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Immobilized Respiratory Chain Activities from *Escherichia coli* Utilized to Measure D- and L-Lactate, Succinate, L-Malate, 3-Glycerophosphate, Pyruvate, or NAD(P)H

CLAUDE BURSTEIN,* ELISABETH ADAMOWICZ, KEBIR BOUCHERIT, CATHERINE RABOUILLE, AND JEAN-LOUIS ROMETTE†

Laboratoire des Biomembranes, Institut Jacques Monod, Centre National de la Recherche Scientifique, Université Paris VII—Tour 43, 2, place Jussieu, 75251 Paris Cédex 05, France; and †Laboratoire de Technologie Enzymatique, Université de Technologie de Compiègne, 60206 Compiègne, France

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ABSTRACT

The respiratory chain (membranous, multienzymatic system) from *Escherichia coli,* was coimmobilized with gelatin and insolubilized in film form by tanning with glutaraldehyde. The film was fixed onto an oxygen sensor. The enzyme electrode can be used for measuring NAD(P)H, D- and L-lactate, succinate, L-malate, 3-glycerophosphate, or pyruvate. The range of metabolites concentrations was from 1 to 50 m*M*.

It was possible to dicriminate between the different metabolites (if mixed):

(1) By inducing during bacterial growth the specific flavoproteins necessary for L-lactate, succinate, L-malate, and 3-glycerophosphate respirations. The constitutive activities are unaltered on glucose or glycerol, namely D-lactate, NAD(P)H, and pyruvate respiration.

*Author to whom all correspondence and reprint requests should be addressed.

2. When intact bacteria were immobilized (with or without induction), D- and L-lactate, succinate, 3-glycerophosphate, and L-malate respiration were measured, no activities of pyruvate and NAD(P)H respiration were obtained. For these last activities, French press breakage (*see* section on Membrane Preparations) of bacteria prior to immobilization was necessary.
3. Products of reactions can be used as enzyme inhibitors: Pyruvate inhibits D- and L-lactate; fumarate inhibits succinate, and oxaloacetate inhibits L-malate respirations.
4. Heat denaturation of the bacteria at 55°C for 1 h maintains full activity of succinate and pyruvate respiration. On the other hand, no activity of D- and L-lactate, L-malate, or NAD(P)H respiration was measurable.

These enzyme electrodes have many applications in basic and applied research.

Index Entries: Enzyme electrode; immobilized respiratory chain; *Excherichia coli;* immobilized bacteria; immobilized membrane; flavoproteins; glutaraldehyde tanning; immobilized enzymes; bacterial respiration.

INTRODUCTION

The utilization of active enzymes after chemical immobilization is a valuable tool for analytical methods because of their specificity and stability. Furthermore, continuous and instantaneous chemical determinations of enzyme effectors (substrates, products, and inhibitors) are possible and easy to perform with enzyme electrodes. The use of an enzyme as a functional element of an electrochemical device was first reported by Clark and Lyons (*1*). Subsequently, Updike and Hicks (*2*) described an electrode incorporating an immobilized enzyme. Specific enzymatic films are tightly fixed onto probes, allowing quantitative assays of the enzyme effectors. At least 60 different enzyme probes have already been described (*3,4*), using various sensors as Pt, NH_4^+, CO_2, H_2O_2, or O_2. In the field of oxygenases, an oxygen probe measuring consumption of oxygen appears to be facilitated by the use of gelatin film. Oxygen dissolved in gelatin is about 20 times higher than in usual buffers (*5*). This property renders the measurement independent of the amount of oxygen in the solution tested.

The enzymes of the respiratory chain are a useful tool for analysis of their substrates and products. In a previous paper we described the immobilization of intact bacteria or inverted vesicles of plasma membrane with respiratory chain activity in *Escherichia coli* (*6*). Similar preparations were able, at a more acidic pH of 5.8, to utilize NADPH after immobilization (*7*). Respiration of NADH with immobilized thermophilic bacteria (PS_3) was also obtained (*8*).

Two recent reviews of bacterial respiration (*9,10*) clearly show its location in the plasma membrane. The first enzymes of the respiratory chain, usually termed dehydrogenase (principally with FAD as coenzyme), face the cytoplasm and vary with growth conditions: Growth on D-, L-lactate, succinate, or glycerol increases, respectively, L-lactate-, succinate-, or 3-glycerophosphate-dehydrogenase. D-lactate- and NADH-dehydrogenase are present regardless of the carbon source.

Quantitative measurements of the dehydrogenases were performed in the presence of a hydrogen acceptor, such as ferricyanide. Complete respiration was measured by oxygen consumption, and kinetic data suggest that the limiting step of the respiratory chain is the first. Thus, we propose the use of the respiratory chain to measure D- and L-lactate, succinate, 3-glyclerophosphate, and NADH. In this paper we also describe the measurement of L-malate, pyruvate, and NADPH. The multienzymatic respiratory chain from *E. coli* was immobilized in a film of tanned gelatin mounted onto an oxygen sensor. Analytical characteritics of these enzyme electrodes were studied. Increased specificity was introduced by culture conditions, differential permeability, inhibition, and heat denaturation.

