Diffusion loading conditions determine recovery of protein synthesis in electroporated P3X63Ag8 cells

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Summary. Using the suspension cell line P3X63Ag8 we have studied the impact of the composition of the diffusion medium on cellular protein synthesis under standard electroporation conditions in TBS-Na. This buffer contains the high saline concentration usually present in electroporation-mediated DNA transfection. Electroporation in the presence of TBS-Na resulted in an immediate shut-off of protein synthesis, even though both FITC-dextran (M_r 40 kD) and Semliki Forest virus core protein (M_r 33 kD) were incorporated efficiently into the cytoplasm across the electropores at 0 °C. Subsequent resealing of the pores was completed after a 5-min incubation at 37 °C. When compared with control cells, overall protein synthesis of electroporated cells recovered slowly to resume a 30% activity after 1 h of incubation at 37 °C. We have determined optimal conditions for diffusion loading (which necessitates the presence of ATP, GTP, amino acids, K^+, Mg^{2+}, and Ca^{2+}) and resealing (in the presence of K^+, Mg^{2+}, and Ca^{2+}), leading to a full and lasting recovery of protein synthesis within 5 min after pore closure.
Key words. Electroporation; diffusion loading; electropore resealing; protein synthesis.

Microinjection of macromolecules into eukaryotic cells is of basic importance in manipulating cellular functions. By artificially introducing DNA, RNA, and protein molecules through the plasma membrane, the cell can be used as a living test tube. Apart from mechanical microinjection [1] a variety of other techniques are used to deliver macromolecules into the cell, such as the Ca^{2+}-phosphate coprecipitation of DNA [2], the application of viral vectors [3], liposomes [4] and erythrocyte ghosts as vehicles for macromolecular transfer [5] and, recently, also electroporation [6–8] and laserporation [9].

Electroporation leads to a permeability increase of the plasma membrane which results in a transient exchange of matter across the locally collapsed membrane structure [6–8]. The advantage of electroporation over other microinjection techniques is its simplicity. Furthermore, uptake of unwanted foreign proteins and lipids (as is the case in erythrocyte-mediated delivery) is excluded. In a single electroporation step, a sufficiently large number of cells can be efficiently loaded with the desired macromolecule. This method is not only suitable for increasing the transformation rate in DNA transfection [10–12] but also allows delivery of inorganic ions [13], and small [14, 15] and large organic molecules [16–18].

The following phases of electroporation-mediated macromolecular transfer are generally distinguished: a) *electroporation:* the application of an electric field pulse leading to pore formation in the plasma membrane; b) *diffusion loading:* the transient exchange of matter across the electropores which remain open at 0 °C; c) *resealing:* the pore closure at 37 °C leading to a fully competent plasma membrane.

Abbreviations: C-protein, core protein of Semliki Forest virus (SFV); DMEM, reinforced Eagle's medium; $NaDodSO_4$, sodium dodecyl sulfate; PIPES, Piperazine-1,4-bis(2-ethanesulfonic acid); TCA, trichloroacetic acid.

Materials and methods

Buffers: TBS-Na: 25 mM Tris/HCl, pH 7.4, 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HPO_4. *TBS-K:* 25 mM Tris/HCl, pH 7.4, 137 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HPO_4. *ATP/GTP-buffer:* 20 mM PIPES, pH 7, 128 mM K-glutamate, 5 mM ATP, 5 mM GTP, 10 µM Ca-acetate, 2 mM Mg-acetate. *ATP/GTP/amino acid buffer:* 20 mM PIPES, pH 7, 128 mM K-glutamate, 5 mM ATP, 5 mM GTP, 10 µM Ca-acetate, 2 mM Mg-acetate. With the exception of methionine (0.01 mM), the amino acid composition and concentration correspond to that of DMEM. *Resealing buffer:* 20 mM PIPES, pH 7.0, 128 mM K-glutamate, 10 µM Ca-acetate, 2 mM Mg-acetate. *Lysis buffer:* 1 × TBS-Na; 0.5% $NaDodSO_4$.

