Acknowledgments. We thank Prof. W. Schaffner, ETH Zürich, for providing us with the P3X63Ag8 cell line, Dr H. Gerber, University of Bern, for the anti-fluorescein antibodies, Prof. W. Schaffner and Dr K. Shine, ETH Zürich, for the critical reading of the manuscript. This study was supported by grant 3.303-0.85 to M.R.M. from the Swiss National Science Foundation and by grants of the Roche Research Foundation and Sandoz Research Foundation to M.R.M. and C.K.

1 Graessmann, M., and Graessmann, A., Proc. natl Acad. Sci. USA *73* (1976) 366.
2 Graham, F. L., and van der Eb, A. J., Virology *52* (1973) 456.
3 Dick, J. E., Magli, M. C., Phillips, R. A., and Bernstein, A., Trends Genet. *2* (1986) 165.
4 Gregoriadis, G., and Buckland, R. A., Nature *244* (1973) 170.
5 Furusawa, M., Nishimura, T., Yamaizumi, M., and Okada, Y., Nature *249* (1974) 449.
6 Baker, P. F., and Knight, D. E., Meth. Enzymol. *98* (1983) 28.
7 Knight, D. E., and Scrutton, M. C., Biochem. J. *234* (1986) 497.
8 Zimmermann, U., Scheurich, P., Pilwat, G., and Benz, R., Angew. Chem. Int. (Ed. Engl.) *20* (1981) 325.
9 Kurata, S.-I., Tsukakoshi, M., Kasuya, T., and Ikawa, Y., Expl Cell. Res. *162* (1986) 372.
10 Chu, G., Hayakawa, H., and Berg, P., Nucl. Acids Res. *15* (1987) 1311.
11 Neumann, E., Schaefer-Ridder, M., Wang, Y., and Hofschneider, P. H., EMBO J. *1* (1982) 841.
12 Stopper, H., Zimmermann, U., and Wecker, E., Z. Naturforsch. *40c* (1985) 929.
13 Baker, P. F., Knight, D. E., and Umbach, J. A., Cell Calcium *6* (1985) 5.
14 Knight, D. E., and Scrutton, C., Eur. J. Biochem. *160* (1986) 183.
15 Tolleshaug, H., and Seglen, P. O., Eur. J. Biochem. *153* (1985) 223.
16 Michel, M. R., Elgizoli, M., Kempf, C., and Koblet, H., Experientia *43* (1987) 676.
17 Vienken, J., Jeltsch, E., and Zimmermann, U., Cytobiologie, Eur. J. Cell Biol. *17* (1978) 182.
18 Zimmermann, U., Riemann, F., and Pilwat, G., Biochim. Biophys. Acta *436* (1976) 460.
19 van Renswoude, J., Bridges, K. R., Harford, J. B., and Klausner, R. D., Proc. natl Acad. Sci. USA *79* (1982) 6186.
20 Karonen, S.-L., Mörsky, P., Siren, M., and Seuderling, U., Analyt. Biochem. *67* (1975) 1.
21 Schaefer, A., Kuehne, J., Zibirre, R., and Koch, G., J. Virol. *44* (1982) 444.
22 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
23 Teissie, J., and Rols, M. P., Biochem. biophys. Res. Commun. *140* (1986) 258.
24 Henderson, R., and Unwin, P. N. T., Nature *257* (1975) 28.
25 Boggs, S. S., Gregg, R. G., Borenstein, N., and Smithies, O., Expl Hemat. *14* (1986) 988.
26 Heppel, L. A., Weisman, G. A., and Friedberg, I., J. Membr. Biol. *86* (1985) 189.

0014-4754/88/030199-05$1.50 + 0.20/0

Ambiquitous behavior of rabbit liver lactate dehydrogenase

M. C. Sanz and C. Lluis

Department of Biochemistry and Physiology, Faculty of Chemistry University of Barcelona, E-08028 Barcelona (Spain)
Received 18 September 1987; accepted 25 November 1987

Summary. Rabbit liver mitochondrial fraction shows lactate dehydrogenase activity. The enzyme can be released from particles by increasing the pH and the ionic strength of the medium. There is a narrow range of pH (6.8–7.4) and ionic strength (20–50 mM NaCl) in which the solubilization sharply increases. It has been shown that divalent anions (SO_4^{2-}) and cations (Mg^{2+}, Ca^{2+}) are highly effective specific solubilizing agents. NADH (1.5 mM) and ATP (1.0 mM) were effective in solubilizing 50% of the enzyme bound, whereas the same concentrations of the analogs NAD^+ and ADP had little effect. Cytosolic lactate dehydrogenase bound to the mitochondrial fraction and a saturation of particles by enzyme was observed in all experiments performed. The in vitro binding requires a short period of incubation between the enzyme and particles and the binding is independent of the temperature in the 0–37 °C range. Binding was prevented by 0.15 M NaCl. The bound enzyme is approximately 20% less active than the soluble one. The results described give support to the proposal that rabbit liver lactate dehydrogenase has an ambiquitous behavior, like other glycolytic enzymes, which have not a fixed intracellular localization.
Key words. Lactate dehydrogenase; glycolytic enzyme; subcellular localization; membrane binding; mitochondria.