MATERIALS AND METHODS

Biological Material

Bacteria

Escherichia coli K12 strain 3300 (constitutive for the lactose operon) was grown exponentially at 37°C in aerated medium (63) × 2 of the following composition: 10.8 g/L of KH_2PO_4, 21.2 g/L of K_2HPO_4, 4 g/L of $(NH_4)_2SO_4$, 0.4 g/L of $MgSO_4-7H_2O$, and 0.001 g/L of $FeSO_4 \cdot 7H_2O$. The culture medium was supplemented with 1 mg/L thiamine and 10 g/L of glucose, glycerol, D,L-lactate, succinate, or D,L-malate and adjusted, if necessary, to pH 7.0 with KOH.

Bacteria were collected by centrifugation and resuspended at 10 mg/ml of bacteria protein in medium (63) × 2 at 37°C. If intact bacteria were used directly for measurements of respiration, they were aerated for 45 min to remove most of the endogenous substrates (*11*). Bacteria were usually washed three times with 200 m*M* K-phosphate buffer at pH 7.6 and finally resuspended at 50 mg/mL of bacterial protein in 50 m*M* of the same buffer before freezing at −80°C. Thawing was rapidly achieved at 37°C only once. Storage at −80°C kept the respiratory activity for at least 1 yr.

Intact bacteria can be used for respiration of D- and L-lactate, succinate, L-malate, and 3-glycerophosphate.

Membrane Preparations

For measurements of NAD(P)H, bacteria were broken either by sonication or French press. For pyruvate measurements, respiration was obtained only with the enzyme electrode prepared with bacteria broken with the French press.

The bacterial suspension described above were broken to obtain inverted vesicles of the inner membrane by one of the two following methods:

(a) Sonication: The bacterial suspension (2–5 mL) was sonicated three times for 10 s at 0°C with an MSE sonicator, using a 7 mm external diameter probe.
(b) French press extrusion: The bacterial suspension (100 mL) was extruded under a pressure of 20,000 psi (approximately 1600 bars) in a Ribi cell fractionator (Sorvall) at 15°C (*12*).

Unbroken bacteria and cell wall fragments were eliminated by 20 min centrifugation at 60,000*g*.

Immobilization

Immobilization was performed by mixing at 40°C the equivalent of 25 mg/mL (bacterial protein), final concentration, of intact bacteria or inverted vesicles with 5% gelatin (250 blooms—bone or pig skin gelatin from Rousselot Chemical Company, France) (*5*). The mixture was gently agitated, and rapidly poured onto a glass plate, and covered with a gas selective hydrophobic film (polypropylene from Bollore Inc., Paris, France) with a thickness of 6 μm. A 1-mL mixture was spread on 40 cm^2 and dried for 5 min at 4°C (or preferably overnight at 4°C). The film was then tanned with 2% glutaraldehyde (a 25% solution from Merck was diluted at 0°C in distilled water just before use) for 3 min. The film was washed three times with 0.1*M* lysine, pH 7.6, to destroy the unreacted glutaraldehyde, followed by three washings with 50 m*M* K-phosphate buffer, at pH 7.6. The enzymatic film was finally stored, anaerobically, in the last buffer and supplemented with 0.5 m*M* azide (to avoid bacterial contamination) at 4°C. The films remained active for at least 3 mo.

The yield of activity obtained (the ratio of activity before and after immobilization) was approximately 30%.

Respiration Measurements

The oxygen consumption (*5*) was measured with a Clark electrode (Radiometer E5046). The sensitive part of the oxygen electrode has a diameter of 0.7 cm.

The probe was tightly covered with the polypropylene film (facing the electrode) and the enzymatic film (Fig. 1a).