Cells: The mouse myeloma suspension cell line P3X63Ag8 was grown in RPMI-1640 medium supplemented with 10% fetal calf serum. CV-1, HeLa, and primary and secondary mouse kidney cells were grown in DMEM containing 10% fetal calf serum.

Electroporation: The P3X63Ag8 cells and trypsinized CV-1, HeLa, or secondary mouse kidney cells were centrifuged at 250 × g, resuspended and washed in the appropriate electroporation buffer by recentrifugation. FITC-dextran or SFV-C-protein were added to the cell suspension before electroporation. The electroporation chamber of the electric discharge circuit (Haefliger, Herbaville, France), cooled to 0 °C, was filled with 0.1 ml cell suspension (10^5 cells), the width of the electroporation chamber was at a distance of 2 mm. The electric pulses were applied by a Buechler electrophoresis power supply (Fort Lee, N.J., USA) with the voltage setting at 800 V, corresponding to a field strength of 4 kV/cm. The capacitor was set to 169 nF and 5 successive pulses were applied at 10-s intervals. The field decay constant (τ) was calculated using the formula $RC = \tau$. For C (capacitance) = 169 nF and R (resistance) = 76.9 Ω, τ was found to be 13 µs. Thereafter, the cells were kept for 10 min at 0 °C (diffusion loading time). They were then subjected to a short centrifugation (5 s, reaching 1500 rpm at the end of acceleration) in a 'Biofuge' centrifuge (Heraeus, FRG) before they were suspended in the appropriate resealing buffer or growth medium.

Fluorescence microscopy was performed with a Nikon fluorescence microscope.

Delivery of FITC-dextran and SFV-C-protein: 25 µg FITC-dextran (Sigma, USA, M_r 40 kD) were added before electroporation to 10^5 cells suspended in TBS-Na. After the diffusion loading (10 min at 0 °C) the cells were diluted with 1.4 ml pre-warmed TBS-Na, resealed for 10 min at 37 °C, and centrifuged as indicated above. To suppress extracellular fluorescence, the pelleted cells were resuspended in 250 µl TBS-Na. 50 µl of anti-fluorescein antibody solution (protein A-Sepharose purified IgG fraction) at saturating conditions (6.7 mg antibodies per ml) were added [19]. The mixture was kept at 0 °C for 1 h before washing. All fluorescence measurements were done with a Perkin-Elmer LS-5B spectrofluorometer (excitation: 490 nm; emission: 520 nm). We noted that autofluorescence was negligible. Highly purified C-protein was iodinated using a modified [^{125}I]enzymobead method [20]. 6 µg were added to 10^5 cells before electroporation. To remove excess C-protein after resealing of the electropores, the cells were centrifuged in a 0.4-ml Eppendorf tube through a cushion of dibutylphthalate [21] layered on top of 15% sucrose in TBS-Na. The tip of the Eppendorf tube containing the cell pellet was cut

off and the radioactivity was counted in a GAMMAmatic counter (Kontron, Switzerland).

[^{35}S]Methionine incorporation into protein: To determine cellular protein synthesis, the cells were labeled for 30 min at 37 °C with 30 µCi of [^{35}S]methionine (NEN, 1120 Ci/mM) in 100 µl medium (5% serum) which contained only one-fifteenth of the regular unlabeled methionine concentration. The cells were pelleted, suspended in 1.5 ml DMEM, washed by recentrifugation and lysed in lysis buffer. The protein concentration of the cellular extract was determined according to Lowry[22]. Incorporation of [^{35}S]methionine into newly synthesized protein was determined by precipitation with 10% TCA containing 1.5 mg cold methionine/ml (final concentration). The precipitate was filtered through GFC-Whatman filters which were washed with a 100-fold volume of 5% TCA containing 0.5% casaminohydrolysate. The filters were then further washed with ice-cold ethanol, dried, and counted in a Packard β-scintillation counter.
To assess the amount of ^{35}S-label present in methionyl-tRNA, samples were subjected to mild alkali treatment (incubation at 37 °C for 20 min in 0.66 M NaOH, 0.33 M H_2O_2, and 0.7 mg/ml L-methionine). The TCA-insoluble counts were then measured as indicated above. The results showed that the amount of ^{35}S present in methionyl-tRNA compared to the radioactivity incorporated into proteins after a A30-min pulse could be neglected.