Introduction

Classically, the cytosolic subcellular localization of glycolytic enzymes has been considered an invarian characteristic of these enzymes, but, actually, theoretical[1] and experimental[2–12] evidences have been accumulated indicating that, in vitro, glycolytic enzymes interact with structural proteins of muscle, particularly with those containing actin[2–4,12] or membranous subcellular structures in muscles[11], brain[8–10] and erythrocytes[5–7]. The reversible association of enzymes onto the structural components of the cell might act as a new mechanism for regulating their activity. This type of behavior have been called ambiquitous[13,14] and has two fundamental requirements: 1) There must be some mechanism by which the strength of the enzyme-particles interaction can be modulated, (for instance, modulation by parameters which reflect the metabolic status of the cell) and 2) the soluble and bound forms must exhibit different kinetic properties. Also, there must be complementary recognition signals on both enzyme and particles allowing specific interactions. There-

fore, the component(s) of the particles responsible for binding the enzyme should be known. But identification of the subcellular components to which glycolytic enzymes bind has only been possible in a small number of cases[15-18].

The ambiquitous behavior of lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase EC 1.1.1.27), the last enzyme in the glycolytic pathway, has not been extensively studied. The enzyme exist in an equilibrium between soluble and bound forms in muscular tissues[19-22] and brain[8,9]. In all cases the in vitro equilibrium has been very strongly governed by the pH, by the ionic strength, by the NADH concentration and by the concentration of particles in the medium[8,9,19-22]. It has been demonstrated[23] that the binding capacity of lactate dehydrogenase to rabbit muscle mitochondria depends on the net protein charge and not on the type of subunit. Thus, isoenzymes with alkaline pI, either H or M, can be bound. Kinetic differences were observed between soluble lactate dehydrogenase and the enzyme bound to rabbit[20] or chicken[19,24,25] muscle particulate fraction or to chicken liver mitochondrial fraction[39]. In general, the bound enzyme is less active than the soluble one. As reported by Hultin's group[24,25] the equilibrium between soluble and bound forms of lactate dehydrogenase in muscular tissues would provide a mechanism for maintaining glycolysis under anaerobic conditions and, under these conditions, binding would minimize competition for electrons between lactate dehydrogenase and mitochondria. Recently, Masters et al.[26] postulated that in cellular situations requiring sustained anaerobic energy production, like muscular tissues, lactate dehydrogenase would need to be localized near the other glycolytic enzymes via enzyme-actin containing filaments interaction in order to convert NADH back to NAD^+ and to be suppled with pyruvate. On the contrary, lactate dehydrogenase may also act independently of other glycolytic enzymes when localized externally bound to mitochondria, capturing pyruvate or lactate and so play a role in feeding these substrates to the mitochondria.

Besides the existence of lactate dehydrogenase externally associated to the mitochondria, the localization of this enzyme inside the mitochondria from many sources has been described[27-31] but controversy exist at this point since the physiological role of intramitochondrial enzyme is not evident.

In this report, we have demonstrated that rabbit liver mitochondrial fraction has externally bound lactate dehydrogenase. In vitro the enzyme can be reversibly bound to the mitochondrial fraction. The equilibrium between bound and free enzyme is affected by parameters or metabolites that can reflect metabolic changes in the cell and that the activity of bound lactate dehydrogenase is different from soluble one. These results give support to the proposal that rabbit liver lactate dehydrogenase in vitro has an ambiquitous behavior and suggest that ambiquity also may play a role in the regulation of glycolysis in liver tissue.

Materials and methods

Materials. All solutions were freshly prepared in the appropiate buffer. ATP, ADP, glucose-6-phosphate and phosphoenolpyruvate were purchased from Sigma Chemical Co.; 50% sodium D,L-lactate, sodium pyruvate and the neutral salts used were purchased from Merck Darmstadt. NADH and NAD^+ were purchased from Boehringer Mannheim. Other reagents and solvents, obtained from Merck or Boehringer, were reagent grade.

Preparation of rabbit liver mitochondrial and cytosolic fractions. Rabbit liver mitochondrial fraction was prepared by differential centrifugation using the method described by Cercek and Houslay[32] in a 0.25 M sucrose solution prepared in 5 mM sodium phosphate buffer pH 6. Three consecutive washes of the first mitochondrial pellet were carried out by homogenization with half the original volume of separation medium and centrifugation at 10,000 × g, 10 min at 2 °C. The particles (1 g) were then washed three times with 30 ml of 5 mM sodium phosphate buffer until no release of xanthine dehydrogenase (used as cytosolic marker enzyme) and lactate dehydrogenase was detected. After each wash, the supernatants were checked for glutamate dehydrogenase and adenylate kinase release as indication of mitochondrial break. The activity of these enzymes was less than 5% or 8% of total activity respectively. The final pellet showed less than 10% of total glucose-6-phosphatase activity (microsomal fraction marker enzyme) and any at all activity of xanthine dehydrogenase was detected. In routinary experiments it was tested that ultrasonic disrupted mitochondrial fraction had 85–90% of total glutamate dehydrogenase activity (mitochondrial matrix marker enzyme).