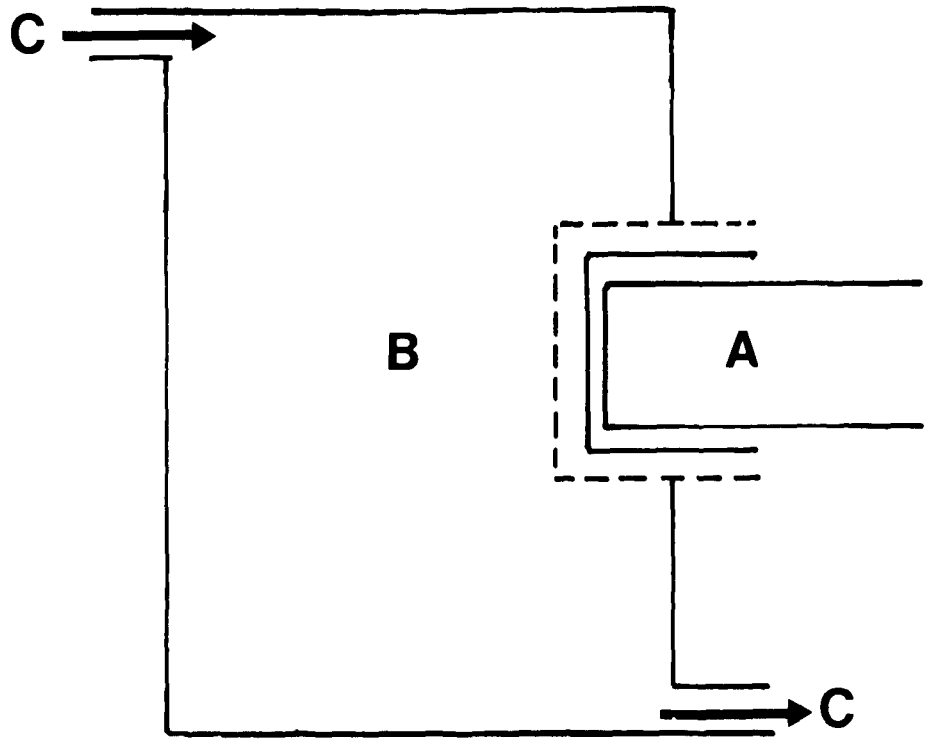

Fig. 1a. Enzyme electrode using the immobilized respiratory chain from *Escherichia coli*. Bacteria grown on D,L-lactate (as example) were included in gelatin at 40°C and spread on a film of polypropylene (—). The film was tanned with glutaraldehyde (Materials and Methods). The enzymatic film (---) was mounted tightly on an oxygen probe (A). This probe is settled in a 70-μL thermostated cell (B), allowing flow measurements (C).

For automatic measurements, the sensor bearing the selective active enzymatic film was placed in a thermostated cell (Radiometer D616), total vol 70 μL, which allowed for flow measurements. A microcomputer controlled the fluidic circuit and performed the signal treatment. The electrode was sequentially exposed (*13*):

(1) To rinse with the above buffer,
(2) To air (to facilitate oxygen saturation),
(3) To sample, containing one substrate at a given concentration at 30°C in 0.1*M* K-phosphate buffer at pH 7.6, and saturated by air at 30°C. For NADPH and pyruvate measurements, the buffers were, respectively, 0.1*M* K-acetate, pH 5.8, and K-phosphate, pH 6.0.
(4) The slope of the signal at inflection point (*14*) was measured (Fig. 1b) and compared automatically to the calibration curve for various respiratory substrates (Fig. 2).

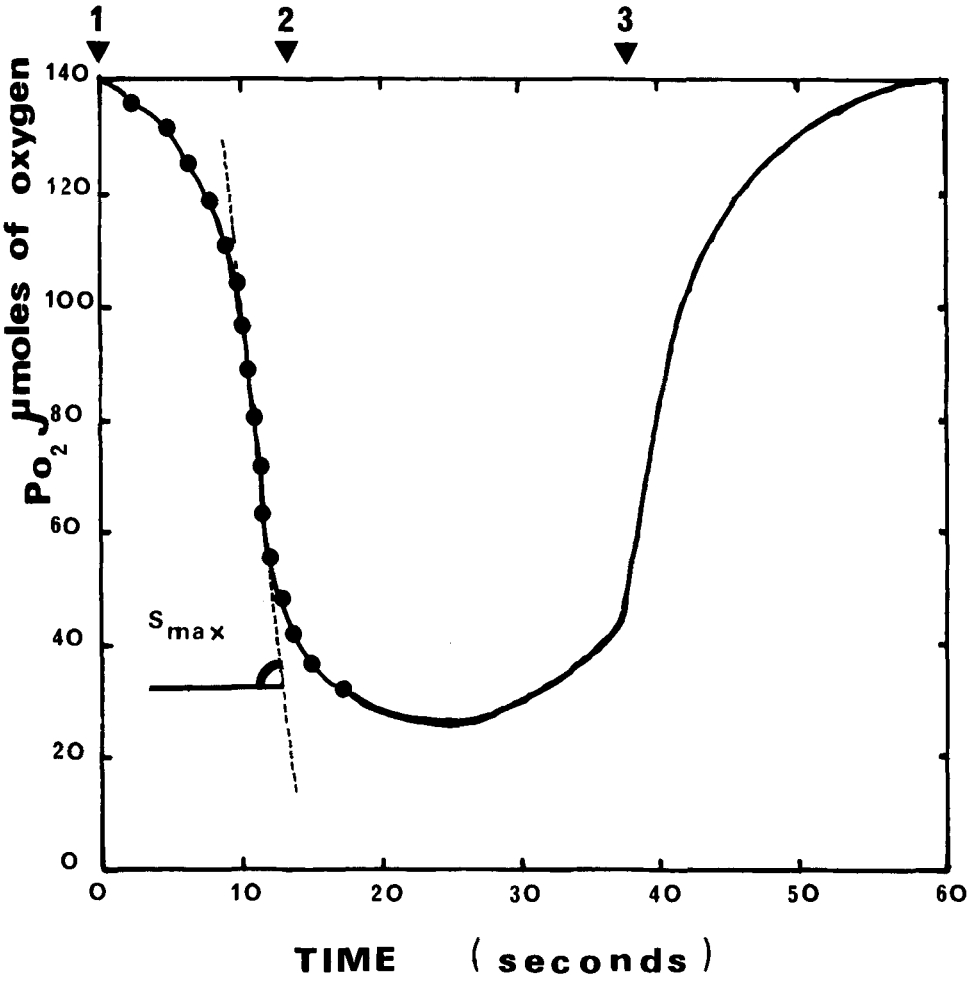

Fig. 1b. At time (1), 50 m*M*, the saturation concentration of L-lactate was introduced in the cell with 0.1*M* K-phosphate buffer, pH 7.6. The maximal slope at the inflection point (S_{max}) of oxygen consumption was measured. At time (2), the cell was rinsed with buffer. At time (3), air was introduced into the cell in order to restore 100% oxygen saturation of the gelatin.