Results

Kinetics of electropore resealing: The resealing of the electropores of P3X63Ag8 cells at 37 °C is a rapid process. Figure 1 shows an experiment in which the kinetics of the pore closure was studied by trypan blue exclusion. Trypan blue (M_r 961) was added at different times during diffusion loading and resealing of the electropores. If trypan blue was present during electroporation and left for 5 min at 0 °C, only about 5% of the cells showed trypan blue exclusion; thus about 95% of the cells exhibited electropores allowing trypan blue to enter. If, however, the cells were shifted after the diffusion loading from 0 °C to 37 °C, a rapid resealing of the electropores was observed. After 30 s of incubation about 60% of the cells showed trypan blue exclusion which reached 100% between 3 and 5 min. In addition, we found that about the same low number of CV-1, HeLa, and secondary mouse kidney cells showed trypan blue exclusion after electroporation and that their resealing kinetics were comparable with that of P3X63Ag8 cells.

Diffusion loading of FITC-dextran and SFV C-protein: Higher M_r macromolecules, e.g. FITC-dextran, were shown to permeate across the electropores during diffusion loading at 0 °C. If FITC-dextran was added before electroporation to P3X63Ag8 cells and was present during the diffusion loading, most of the cells exhibited a diffuse cytoplasmic fluorescence after resealing (not shown). To discriminate quantitatively between FITC-dextran molecules incorporated into the cytoplasm and molecules adsorbed to the surface, the percentage of incorporated and surface bound molecules was determined. Since binding of anti-fluorescein antibodies to FITC-substituted protein was shown to result in a dramatic quenching of the FITC quantum yield[19], we used these antibodies to suppress extracellular fluorescence. Quenching efficiency was tested with FITC-dextran in the absence of added cells. After electroporation and diffusion loading followed by resealing, the cells were incubated in the presence of a saturating amount of anti-fluorescein antibodies. The signal reduction at 520 nm was then measured in the fluorescence spectrophotometer and compared with the signal emission of electroporated cells which were not treated

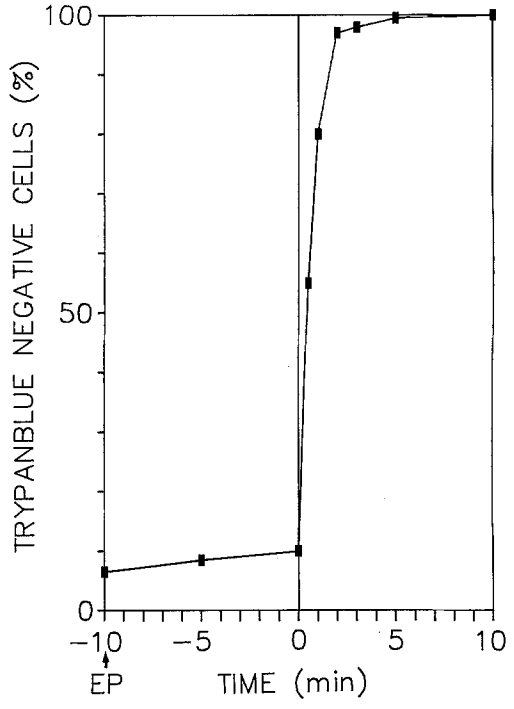

Figure 1. Kinetics of trypan blue exclusion. 10^5 P3X63Ag8 cells (logarithmic phase of growth), suspended in TBS-Na, were electroporated as indicated in 'Materials and methods'. Trypan blue (0.05% final concentration) was added either just before electroporation or at various times as indicated during diffusion loading at 0 °C or during resealing at 37 °C. The dye was allowed to react with the cells during 5 min and percentages of trypan blue negative cells were counted under a microscope. A minimum of 500 cells for each time point was scored. The curve represents the arithmetic mean of four independent experiments performed in duplicate. EP = electroporation.