Mitochondrial pellet depleted of the externally bound lactate dehydrogenase was obtained from 1 g of active mitochondrial fraction by two successive washes with 30 ml of 0.15 M NaCl (pH 6) and three washes with 5 mM sodium phosphate buffer pH 6. Mitochondrial pellet without any apparent activity was obtained. Also in these cases, after each wash with salt or buffer the supernatants were checked for glutamate dehydrogenase and adenylate kinase release as indication of mitochondrial break. The activity of both enzymes was always less than 10% of total.

Rabbit liver cytosolic fraction was obtained from the post mitochondrial supernatant by centrifugation at 70,000 × g, 60 min at 2 °C or at 105,000 × g, 60 min at 2 °C since no differences in binding experiments were observed.

Enzyme activities. The lactate dehydrogenase activity was determined by measuring absorbance changes at 340 nm for periods less than 2 min with a PYE Unicam SP-1700 recording spectrophotometer in 1 cm light-path cells. The reaction was started by the addition of 0.2 ml of the enzyme solutions or particulate suspensions. Final concentrations of substrates (2.5 ml) were 0.3 mM pyruvate and 0.1 mM NADH in 5 mM sodium phosphate buffer pH 6, unless otherwise indicated. The reaction was performed at 30 ± 0.1 °C. An enzyme unit (U) is defined as the amount of soluble or bound enzyme causing the disappearance of 1 μmol NADH/min in the conditions given for activity determination. The particulate suspension absorbance did not vary during the period of enzyme activity determination. In the range of concentrations used in this work suspensions containing particulate fraction were seen to satisfy Lamber-Beer's law and the molar absortivity coefficient for NADH ($6.3 \cdot 10^3\ M^{-1}\,cm^{-1}$) did not vary. The activity of the bound enzyme was proportional to the amount of particles in suspension in the range of concentrations used.

Using Selwyn's method[33] it was established that the soluble and bound enzyme were stable for at least 5 min under the activity determination conditions and within the range of concentrations used. After 2 h at 0 °C (ice-water bath) the most diluted concentrations of soluble or bound lactate dehydrogenase lost less than 3% of its activity.

Adenylate kinase activity was assayed by the method of Bergmeyer et al.[34]. Glucose-6-phosphatase activity was determined by the method of Houslay et al.[35]. The method of Schmidt[36] was used for determining the activity of glutamate dehydrogenase. Xanthine dehydrogenase activity was assayed by the method of Canela et al.[37].

Binding experiments. In vitro binding of lactate dehydrogenase to the mitochondrial fraction was carried out by incubation of rabbit liver cytosolic lactate dehydrogenase and particles in 5 mM sodium phosphate buffer pH 6 at a tem-

perature stated in each case. After a defined period of time (see results) incubation mixture was centrifuged at 25,300 × g, 30 min at 2 °C and supernatant lactate dehydrogenase activity was determined, it corresponded to the unbound soluble enzyme. The sediment was homogenized with the same initial volume of buffer and thereafter centrifuged under the same conditions in order to eliminate the unbound enzyme retained in the precipitate. The final pellet was homogenized in the same volume of buffer and tested for activity. Control samples were prepared in parallel containing the same amount of soluble enzyme without particles or particles without soluble enzyme. In vitro bound lactate dehydrogenase was calculated as bound units/g fresh weight of pellet and this is the difference between the activity of the final and initial pellets.

Results

Rabbit liver mitochondrial fraction was obtained and washed as indicated in 'methods' until no release of lactate dehydrogenase activity was observed and a constant enzyme activity of the pellet was obtained, 51.6 ± 13.0 U/g fresh weight (average of 14 values obtained from different mitochondrial fraction preparations). This represents about 5–10% of cytoplasmic lactate dehydrogenase activity. This bound enzyme activity remained unaltered for at least two days at 4 °C. Solubilization of this mitochondrial bound enzyme by varying different parameters was tested.

Influence of pH and ionic strength on the lactate dehydrogenase solubilization. Results of the effect of variations in the pH of the washing medium on the enzyme solubilization are given in figure 1. A sigmoidal dependence was observed between solubilization and pH. A sudden release occurred in the pH range 6.8–7.4. When mitochondrial fraction suspension (as indicated in fig. 1) was prepared at pH > 7.1 a release of glutamate dehydrogenase activity (mitochondrial matrix marker enzyme) was also observed (50–70% of total activity at pH 7.4) indicating that in this condition a mitochondria break is produced. No glutamate dehydrogenase release was produced when pH 7.

The ionic strength of the washing solution induced lactate dehydrogenase solubilization as shown in figure 2. First of all, for each salt used a sigmoidal dependence between solubilization and ionic strength were observed. Second, at a constant ionic strength, a specific effect of anions and cations was shown. The effectiveness as solubilizing agents of divalent anions and cations proved to be very high. Ca^{2+} and Mg^{2+} appeared as very specific solubilizating agents.

