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Cytosolic malic dehydrogenase activity is associated with a putative substrate for the transforming gene product of Rous sarcoma virus

(M_r 38,000 phosphoprotein/pp60^{src} substrate/metabolism/malate–aspartate shuttle/NADH/NAD ratios)

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ABSTRACT **A cellular protein of apparent M_r 34,000–36,000 was suggested as a possible physiological substrate for the protein kinase (EC 2.7.1.37) activity associated with the transforming gene product of Rous sarcoma virus. We find this protein to migrate with an apparent M_r of 38,000 in NaDodSO$_4$/polyacrylamide gels. It was not separable from cytosolic malic dehydrogenase activity when purified by chromatography on DEAE-Sephacel, hydroxylapatite, poly(A)-Sepharose, and blue Sepharose, by gel filtration, and by isoelectric focusing. The M_r 38,000 protein as well as cytosolic malic dehydrogenase activity focused with a pI of 7.5. In gel filtration experiments, both displayed an apparent native M_r of 68,000. The malic dehydrogenase activity contained in homogeneous preparations of the M_r 38,000 protein had a specific activity of up to 130 units/mg of protein. The recovery of the enzyme was 5–10% of the activity in the extract. Antiserum against the M_r 38,000 protein inactivated the malic dehydrogenase activity associated with the M_r 38,000 protein.**

Rous sarcoma virus (RSV) induces tumors in several species and is able to transform various cultured cells *in vitro* rapidly and efficiently. A single viral gene, termed *src*, contains the information for the transforming capacity of the virus (1, 2). Replication-competent RSV-associated virus (RAV) lacks this gene and provides a nontransforming control virus. The product of *src* has recently been identified by Erikson and coworkers as a phosphoprotein of M_r 60,000 (pp60^{src}) (3–5), associated with a cAMP-independent protein kinase activity (6). This protein kinase activity is likely to be a true enzymatic function of pp60^{src} (7–9) and is peculiar in that tyrosine serves as the phosphate-accepting amino acid (10). Cells transformed by RSV display an 8-fold overall increase in tyrosine phosphorylation (11); hence a protein that is phosphorylated in tyrosine after transformation by RSV is a likely proximal or distal target of pp60^{src} kinase.

By using mutants that are temperature-sensitive for transformation [ts-td mutants (12)], such a protein has been identified. A protein of M_r about 34,000–36,000 was observed to be phosphorylated in tyrosine within 1 hr after a shift from the nonpermissive to the permissive temperature (13–15). Its purification has been described (15). The protein is abundant [0.25% of methionine-containing cellular protein (14)], highly conserved during evolution, cytosolic, and unglycosylated (15). *In vitro* it can be phosphorylated on tyrosine by pp60^{src} (15).

We were interested in the question of whether this protein could be the subunit of one of the enzymes of glycolysis or intermediary metabolism. Because tumor cells are known to have undergone changes in glycolysis (16–18), intermediary metabolism (19), and NADH/NAD ratios (20), identification of an enzyme involved in these reactions and modified after transformation would be of great importance for the understanding of oncogenesis. In this communication, we present evidence that the M_r 34,000–36,000 protein is most likely the subunit of cytosolic malic dehydrogenase.

MATERIALS AND METHODS

Cells and Viruses. The Schmidt–Ruppin strain of RSV (subgroup A), its transformation-defective temperature-sensitive mutant (NY68), RAV type 1 (RAV-1) (subgroup A), and myelocytomatosis virus [MC29 (RAV-1)] were used. Cell culture was performed as described (8).

Radioactive Labeling of Cells and Extraction. Labeling with [^{32}P]orthophosphate was carried out as described (8). For harvesting, the cells were washed with ice-cold phosphate-buffered saline, immediately lysed in 10 mM Tris·HCl, pH 7.2/1 mM EDTA/1 mM 2-mercaptoethanol/1 mM NaF/0.05% Triton X-100 and centrifuged essentially as described (15).

Materials. All salts were purchased from Merck (Darmstadt) and were analytical grade; 2-mercaptoethanol and hydroxylapatite were obtained from Bio-Rad; glycerol (double distilled) was from Serva, Heidelberg; DEAE-Sephacel, poly(A)-Sepharose, blue Sepharose, and Sephacryl S-200 were from Deutsche Pharmacia (Freiburg).

Gel Electrophoresis and Autoradiography. All samples (maximum, 20 μl) were analyzed on 12% NaDodSO$_4$/polyacrylamide gels (0.7 mm thick; 13 cm long) run for 6–8 hr at 80–90 V; the gels were stained, destained, and dried according to conventional procedures. For autoradiography, Kodak XR-5 film was exposed to the dried gels with Cronex Lightning Plus screens. The molecular weights of the proteins in the gels were calculated by using the Pharmacia calibration kit.