Discrimination between cell-incorporated and membrane-adsorbed FITC-dextran molecules using anti-fluorescein antibodies. FITC-dextran (250 µg/ml) was added to P3X63Ag8 cells (10^5 cells/assay) suspended in TBS-Na and the cells were either electroporated or non-electroporated. After 10-min-diffusion loading at 0 °C followed by centrifugation and a wash step the pelleted cells were suspended either in the presence or absence of a saturating amount of anti-fluorescein antibodies (6.7 mg/ml). The fluorescence intensity was measured at 520 nm as described in 'Materials and methods'. Electroporated cells not treated (*a*) and treated (*b*) with antibodies; control cells not treated (*c*) and treated (*d*) with antibodies. EP = electroporation; ab = antibodies.

Treatment of cells	Fluorescence intensity (arbitrary units)
a) + EP, − ab	100
b) + EP, + ab	74
c) − EP, − ab	30
d) − EP, + ab	1

with antibodies. Electroporated cells not treated with antibodies showed a maximum fluorescence and were taken as 100% (table, a). Treatment of the cells with antibodies reduced the fluorescence by 25% when compared with untreated cells (table, b). Non-electroporated control cells which had been exposed to FITC-dextran showed a background signal of about 30% in the absence of antibodies (table, c) which was reduced to almost zero in the presence of antibodies (table, d). This result indicates that about two thirds of the cell-bound fluorescence could be accounted for by molecules which were incorporated into the cell, whereas one third was due to molecules which remained attached to the surface of the plasma membrane.

We also determined the efficiency of SFV C-protein incorporation in the presence of 10^5 P3X63Ag8 cells. A constant number of 10^{14} C-protein molecules was present during electroporation and diffusion loading. It was found that about 6×10^5 molecules per electroporated cell were incorporated, whereas only about 3×10^4 molecules remained at the surface of a non-electroporated control cell. The efficiency of C-protein incorporation at that cell density was thus about 0.06%.

Optimization of protein synthesis following electroporation: Figure 2 shows that the composition of the medium during electroporation and diffusion loading has a significant impact on cellular protein synthesis. P3X63Ag8 cells were electroporated in the presence of various buffers. After the 10-min diffusion loading at 0 °C, the cells were transferred to the resealing medium and incubated at 37 °C for various time periods. Following incubation, protein synthesis was monitored by the incorporation of [^{35}S]methionine during 30 min into TCA-precipitable material. When the cells were electroporated in the presence of TBS-Na, and [^{35}S]methionine incorporation was measured immediately after the 10 min diffusion loading time, protein synthesis was less than 5% if compared to non-electroporated control cells. Upon resealing of the electropores at 37 °C in TBS-Na, protein synthesis resumed slowly and reached a 30% recovery 1 h after electroporation. Full activity was achieved only after about 4 h of incubation at 37 °C in DMEM supplemented with 5% fetal calf serum (not shown). If, however, the sodium present in TBS-Na was replaced by potassium, the rate of recovery of protein synthesis was faster. Immediately after diffusion loading protein synthesis was about 10% and reached a 40% recovery after 1 h of incubation at 37 °C (not shown). The presence of ATP, GTP, and of potassium instead of sodium during diffusion loading together with the omission of ATP and GTP during resealing markedly changed the kinetics of protein synthesis recovery. From an original low level of 20%, protein synthesis recovered during the first 15 min of incubation at 37 °C to about 60% and resumed an 80% activity after 1 h. In addition, figure 2 also demonstrates that the composition of the resealing medium for the recovery of protein synthesis was important. P3X63Ag8 cells were electroporated in the presence of ATP, GTP, K^+, Mg^{2+} and Ca^{2+}. If the same buffer was present during resealing at 37 °C, protein synthesis did not recover and remained at a constant low level of about 15% when compared with control cells.