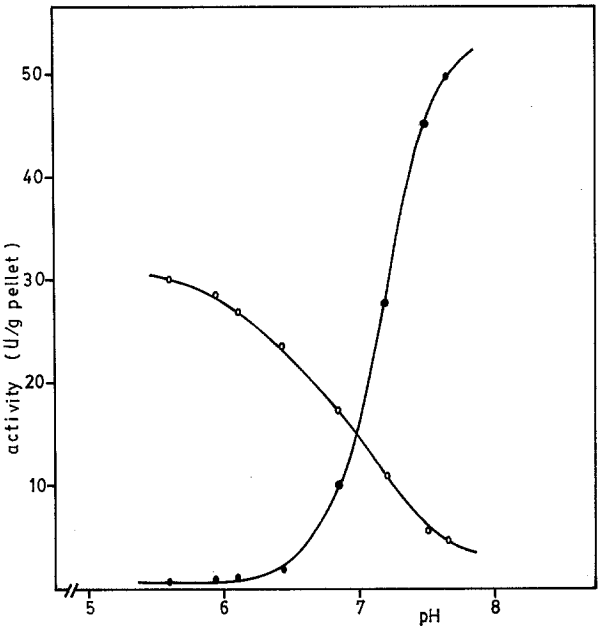

Figure 1. Effect of pH on lactate dehydrogenase solubilization from mitochondrial fraction. Suspensions containing 60 mg of mitochondrial fraction in 10 ml of 5 mM sodium phosphate buffer pH 5.5–8 were prepared and the final pH measured. After 5 min incubation at 0 °C, suspensions were centrifuged at 25,300 × g, 30 min at 2 °C. Activity of 0.2 ml of pellets homogenized with 10 ml of 5 mM sodium phosphate buffer pH 6 was determined in the same buffer medium (o). Activity (expressed as units solubilized/g pellet initially present) of 0.2 ml of supernatants was determined in 50 mM sodium phosphate buffer pH 6 (•), under these conditions the activity determination pH did not change and the activity in this medium is the same as when determined in 5 mM buffer.

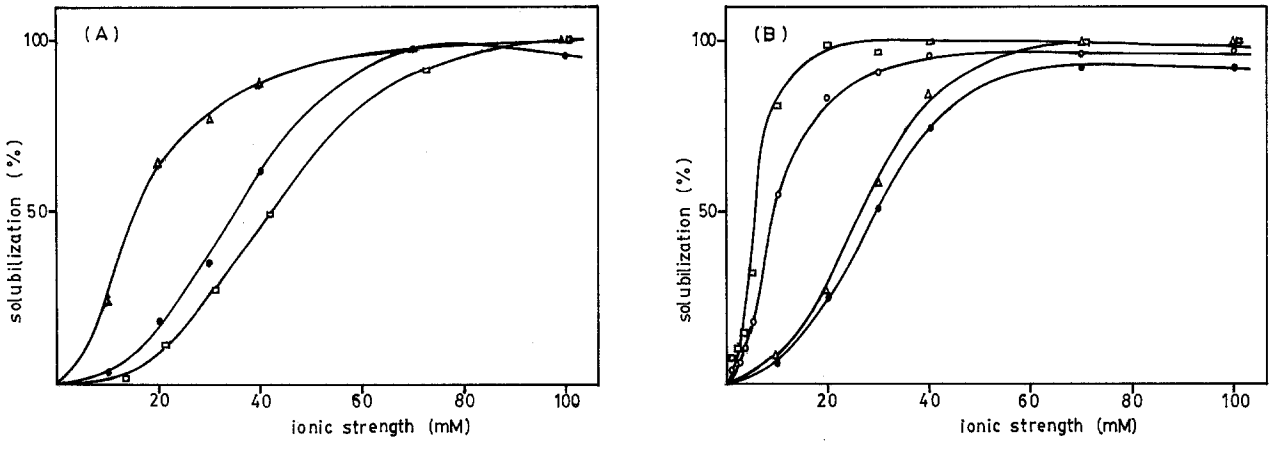

Figure 2. Salt-induced solubilization of mitochondrial bound lactate dehydrogenase. 10 ml of suspensions containing 60 mg of mitochondrial fraction in 5 mM sodium phosphate buffer pH 6 (A) or 5 mM imidazole-HCl buffer pH 6 (B) were prepared containing different salt concentrations in order to give the indicated ionic strength. Suspensions were centrifuged at 25,300 × g, 30 min at 2 °C and 0.2 ml of supernatants were used to determine their lactate dehydrogenase activity. Controls were made to be sure soluble lactate dehydrogenase activity was not modified when the ionic strength in the reaction medium varied in the same interval as the one obtained in the activity determination of the supernatants. Salt added: *A* Na_2SO_4 (△), NaCl (•) and $NaCH_3COO$ (□); *B* $MgCl_2$ (□), $CaCl_2$ (o), KCl (△) and NaCl (•).