RESULTS AND DISCUSSION

Analytical Properties of the Enzyme Electrodes

Measurements

The calibration curve was obtained with various known concentrations of the metabolites (an example of titration curve is shown in Fig. 3). When saturating amounts of substrate were added, S_{max} was obtained, and the concentration giving half S_{max} was termed "apparent K_M." The K_Ms were determined (*see* Table 1) for various respiratory substrates.

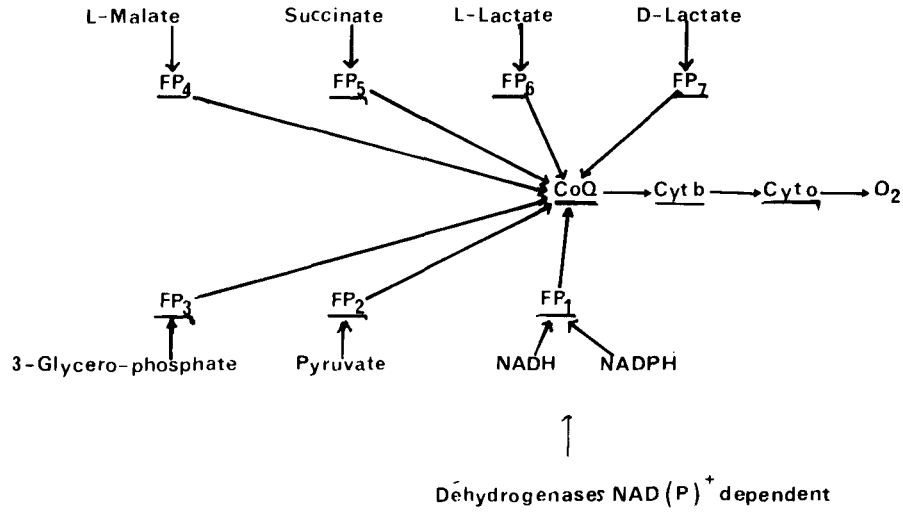

Fig. 2. *Escherichia coli* respiratory chain. FP: flavoprotein; CoQ: coenzyme Q; cytb: cytochrome b; cyto: cytochrome o. Underlined proteins are located on the plasmic membrane. Flavoproteins seem to be different to each pathway (except for NADH and NADPH).

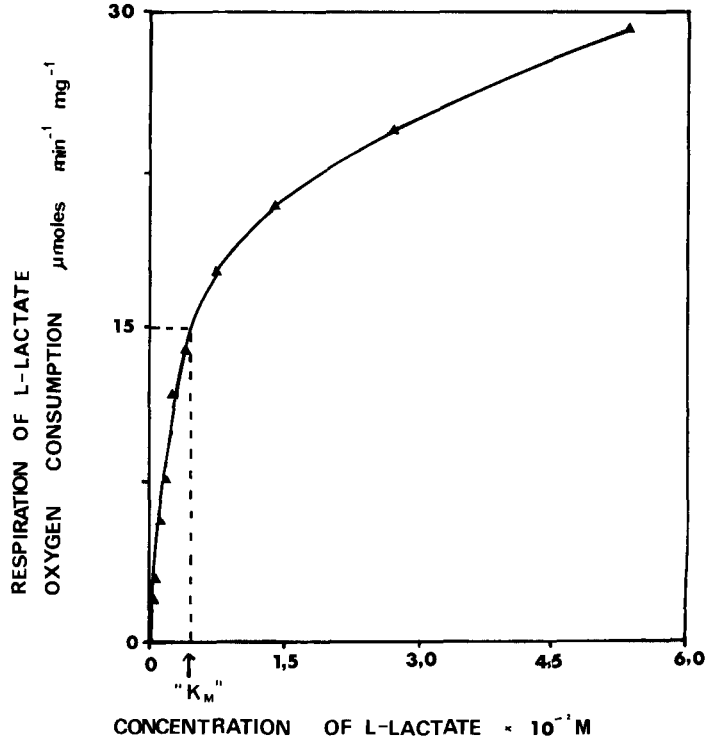

Fig. 3. Calibration of L-lactate. L-lactate respiration was measured, as described in Figs. 1a,b, with the enzyme electrode at various L-lactate concentrations.