Complete recovery of protein synthesis was achieved by adding amino acids to the ATP and GTP present during diffusion loading and omission of ATP, GTP and amino acids during resealing. Figure 3 shows that immediately after diffusion loading protein synthesis dropped by about 10% and recovered fully after 5 min of resealing. If ATP and GTP were omitted during diffusion loading but the amino acids were present, initial protein synthesis was again less than 20% and reached about 50% after 10 min, then decreased steadily, leveling off at about 20% after 60 min of incubation at 37 °C. This figure demonstrates that diffusion loading either in the presence of ATP/GTP/amino acids or amino acids alone led to similar kinetics of protein synthesis: a sharp increase occurred immediately after resealing, reaching a maximum after 10 min, which was then followed by a renewed decrease. This shows that ATP/GTP and amino acids act synergistically on the fast recovery of protein synthesis during diffusion loading but are not responsible for its maintenance.

The inset of figure 3 indicates that both ATP and GTP are mandatory for a full recovery of protein synthesis. If ATP alone was present together with the amino acids, the recovery of protein synthesis was 43% after 10 min of resealing and reached 57% after 30 min. In contrast, recovery of protein synthesis in presence of GTP and amino acids showed higher values: 55% after 10 min and 79% after 30 min.

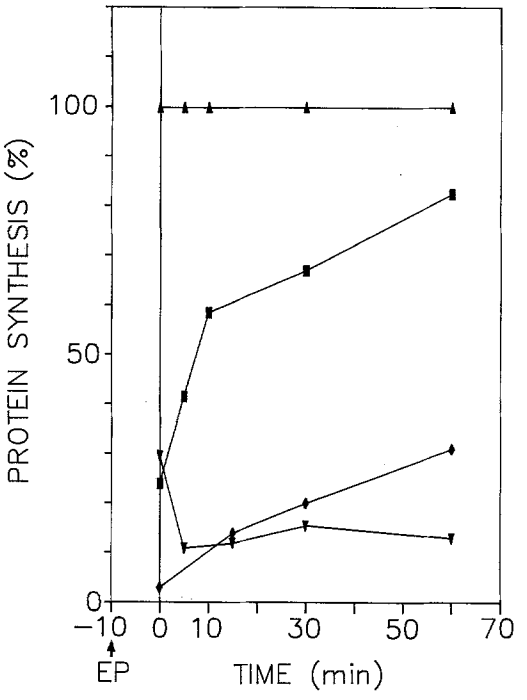

Figure 2. The presence of ATP and GTP during diffusion loading is important for recovery of overall protein synthesis. P3X63Ag8 cells (10^5/assay) were electroporated in the presence of various diffusion loading buffers. After diffusion loading (10 min at 0 °C) the cells were centrifuged and they were then suspended in the corresponding resealing buffer and incubated at 37 °C for various time periods as indicated. The cells were centrifuged, suspended in medium containing [^{35}S]methionine and incubated for another 30 min at 37 °C. TCA-precipitable counts were determined as described in 'Materials and methods'. Non-electroporated control cells were assayed in parallel at each time point and taken as 100%. ▲, non-electroporated control cells; ◆, electroporated cells, diffusion loading and resealing in TBS-Na^+; ▮, electroporated cells, diffusion loading in presence of ATP, GTP, K^+, Mg^{2+}, Ca^{2+}; resealing in K^+, Mg^{2+}, Ca^{2+}; ▼, electroporated cells: diffusion loading and resealing in presence of ATP, GTP, K^+, Mg^{2+}, Ca^{2+}. EP = electroporation.