Determination of Enzyme Activities. Malic dehydrogenase (MDH; EC 1.1.1.37) activity was measured in a total volume of 1.35 ml in 50 mM potassium phosphate, pH 7.2/1 mM EDTA/1 mM dithiothreitol/0.5 mM NADH/0.2 mM oxalacetate. The oxalacetate was dissolved and neutralized with KOH immediately before the assay. Lactate dehydrogenase (LDH; EC 1.1.1.27), 3-phosphoglycerate kinase (GK; EC 2.7.2.3), and pyruvate kinase (PK; EC 2.7.1.40) were determined as described (21). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) was determined in a total volume of 1.35 ml in 50 mM Tris·HCl, pH 7.5/1 mM ATP/6 mM glycerate-3-phosphate/1 mM EDTA/1 mM dithiothreitol/2 mM MgSO$_4$ containing GK at 10 units/ml.

All enzymatic activities and kinetic values were measured at 37°C. One unit of enzyme is defined as the amount of protein

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Abbreviations: RSV, Rous sarcoma virus (transforming); RAV, RSV-associated virus (nontransforming); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GK, 3-phosphoglycerate kinase; LDH, lactate dehydrogenase; MDH, malic dehydrogenase; PK, pyruvate kinase.

catalyzing the consumption of 1 μmol of substrate per min under the above conditions.

RESULTS

Erikson and Erikson (15) purified the M_r 34,000–36,000 protein by passing a cellular extract through DEAE-Sephacel, followed by chromatography on hydroxylapatite and gel filtration on a Sephacryl S-200 column. We performed the first two steps of this procedure and determined the activities of three cytosolic enzymes of similar size, MDH, GAPDH, and LDH. As a control, the activities of two other cytosolic enzymes, PK and GK, were also followed. The DEAE-Sephacel flow-through from ortho-$^{32}PO_4$-labeled RSV-transformed cells contained 80–90% of the initial activities of all five enzymes (data not shown).

Upon chromatography on a hydroxylapatite column (Fig. 1*a*), a phosphorylated protein of M_r 38,000 (pp38) was found in two peaks, one with a maximum in fraction 39 and the other with a maximum in fraction 46 (chromatography on hydroxylapatite with a continuous 0–500 mM phosphate gradient showed an identical separation of pp38 into two peaks; rechromatography of the pp38 fraction eluting after 250 mM phosphate showed that this behavior was reproducible). This protein, reproducibly migrating with an apparent M_r of 38,000 (±500) has the characteristics of the M_r 34,000–36,000 putative pp60^{src} substrate (13–15) as will be shown below.

A parallel experiment with RAV-infected cells (data not shown) demonstrated that a weakly phosphorylated M_r 38,000 protein from untransformed cells was found in fraction 39 but was absent from fraction 46. A transformation-specific phosphorylation of a M_r 120,000 minor protein, migrating between the two peaks of pp38, was regularly observed. Upon long exposure of the film, we noticed that 62,000-, 60,000-, and 43,000-dalton proteins were weakly phosphorylated specifically in the transformed cell. Of these, PK (62,000–60,000 daltons) and GK (43,000 daltons) have been tentatively identified on the basis of the comigration of phosphate label and enzyme activity in additional purification steps.