Table 1
"Apparent K_M" of the Respiratory Chain in Suspension and After Immobilization Onto the Oxygen Electrode[a]

Growth medium	Substrates	Bacteria	Apparent K_M, mM	
			In suspension	Immobilized
Glycerol	D-Lactate	Whole	1.0	10.0
D,L-Lactate	L-Lactate	Whole	10.0	3.0
Succinate	Succinate	Whole	1.0	10.0
D,L-Malate	L-Malate	Whole	2.0	20.0
Glycerol	Pyruvate	Broken	—	30.0
Glycerol	NADH	Broken	0.4	1.0
Glycerol	NADPH	Broken	0.7	2.0
Glycerol	3-Glycerophosphate	Whole	1.0	5.0
D,L-Malate	Fumarate	Whole	0.5	3.0

[a]In suspension, whole or broken bacteria were used after growth in a medium supplemented by various carbon sources. Immobilization was obtained in gelatin tanned with glutaraldehyde. The films were tightly mounted on the oxygen probe, as shown in Fig. 1a. Measurements were performed with 0.1*M* K-phosphate buffer, pH 7.6 (except for NADPH, pH 5.8, and for pyruvate, pH 6.0). The maximal slope at the inflection point of oxygen consumption was measured at variable substrate concentrations.

The approximate apparent K_M could be determined.

Figure 4 is another type of calibration, i.e., pyruvate inhibition of the L-lactate respiration. This curve may be used for pyruvate assay by lactate inhibition (*see* below). Similar results were obtained with fumarate for succinate respiration and oxaloacetate for L-malate respiration.

Stability of the Enzyme Electrode Activity

The operational stability of the L-lactate respiration activity was measured automatically and repetitively with a saturated concentration of L-lactate (as example). One measurement was performed each 5 min at 30°C; less than 20% of the initial activity was lost after 900 measurements. Reproducibility of the measurements was better than 2% for the first 100 assays (Fig. 5).

Synthesis of Various Specific Flavoproteins

Activity of various specific flavoproteins was dependent on the carbon sources of the culture and on the use of intact bacteria or broken bacteria that yield inverted membrane vesicles (Table 2).

Use of Induction for Discrimination of the Various Substrates

The flavoproteins responsible for the specific respiration of L-lactate, succinate, 3-glycerophosphate, and L-malate appear to be induced, respectively, by D,L-lactate, succinate, glycerol, or D,L-malate (*see* Table 2).

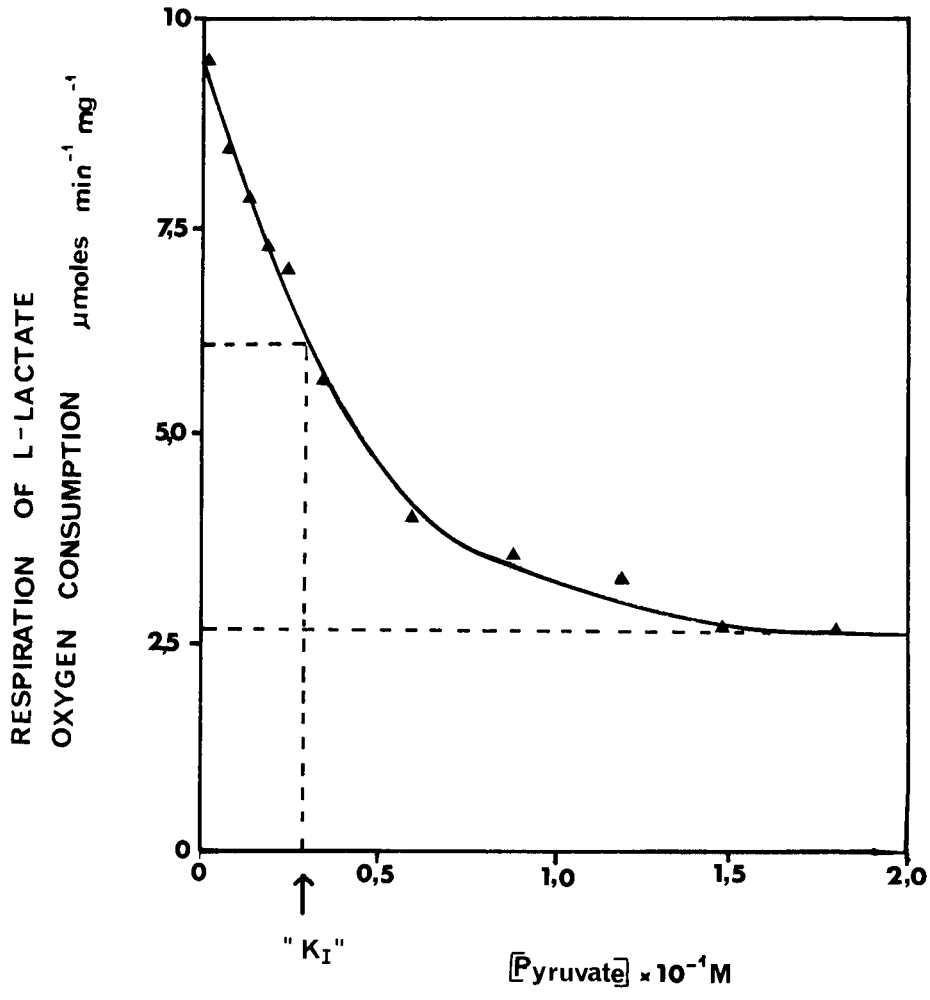

Fig. 4. Calibration of pyruvate by inhibition of L-lactate respiration. Measurements were performed, as described in Figs. 1a,b. Bacteria grown on D,L-lactate were immobilized. L-Lactate at $1K_M$ was added for each measurement with various concentrations of pyruvate.