It is noteworthy that electroporation in the presence of ATP/GTP and amino acids did not change significantly the number of cells showing trypan blue exclusion, and the resealing kinetics of the various cell lines tested were closely similar when compared with those for TBS-Na. If TBS-Na was replaced by ATP/GTP and amino acids during diffusion loading, about twice as many C-protein molecules were incorporated per P3X63Ag8 cell.

The plating efficiencies of electroporated CV-1 and HeLa cells were compared with those of non-electroporated control cells to demonstrate that electroporated and resealed cells were viable. After diffusion loading (in the presence of ATP/GTP and amino acids) and resealing, the cells were plated in the presence of DMEM supplemented with 10% fetal calf serum. The cells attached to the culture plate were trypsinized and counted in the presence of trypan blue 12 and 24 h after plating. The growth rates and plating efficiencies of both electroporated and control cells were practically the same (not shown) and both cell types finally grew out to confluent monolayers. Similar results were also obtained with electroporated secondary mouse kidney cells and P3X63Ag8 suspension cells. These results strongly support

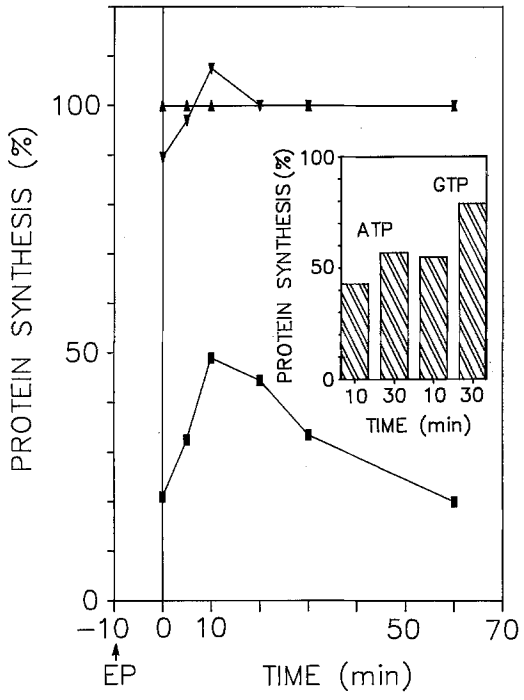

Figure 3. Fast recovery of protein synthesis depends on the presence of ATP, GTP and amino acids during diffusion loading. P3X63Ag8 cells (10^5/assay) were electroporated either in the presence of ATP, GTP, amino acids, K^+, Mg^{2+}, and Ca^{2+} or only in the presence of added amino acids, K^+, Mg^{2+}, and Ca^{2+}. After the diffusion loading the cells were resealed in buffer containing K^+, Mg^{2+}, and Ca^{2+}. The incorporation of [^{35}S]methionine into newly synthesized protein after the various resealing periods was determined as described in figure 2. ▲, non-electroporated cells; ▼, electroporated cells, diffusion loading in presence of ATP, GTP, amino acids, K^+, Mg^{2+}, Ca^{2+}; ▮, electroporated cells, diffusion loading in presence of amino acids, K^+, Mg^{2+}, and Ca^{2+}. Inset: diffusion loading in presence of ATP or GTP, respectively, amino acids, K^+, Mg^{2+}, and Ca^{2+}. EP = electroporation.

the notion that, under the electroporation conditions used, the temporarily permeabilized plasma membrane was completely regenerated, rendering the cells viable.

Discussion

In this paper we present evidence that using our electroporation conditions, about 95% of the cells exhibited electropores large enough to allow penetration of macromolecules across the plasma membrane. We found that the resealing kinetics were closely similar for the various cell lines tested and that the integrity of their plasma membranes was fully restored.