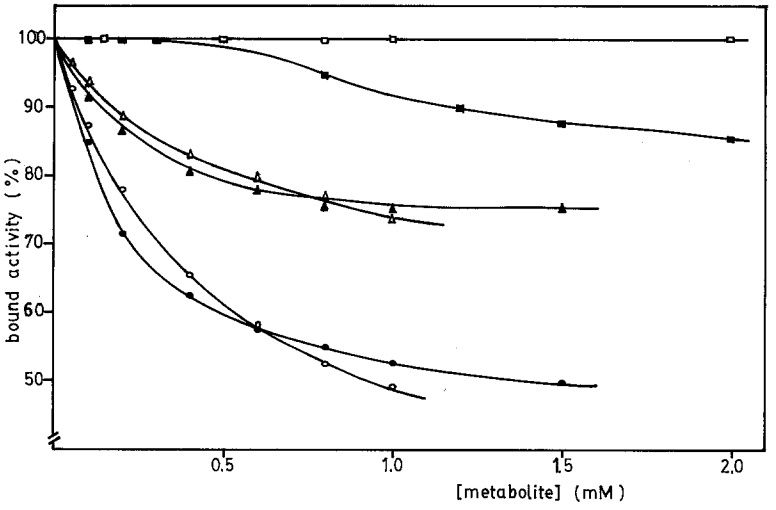

Figure 3. Effect of metabolites on lactate dehydrogenase solubilization from mitochondrial fraction. Suspension containing 60 mg of mitochondrial fraction in 10 ml of 5 mM sodium phosphate buffer pH 6 were prepared in absence or in presence of metabolites indicated. The suspensions were centrifuged at 25,300 × g, 30 min at 2 °C. Pellets were homogenized with 10 ml of the same buffer and their lactate dehydrogenase activity was determined. The results are expressed as a percentage of the enzyme bound when no metabolites were added. ATP (o), NADH (•), NAD (△), ADP (▲), pyruvate (■) and coincident values for D,L-lactate, phosphoenolpyruvate and glucose-6-phosphate (□).

This differential effect among ions was specially evident at the lower ionic strength. It should be noted that enzyme solubilization by salt was accompanied by a logical decrease in the activity of the particulate fraction (results not shown).

Specificity of metabolites for lactate dehydrogenase solubilization. The results of the effect of some metabolites on the lactate dehydrogenase solubilization from mitochondrial fraction are given in figure 3. At the metabolites concentration range used, the ionic strength of the medium was, in all cases, less than those producing lactate dehydrogenase solubilization (see above). Neither D,L-lactate, glucose-6-phosphate or phosphoenolpyruvate produced enzyme solubilization. Pyruvate appears to be a poor solubilizing agent since the highest pyruvate concentration used (2 mM) only produced 15% of lactate dehydrogenase release. In contrast, NADH seems to be more effective because concentrations up to 1.5 mM removed up to half the enzyme bound. One of the most important facts derived from figure 3 is that ATP can solubilize lactate dehydrogenase with the same effectiveness as NADH; thus, a concentration of 1.0 mM ATP is required for removing 50% of bound lactate dehydrogenase. In contrast, the analogs ADP and NAD^+ only produced a maximum of 25% of lactate dehydrogenase solubilization at the same concentration.

Lactate dehydrogenase binding to the particulate fraction. In vitro binding was performed using the procedure described in 'methods'. In figure 4 the obtained results are shown when binding was carried out by incubation of soluble enzyme and particles at 0 °C during 115 min, showing that a short period of time is sufficient to allow binding. In vitro binding of lactate dehydrogenase is possible for both mitochondrial washed fraction (which has lactate dehydrogenase activity) and mitochondrial pellet depleted of lactate dehydrogenase activity (see methods). Mitochondrial washed fraction can bind up to 300 U/g, 6–7-fold more than the initial activity of the fraction (fig. 4, continuous line). The mitochondrial pellet depleted of externally bound lactate dehydrogenase can bind 400 U/g (fig. 4, continuous line). This difference of 100 U/g is in good agreement with the value of 116 ± 36 U/g (average of 10 values) of lactate dehydrogenase solubilized by five consecutive washes of active mitochondrial fraction with 0.15 M NaCl.