Bacteria growing exponentially on medium (63) × 2 with 10 g/L glucose were centrifuged and quickly resuspended at 37°C on medium (63) × 2 supplemented with 1 g/L glucose and 10 g/L D,L-lactate (as example). Exponential growth resumes and stops when all the glucose is consumed (results to be published). After a lag period of about 2 h, the growth restarted on D,L-lactate. L-lactate respiration appeared about 1 h after the exhaustion of glucose. Similar results were previously described with a mixture of glucose and lactose as part of the demonstration of the induction of the enzymes of the lactose operon.

Induction of specific flavoproteins for L-lactate, succinate, 3-glycerophosphate, and L-malate are most probable because of the appear-

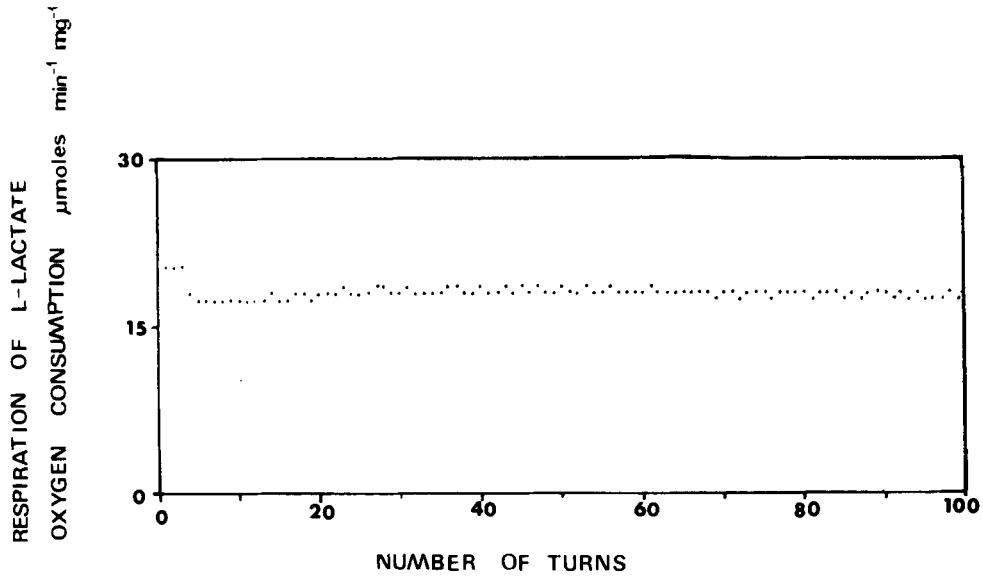

Fig. 5. Repeated measurements of L-lactate (as example). Repeated measurements of L-lactate were obtained automatically with an L-lactate enzyme electrode, as described in Figs. 1a,b, except that L-lactate concentration was around K_M (3 m*M*).

ance of activity upon culture with specific substrate and diauxie observed upon culture with limiting amount of glucose supplemented with the various inducers.

Table 2
Respiratory Activities Vary with Growth Conditions and the Polarity of the Membrane of the Bacteria[a]

Growth medium	Bacteria	Respiratory activity, µmol O_2/min/mg of proteins						
		NADH	NADPH	D-Lactate	L-Lactate	Succinate	L-Malate	Pyruvate
Glycerol	Whole	0.3	<0.1	1.5	0.3	1.5	0	0
	Broken	6.0	1.5	1.5	0.3	2.2	0	15
Glucose	Broken	*6.0*	*1.5*	0.5	0.1	0.3	0	*10*
D,L-Lactate	Whole	0.4	<0.1	1.5	30	3.0	0	0
Succinate	Whole	0.4	<0.1	0.7	3.7	3.7	0	0
D,L-Malate	Whole	0.1	<0.1	6.0	5.4	5.4	6.7	0

[a]Bacteria were grown in a medium supplemented with various 10 g/L carbon sources. Whole or broken bacteria were immobilized.

Respiratory activities were measured with the enzyme electrode after immobilization of the respiratory chain on the oxygen probe as, shown in Fig. 1a. S_{max} was determined with 50 m*M* of various substrates, as shown in Fig. 1b.

The figures underlined are the best conditions used for each substrate of the respiratory chain.

It appears that D-lactate, pyruvate, and NAD(P)H respirations are constitutive activities (Table 2).

Use of Intact or Permeabilized Bacteria for Discrimination of Various Subtrates

Intact immobilized bacteria were unable to respire NAD(P)H or pyruvate unless broken by French press. Intact, immobilized bacteria were able to respire D- and L-lactate, succinate, L-malate, and 3-glycerophosphate (Table 2).