It has been shown that centrifugation (5 min, 800 rpm) of electroporated CHO cells resulted in a draining of the cytoplasm (as judged by an increased optical density at 260 nm of the supernatant) and ultimately to cell death [23]. In agreement with this observation we noticed a slight increase in optical density at 260 nm when the cells were electroporated in the presence of TBS-Na. However, the majority of these cells turned out to be viable. If TBS-Na was replaced by ATP/GTP and amino acids during electroporation, there was no measurable increase in optical density and the short centrifugation step involved after diffusion loading had no influence on the survival rate of the various cell lines tested. Furthermore we demonstrate that SFV C-protein and FITC-dextran cross the electropores and are incorporated into the cytoplasm of the resealed cells. These molecules have estimated unhydrated sphere radii of 2.18 nm and 2.32 nm (from 0.00151 nm^3/Da) [24], respectively. We therefore estimate that the electropores have a diameter of at least 5 nm.

Our observation that electroporated cells entrap macromolecules efficiently is in agreement with the finding that electroporated human erythrocyte ghosts could be loaded with enzymes such as urease (M_r 489 kD) [18]. Vienken et al. [17] could demonstrate that electroporated human erythrocytes were able to entrap ferritin and latex particles with diameters up to 0.176 μm.

We determined the efficiency of incorporation of macromolecules in TBS-Na with a constant concentration of ^{125}I-labeled C-protein (10^{14} molecules per 10^5 cells) and we estimated it to be about 6×10^5 molecules per cell. Compared with the delivery by red cell mediated microinjection (about 10^7 molecules per cell), electroporation is less efficient and can be compared with that of liposome-mediated delivery (manuscript in preparation).

It has been emphasized by Knight and Scrutton [7] that the composition of the medium for electroporation is of importance, since on permeabilization it will enter the cytosol and dilute low-M_r constituents in this compartment. These authors recommend an electroporation medium which mimics the composition of the cytosol, or at least has no measurable adverse effect on the cell metabolism. Hence, the medium should contain a high concentration of K^+, low concentrations of Na^+ and Cl^-, millimolar concentrations of Mg^{2+} and ATP^{2-}, and a micromolar concentration of Ca^{2+}. The salient finding of our report confirms and extends the above mentioned notion and in addition shows that the medium present during electroporation, diffusion loading and resealing is of paramount importance for the protein synthesis. If the medium consisted of TBS-Na (a buffer containing a high saline concentration usually present in electroporation-mediated DNA transfection; ref. 25, 11, and W. Schaffner, pers. comm.), cellular protein synthesis measured immediately after resealing amounted to only about 5% when compared with control cells. The recovery of protein synthesis was slow (fig. 2). This rapid decrease in protein synthesis can be explained by the flooding of the cytosol with Na^+ and the increased need for ATP used by the Na^+/K^+-pump to counterbalance the disturbed Na^+/K^+-equilibrium. Since the presence of ATP and GTP is mandatory for protein synthesis, an inadequate supply of these nucleotides could explain the initial drop in protein synthesis. Addition of ATP and GTP to the electroporation and diffusion loading medium clearly showed that these components play a crucial role in the recovery of protein synthesis. These findings suggest that intracellular ATP and GTP might diffuse into the surrounding medium. If, however, ATP and GTP remained present during resealing at 37 °C protein synthesis did not recover (fig. 2). The high negative charge of these nucleotides apparently leads to an additional permeabilization for low molecular weight components [26].

A minimal drop in initial protein synthesis and an optimal recovery was observed by the addition of amino acids together with ATP and GTP during diffusion loading, suggesting that these components act synergistically in the recovery of protein synthesis in its initial phase, yet not in its maintenance (fig. 3). In conclusion we present evidence that electroporation performed under non-optimized diffusion loading conditions profoundly perturbs cellular protein synthesis for at least 4 h. Thus, optimized electroporation conditions are a prerequisite in studying e.g. the turnover of delivered macromolecules, and/or their impact on cell metabolism or cell morphology. Furthermore, the possibility remains open that the relatively small increase in the transformation rates obtained so far in electroporation-mediated DNA transfection over the classical Ca^{2+}-phosphate coprecipitation could be due to the phenomenon described above.

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Ambiquitous behavior of rabbit liver lactate dehydrogenase

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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-