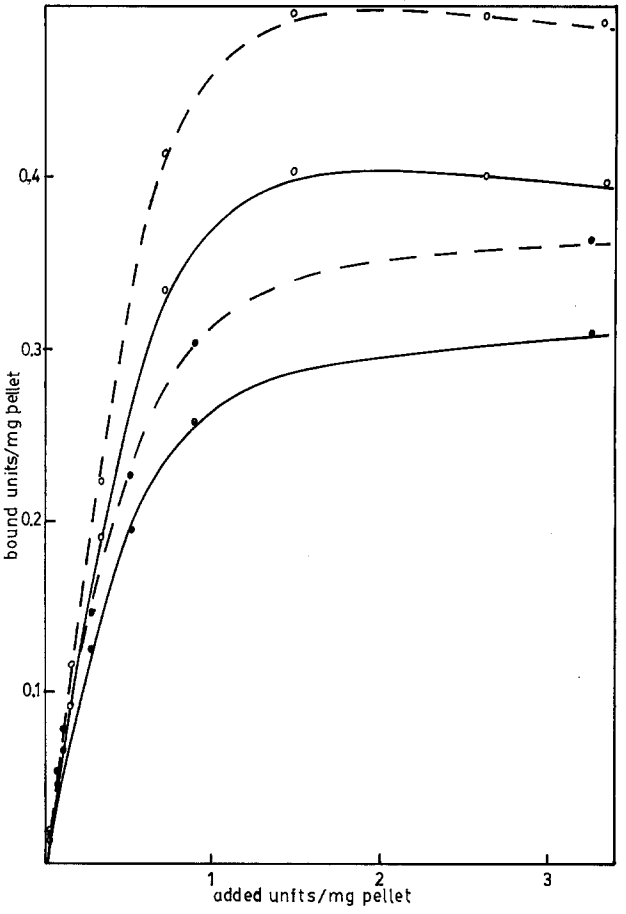

Figure 4. Lactate dehydrogenase binding to particulate fraction. Binding experiments were performed as described in methods. Incubation samples were prepared in 10 ml of 5 mM sodium phosphate buffer pH 6 containing the soluble enzyme units indicated along the abcissa and 60 mg of (•) crude mitochondrial fraction with an initial activity of 40 U/g or (o) mitochondrial pellet free from externally bound lactate dehydrogenase. Continuous lines represent the amount of the enzyme bound measured as the activity increase of pellet. Discontinuous lines represent the amount of the enzyme bound measured as the difference between enzyme units added to the incubates and the enzyme units found in the supernatants after centrifuging the incubates. mg pellet is referred as net weight.

When NaCl was present in the incubation mixtures in a concentration of 0.15 M, the in vitro binding was totally prevented. This is in agreement with the lactate dehydrogenase solubilization caused by this salt (see fig. 2).

The temperature dependence of binding was also tested. Incubation samples as described in figure 4 were prepared containing 0.136 U/mg of soluble enzyme. The enzyme bound to the active mitochondrial pellet (80 ± 0.2 U/g) was the same for all temperatures tested (0, 26, 30 and 37 °C).

One of the most interesting results derived from the binding experiments was that the bound enzyme is less active than the soluble one. Effectively, in the experiments described above, the amount of the enzyme bound was calculated, as indicated in 'methods', by measuring the activity increase of pellet (continuous lines of fig. 4); however, the amount of the enzyme bound may also be calculated in an alternative way by measuring the difference between the units of soluble enzyme added to the incubates and the units found in the supernatants after centrifuging the incubates (discontinuous lines of fig. 4). If the activity of the enzyme in the soluble and bound forms is equal the values calculated by these two procedures should coincide. However, the values calculated by the latter process are always lower than those obtained by the former process (fig. 4). The enzyme bound to the active mitochondrial fraction is 18 ± 4% (average of 12 values) less active than the soluble one. The lactate dehydrogenase bound to the inactive mitochondrial pellet is 24 ± 7% (average of 22 values) less active than the soluble one.

The Scatchard plots of binding show deviations from linearity. Also, experimental data obtained from binding curves did not obey the simple equation of Langmuir isotherm [38], showing that binding is not a simple process.

Discussion

Lactate dehydrogenase is associated with the mitochondrial fraction of rabbit liver, like subcellular particulate fractions from other tissues [10, 20, 22, 39]. We have observed that there is a short range of pH and ionic strength in which lactate dehydrogenase release from rabbit liver mitochondria increases from very little to almost a maximum, indicating that ionic interactions are important in the binding. At pH > 7.1 (not a pH lower than 7) we have observed (unpublished results) that a release of glutamate dehydrogenase activity (mitochondrial matrix marker enzyme) is produced when a suspension of mitochondrial fraction was prepared in the conditions given in figure 1. This seems to suggest that at higher pH broken mitochondria are present and the study of dissociation of lactate dehydrogenase-mitochondrial complex by high pH was done in this system. Nevertheless, these results point out that subcellular variations of pH could be of great importance for lactate dehydrogenase-mitochondria complex dissociation. The pH variations in the interval for maximal solubilization described here occur with some metabolic alterations (i.e., ischemia or anoxia) [40, 41] during which variations in the equilibrium between soluble and bound forms of some glycolytic enzymes have been described [4, 42, 43]. On the other hand, at ionic strength higher than 0.05, solubilization of almost all lactate dehydrogenase is observed. This behavior, also reported for other glycolytic enzymes [4, 5], might allow the conclusion that the interaction of glycolytic enzymes are not physiologically significant. However, dissociation of the enzyme-membrane complex in vitro by physiological saline does not exclude the possible interaction between the enzyme and membranes in vivo though it is an open question as to what is the actual ionic strength in the cytoplasm. Now it is accepted that most, if not all, of the ions of the cell are bound to macromolecules [44, 45] making the ionic strength of the cytoplasm lower than 0.1 M. Further, there are various factors which may promote these interactions in vivo, such are the microenvironment around the enzyme, including the concentration of proteins and ligands. Thus, some glycolytic enzymes, including lactate dehydrogenase, bind to muscle structural proteins at 'physiological' ionic strength (0.15 M NaCl) when the protein concentration approach those occurring in vivo [3, 22].

