237, 569–576
22 Siegel, L. M. and Davis, P. S. (1974) J. Biol. Chem. 249, 1587–1598
23 Singer, T. P. and Gutman, M. (1970) in Pyridine Nucleotide-dependent Dehydrogenases (Sund, H., ed.), pp. 375–390, Springer, Berlin
24 Konings, W. N., Van Driel, R., Van Bruggen, E. F. J. and Gruber, M. (1969) Biochim. Biophys. Acta 195, 55–66
25 Singer, T. P. and Gutman, M. (1971) Adv. Enzymol. 34, 79–154
26 Hatefi, Y. (1968) P.N.A.S. (U.S.) 60, 733–744
27 Horgan, D. J., Singer, T. P. and Casida, J. E. (1968) J. Biol. Chem. 243, 834–843
28 Palmer, B., Horgan, D. J. Tisdale, H., Singer, T. P. and Beinert, J. (1968) J. Biol. Chem. 243, 844–847
29 Lardy, H. A., Johnson, D. and McMurray, W. C. (1958) Arch. Biochem. Biophys. 78, 587–597
30 Chance, B. (1961) J. Biol. Chem. 236, 1569–1576
31 Ohnishi, T. (1973) Biochim. Biophys. Acta 301, 105–128
32 Ohnishi, T., Asshura, T., Yonetani, T. and Chance, B. (1971) J. Biol. Chem. 246, 5960–5964
33 Beinert, H., Palmer, G., Cremona, T. and Singer, T. P. (1965) J. Biol. Chem. 240, 475–480
34 Hatefi, Y. and Stempel, K. E. (1969) J. Biol. Chem. 2350–2357
35 Matz, L. L. (1972) Bacteriol. Proc. 72, 159
36 Jinks, D. C. and Matz, L. L. (1974) Bacteriol. Proc. 74, 170