Use of Inhibitors for Discrimination of Various Substrates

The products of the reactions are reversible inhibitors. Measurements of respiration activity at one concentration of substrate (around apparent K_M) and various concentrations of products (around apparent K_I) permit the determination of the approximative value of apparent K_I (Fig. 4). An excess of product ($10 \times K_I$) in the presence of 1 K_M of substrate gave good inhibition. These inhibitions can be used to increase the specificity of the enzyme elecrode: pyruvate inhibits D- and L-lactate oxidation; fumarate inhibits succinate; and oxaloacetate inhibits L-malate respiration (Table 3).

Differentiation of Respiratory Activities and NAD(P)$^+$-Dependent Dehydrogenases

NAD(P)$^+$ addition to inverted membrane vesicles (obtained with bacteria broken by French press) did not increase the respiration activity measured on the enzyme electrode when D- or L-lactate, succinate, L-malate, 3-glycerophosphate, or pyruvate were added to substrates. No NAD(P)$^+$-dependent dehydrogenases were detectable under our experimental conditions.

Table 3
Measurements of Apparent K_I for Various Competitive Inhibitors[a]

Carbon sources	Substrates	Inhibitors	Apparent K_I, m*M*
Glycerol	D-Lactate	Pyruvate	50
D,L-Lactate	L-Lactate	Pyruvate	30
D,L-Malate	L-Malate	Oxaloacetate	100
Succinate	Succinate	Fumarate	50
Succinate	Succinate	Malonate	20
Succinate	Succinate	Oxalate	100
D,L-Lactate	L-Lactate	Oxamate	200

[a]Inhibition of respiration was measured on the enzyme electrode, as shown in Fig. 4. For one concentration of substrate (near K_M), various concentrtions of inhibitors were added. Approximate determination of apparent K_I was performed.

All these respiratory activities were bound to the inner membrane and were sensitive to 10 m*M* cyanide (the concentration that inhibits membrane-bound cytochrome oxidase in *E. coli*, which is part of the respiratory chain).

Thermoresistance of Respiration of Succinate and Pyruvate

To increase the electrode specificity, bacteria grown on succinate were heated at 55°C for 1 h. With immobilized intact bacteria, mainly succinate respiration was maintained. After similar heat treatment of bacteria grown on glycerol, breakage by French press, and immobilization in the film mounted onto the oxygen probe, pyruvate oxidation was maintained, whereas NADH respiration disappeared.

Specificity of Enzyme Electrode Prepared with the Immobilized Respiratory Chain of Escherichia coli

(1) Inducability was used for L-lactate, L-malate, succinate, and 3-glycerophosphate. Constitutivity was used for D-lactate, pyruvate, and NAD(P)H.
(2) Permeability of the effectors permitted the use of intact bacteria for D- and L-lactate, succinate, L-malate, pyruvate and 3-glycerophosphate. Impermeability for NAD(P)H was observed with intact bacteria (activity was obtained with these substrates after breakage by French press).
(3) Inhibitors were used to decrease the respiration of succinate (by fumarate or malonate) D- or L-lactate (by pyruvate or oxalate), and L-malate (by oxaloacetate).
(4) Thermoresistance of the flavoproteins was used to measure respiration of succinate or pyruvate. Combination of these four conditions allow good specificity of the respiratory chain electrode (when measurements were performed in the presence of a mixture of substrates).

For summary of above, *see* Table 4.

CONCLUSIONS

Excherichia coli was a good source for the immobilized respiratory chain. Inclusion in gelatin tanned with glutaraldehyde allowed the use of enzyme electrodes consuming oxygen when substrates were added. Quantitative measurements of D- and L-lactate, succinate, L-malate, pyruvate, 3-glycerophosphate, and NAD(P)H were performed.

The specificity of the electrode for immobilized respiratory chain was obtained by a combination of various properties of the system (Table 4).

Table 4
Properties of Various Flavoproteins Used for Enzyme Electrode Selectivity

Enzyme electrode for	Inducibility, I; constituitivity, C	Permeability, P; impermeability, i	Inhibitors	Thermosensibility, S; thermoresistance, R
3-Glycerophosphate	I	P	—	—
Succinate	I	P	Fumarate	R
			Malonate	—
L-Lactate	I	P	Pyruvate	S
L-Malate	I	P	Oxaloacetate	S
D-Lactate	C	P	Pyruvate	S
Pyruvate	C	P	—	R
NADH	C	i	NAD^+ non	S
			Inhibitor	—
NADPH	C	i	$NADP^+$ non	S
			Inhibitor	—

[a]Inducibility (I) or constitutivity (C); Permeability of substrates (P) or impermeability (i); Inhibitors, thermosensitivity (S) or thermoresistance (R), are summarized.

More detailed descriptions of the properties of these enzyme electrodes will be published in the near future (*15*).

The advantage of using the respiratory chain from *E. coli* as an enzyme source is obvious. Large amounts of bacteria or broken bacteria are easy to obtain. Furthermore, the presence of oxygen drive the reaction to complete oxidation of the substrate.