The effectiveness of salt in lactate dehydrogenase solubilization from rabbit liver mitochondria is not solely a function of ionic strength but, rather, varies with the salt used. The effectiveness of cations ($Mg^{2+} > Ca^{2+} > K^+ > Na^+$) is not related with the radii of those having similar charge ($K^+ < Na^+$, $Ca^{2+} < Mg^{2+}$). The salt effect seems to be a function of the ion charge. Our results contrast with those of Hultin's group [46] who observed that the solubilization of lactate dehydrogenase from chicken or trout skeletal muscle subcellular particles was independent of the specific cation involved. Perhaps, this may constitute an important difference between muscle and liver.

We have stated that the most effective solubilizing metabolites among those assayed were ATP and NADH. Solubilization by NADH of lactate dehydrogenase bound to chicken muscle particulate fraction was also observed by Ratner et al. [21]. The solubilizing effect of NADH described in this paper occurred at relatively low and physiological concentrations. Although it is very difficult to establish the in vivo fluctuations of ATP concentration in the microenvironment of mitochondrial membranes, it is interesting to note from the results described here that ATP and NADH have a similar solubilizing effect which is higher than the effect observed using the analogs NAD^+ and ADP.

From binding experiments it is deduced that the interaction between lactate dehydrogenase and mitochondria is a reversible process. The crude mitochondrial fraction can bind 6–7 fold more enzyme than the initially bound. This difference may be due to incomplete saturation in the freshly obtained particles. A significant result obtained from binding experiments is that the bound enzyme is approximately 20% less active than the soluble one. We have observed a similar activity decrease when M_4 isoenzyme is bound to beef liver mitochondrial fraction (data in preparation). A similar decrease in activity (15–30%) was observed for chicken liver lactate dehydrogenase bound to mitochondrial fraction [39]. In contrast, for muscular tissues the inactivation of lactate dehydrogenase by binding is very high; thus, lactate dehydrogenase bound to rabbit muscle mitochondrial fraction is 70% less active than the soluble enzyme [20]. Also, 70% inactivation was observed for lactate dehydrogenase bound to particulate fraction of chicken breast muscle [25]. These differences observed between muscle and liver can account for a different role of this enzyme in both tissues.

1 Kurganov, B. I., J. theor. Biol. *111* (1984) 707.
2 Bronstein, W. W., and Knull, H. R., Can. J. Biochem. *59* (1981) 494.
3 Clarke, F. M., and Masters, C. J., Biochim. biophys. Acta *381* (1975) 37.
4 Clarke, F. M., Stephan, P., Huxham, G., Hamilton, D., and Morton, D. J., Eur. J. Biochem. *138* (1984) 643.
5 Jenkins, J. D., Madden, D. P., and Steck, T. L., J. biol. Chem. *259* (1984) 9374.
6 Jenkins, J. D., Kezdy, F. J., and Steck, T. L., J. biol. Chem. *260* (1985) 10426.
7 Kelley, G. E., and Winzor, D. J., Biochim. biophys. Acta *778* (1984) 67.
8 Knull, H. R., J. biol. Chem. *255* (1980) 6439.
9 Knull, H. R., Neurochem. Int. *7* (1985) 379.
10 Knull, H. R., and Fillmore, S. J., Comp. Biochem. Physiol. *81 B* (1985) 349.
11 Pierce, G. N., and Philipson, K. D., J. biol. Chem. *260* (1985) 6872.
12 Westrin, H., and Backman, L., Eur. J. Biochem. *136* (1983) 407.

13 Wilson, J. E., Trends Biochem. Sci. *3* (1978) 124.
14 Wilson, J. E., Biophys. J. *16* (1982) 997.
15 Fiek, C., Benz, R., Ross, N., and Brdiczka, D., Biochim. biophys. Acta *688* (1982) 429.
16 Kliman, H. J., and Steck, T. L., J. biol. Chem. *255* (1980) 6314.
17 Lluis, C., Int. J. Biochem. *16* (1984) 1005.
18 Lluis, C., J. Prot. Chem. *5* (1986) 423.
19 Ehmann, J. D., and Hultin, H. O., Archs Biochem. Biophys. *154* (1973) 471.
20 Lluis, C., Int. J. Biochem. *16* (1984) 997.
21 Ratner, J. H., Nitisewojo, P., Hirway, S., and Hultin, H. O., Int. J. Biochem. *5* (1974) 522.
22 Ross, R. E., and Hultin, H. O., J. Cell Physiol. *105* (1980) 409.
23 Lluis, C., Int. J. Biochem. *17* (1985) 1219.
24 Hultin, H. O., Ehmann, J. D., and Melnick, R. L., J. Food Sci. *37* (1972) 269.
25 Nitisewojo, P., and Hultin, H. O., Eur. J. Biochem. *67* (1976) 87.
26 Masters, C. J., Reid, S., and Don, M., Molec. cell. Biochem. *76* (1987) 3.
27 Brdiczka, D., Krebs, W., and Kloock, P., Biochim. biophys. Acta *297* (1973) 203.
28 Kline, E. S., Brandt, R. B., Laux, J. E., Spainhour, S. E., Higgins, E. S., Roger, K. S., Tinsley, S. B., and Water, M. G., Archs Biochem. Biophys. *246* (1986) 673.
29 Mattison, A. G. M., Johansson, R. G., and Bostrom, S. L., Comp. Biochem. Physiol. *41 B* (1972) 475.
30 Skilleter, D. N., and Kun, E., Archs Biochem. Biophys. *152* (1972) 92.
31 Wilson, J. E., J. Neurochem. *19* (1972) 223.
32 Cercek, B., and Houslay, M. D., Biochem. J. *207* (1982) 123.
33 Selwyn, M. J., Biochim. biophys. Acta *105* (1965) 193.
34 Bergmeyer, H. U., Gawehn, K., and Grassl, M., in: Methods of Enzymatic Analysis, vol. 1, p. 425. Ed. H. U. Bergmeyer. Academic Press, New York 1974.
35 Houslay, M. D., and Palmer, R. W., Biochem. J. *174* (1978) 909.
36 Schmidt, E., in: Methods of Enzymatic Analysis, vol. 2, p. 650. Ed. H. U. Bergmeyer. Academic Press, New York 1974.
37 Canela, E. I., and Nin, C. M., J. Prot. Chem. *4* (1985) 305.
38 Arnol, H., Henning, R., and Pette, D., Eur. J. Biochem. *22* (1971) 121.
39 Sagrista, L., and Bozal, J., Biochimie *69* (1987) 205.
40 Desmoulin, F., Cozzone, P. J., and Canioni, P., Eur. J. Biochem. *162* (1987) 151.
41 Spriet, L. L., Soderlund, K., Bergstrom, M., and Hultman, E., J. appl. Physiol. *62* (1987) 616.
42 Clarke, F. M., Shaw, F. D., and Morton, D. J., Biochem. J. *186* (1980) 105.
43 Wals, T. P., Masters, C. J., Morton, D. J., and Clarke, F. M., Biochim. biophys. Acta *675* (1981) 29.
44 Carpenter, D. O., Hovey, M. M., and Bak, A. F., Ann. N.Y. Acad. Sci. *204* (1973) 502.
45 Cope, F. W., and Damadian, R., Physiol. Chem. Phys. *6* (1974) 17.
46 Melnick, R. L., and Hultin, H. O., J. Food Sci. *35* (1970) 67.

0014-4754/88/030203-06$1.50 + 0.20/0

Spatial variation in response to odorants on the rat olfactory epithelium

D. A. Edwards, R. A. Mather and G. H. Dodd [1]

Olfaction Research Group, Chemistry Department, Warwick University, Coventry CV4 7AL (England)
Received 29 September 1987; accepted 8 December 1987

Summary. We have measured the electro-olfactogram produced by four odorants, nicotine, i-pentyl acetate, i-pentanoic acid and cineole from twelve positions on an in vitro preparation of rat olfactory tissue. Each odorant shows a different pattern of response over the twelve positions which can be explained by differences in olfactory receptor populations between regions of the rat olfactory epithelium.
The result for nicotine is further evidence that there are olfactory receptors which are stimulated by nicotine when it is presented as a vapour.
Key words. Electro-olfactogram; olfaction; spatial patterning; nicotine.

Introduction

The olfactory epithelium in the rat is located on bony turbinate structures and on the septum which separates the two halves of the nasal cavity. This sensory epithelium is the site of a complex series of events following odorant stimulation, culminating in the generation of an action potential in the primary olfactory neurons. These primary neurons synapse in the olfactory bulb where subsequent processing of the information from the epithelium occurs [2, 3].
The mechanisms by which the olfactory system can distinguish between the very large number of 'smells' found in the environment using a finite number of receptors is still not fully understood. One level at which determination of odour quality can occur is at the initial interaction of odorant with the olfactory epithelium. The layer of mucus which covers the olfactory epithelium will affect odorants which dissolve in it in different ways [4, 5] and will therefore affect the rate and concentration at which odorants reach the olfactory receptors. This mode of discrimination between odorants is an example of 'imposed' patterning of the stimulus-olfactory epithelium interaction. A second type of discrimination is more specific, in that the stimulus can be identified through differences in the stimulated receptor populations, in the transduction pathways activated and in the arrangement of neuronal connections to the olfactory bulb.
This specific 'patterning' of the response to odorants has been demonstrated in several ways at different levels in the transduction process. Electrophysiological studies have shown that an odorant stimulates more than one receptor