The use of polypropylene as a gas-selective film greatly improved the performance of the oxygen probe. The high level of oxygen dissolved in the gelatin film (*5*) renders the assay independent of the oxygen content of the measured sample.

Futai (*16*) described an "L-lactate dehydrogenase possibly induced by lactate." With our present results it is clear that "L-lactate dehydrogenase" was massively induced by D,L-lactate. To avoid confusion with NAD^+-dependent dehydrogenases (not measurable in our conditions), we propose to call this protein "the specific flavoprotein necessary to introduce L-lactate in the respiratory chain."

Our results, furthermore, strongly suggest the existence of specific flavoproteins inducible for succinate, 3-glycerophosphate, L-malate, and other flavoproteins constitutive specifically for D-lactate, NADH, NADPH, and pyruvate.

The specific flavoproteins of the respiratory chain (bound to the membrane) are different from the soluble purified flavoproteins with currently known oxidase activities, such as L-lactate oxidase from *Mycobacterium smegmatis* (*17*) or *Pediococcus sp.* (*18*) or pyruvate oxidase from *Pediococcus sp.* (*18*). If the stability of immobilized purified enzymes is

found acceptable, they may prove a good complementary tool for testing the specificity of enzyme electrodes prepared with the immobilized respiratory chain of *E. coli.*

With one particularly easy to prepare film, hundreds of measurements were able to be performed. No special preparation of the sample was necessary (clarification, purification, concentrations, or dilutions). The range of measurable metabolites was close to the concentrations encountered in the cells and in various biological media (blood, yogurt, or wine, for example).

The use of intact bacteria or crude extracts of inverted vesicles of membranes present several advantages:

(1) No purification of enzyme is necessary.
(2) The starting material is very abundant and easy to prepare (bacterial culture).
(3) The respiratory chain linked to the inner membrane seems to be stabilized by biological immobilization prior to technological immobilization in gelatin-tanned films (which further increased the stability of the respiratory chain).
(4) No added cofactors (i.e., NAD^+ or $NADP^+$), coenzymes (i.e., FAD or FMN), or extrahydrogen acceptors (i.e., ferricyanide or DCIP) were needed.

All $NAD(P)^+$-dependent dehydrogenases (which represent about one-third of the known enzymes) are easy to measure in crude turbid extracts because of the formation of NAD(P)H, which can be respired and measured by the enzyme electrode [NAD(P)H respiration].

These probes allow us to understand better the metabolism and regulation of the flavoproteins in the respiratory chain. Many applications in basic and applied research are feasible.

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REFERENCES

1. Clark, L. C., and Lyons, C. (1962), *Ann. NY Acad. Sci.*, **102,** 29.
2. Updike, S. J., and Hicks, J. P. (1967), *Nature,* **44,** 579.
3. Aizawa, M. (1983), in *Proceedings of the Intern Meeting on Chemical Sensors,* Seiyama, T., Fueki, K., Shiokawa, J., and Suzuki, S., eds., Elsevier Kodansha Japan, Fububa, Japan pp. 683–692.
4. Guilbault, G. G. (1984), *Analytical Uses of Immobilized Enzymes.* 3d ed., M. Dekker, NY, NY.
5. Romette, J. L. Froment, B., and Thomas D. (1979), *Clin. Chim. Acta.*, **95,** 249.

6. Burstein, C., Ounissi, H., Legoy, M. D., Gellf, G., and Thomas D. (1981), *Appl. Biochem. Biotechn.* **6,** 329.
7. Chave, E. Adamowicz, E., and Burstein, C. (1982), *Appl. Biochem. Biotechn.* **7,** 431.
8. Estival F., and Burstein, C. (1984), *Enzy. Microb. Technol.* **7,** 29.
9. Bragg, P. D. (1980), in *Diversity of Bacterial Respiratory Systems,* Knowles, C. J., ed., CRC, FL, pp. 116–136.
10. Ingledew, W. J. and Poole, R. K. (1984), *Microbiol. Rev.,* pp. 227–271.
11. Burstein, C., Tiankova, L., and Kepes, A., (1979), *Eur. J. Biochem.,* **94,** 387.
12. Joseleau-Petit, D., and Kepes, A. (1975), *Biochim. Biophys. Acta,* **406,** 36.
13. Romette, J. L. and Yang, J. S. (1983), *Biotechn. Bioeng.,* **25,** 2557–2566.
14. Kernevez, J. P., Konate, L. and Romette, J. L. (1983), *Biotechn. Bioeng.,* **25,** 845.
15. Burstein, C., Adamowicz, E., Boucherit, K., and Rabouille, C. (1986), To be published.
16. Futai, M., and Kimura, H. (1977), *J. Biol. Chem.,* **252,** 5820.
17. Mascini, M., Palleschi, G., and Moscone, D. (1983), in *Proceedings of the Intern Meeting on Chemical Sensors,* Seiyama, T., Fueki, K., Shiokawa, J., and Suzuki, S., eds., Elsevier Kodansha, Japan, Fububa, Japan pp. 603–608.
18. Toyo Jozo Co. 4-5-13 Shibaura, Minato-Ku, Tokyo, Japan.