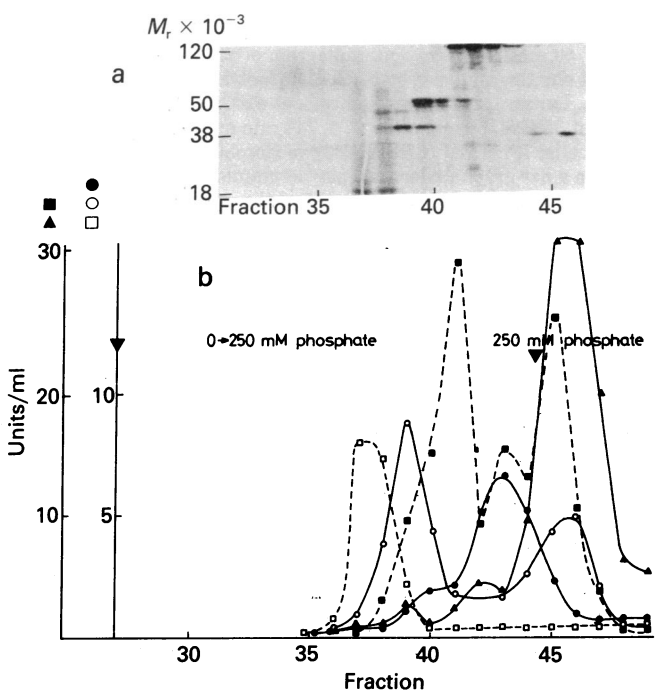

FIG. 1. DEAE-Sephacel and hydroxylapatite chromatography of ortho-$^{32}PO_4$-labeled cell extracts. A lysate from 10 dishes (10-cm) of RSV-transformed cells labeled for 4 hr with $^{32}PO_4^{3-}$ was passed over a DEAE-Sephacel column 1 × 17 cm at a flow rate of 30 ml/hr. The column was washed with 30 ml of buffer I (lysis buffer, containing 10% glycerol). The flow-through fractions were pooled and applied to a hydroxylapatite column (0.5 × 10 cm) which was eluted (15 ml/hr) with a 30-ml gradient of 0–250 mM phosphate in buffer I, followed by 20 ml of 250 mM phosphate. (*a*) NaDodSO$_4$/polyacrylamide gel electrophoresis of fractions from the hydroxylapatite column of SR-1. (*b*) Analysis of hydroxylapatite column fractions for LDH (■), MDH (○), GAPDH (●), PK (▲), and GK (□).

Determination of enzyme activities in the fractions from the hydroxylapatite column (Fig. 1*b*) revealed a broad distribution with partial overlapping. The enzyme that migrated with the two peaks of pp38 was MDH. However, LDH displayed a similar pattern and also overlapped with the pp38 peaks, as well as did part of the GAPDH activity. It was not suprising to observe a heterogeneous chromatographic behavior of these enzymes because MDH and LDH are known to occur in several isoenzymatic forms in the cell (22–24).

In order to achieve a better separation of MDH from GAPDH and LDH, we used chromatography on poly(A)-Sepharose, which had been found to bind GAPDH as well as LDH (25, 26). The fractions eluting from hydroxylapatite with 250 mM phosphate (fraction 42 to the end) were dialyzed and applied to a poly(A)-Sepharose column which was washed with increasing amounts of NaCl. The profile of enzyme activities, the Coomassie blue-stained bands after analysis on NaDodSO$_4$/polyacrylamide gels, and the autoradiogram of the same gel are shown in Fig. 2. pp38 eluted only in fractions 4–10, comigrating with MDH activity and part of the PK activity. Washing the column with 80 mM NaCl yielded LDH, which could be identified with the protein band of M_r 35,000; 160 mM NaCl eluted GAPDH at the same position as the M_r 38,400 protein.

From these results and the fact that the specific activities of the enzymes in the fractions (see legend of Fig. 2) are close to the values for pure preparations (fractions 22–24, 29–32), the three protein bands with M_r in the region of 35,000 observed in the flow-through from DEAE-Sephacel could be assigned to be the subunits of GAPDH (M_r 38,400), MDH (M_r 38,000), and LDH (M_r 35,000). The band of M_r 60,000–62,000 is likely to be the subunit of PK. It should be noted that, when the pp38 peak eluted from the hydroxylapatite column with lower phosphate concentrations (fractions 36–40; Fig. 1) was chromatographed on poly(A)-Sepharose, it was found to behave identically to pp38 from the peak in fraction 46 and to comigrate with MDH activity as well.

Because chromatography on poly(A) had not been used in the purification of the M_r 34,000–36,000 protein (15), a temperature-shift experiment was performed to establish that pp38 was identical. Sister cultures of NY68-infected cells were starved for 4 hr in phosphate-free medium and then labeled for 4 hr at 42°C with $^{32}PO_4^{3-}$, shifted to 35°C, and incubated for a further 20, 40, 60, and 240 min. One plate was kept at 42°C for 240 min. Uninfected chicken embryo fibroblasts and MC29-transformed fibroblasts were labeled the same way at 42°C. MC29-transformed cells have been reported to show no increase in phosphorylation of the M_r 34,000–36,000 protein (13).

The lysates of the seven different cultures were passed in parallel through small DEAE-Sephacel columns. The effluents from these columns were dialyzed and applied to poly(A)-Sepharose columns which were washed with increasing amounts of NaCl. Analysis of three samples (Fig. 3*a*) clearly demonstrated that the pp38 which partly ran through poly(A)-Sepharose and partly eluted with 10 mM NaCl had the properties of the M_r 34,000–36,000 protein: it was phosphorylated 1 hr after shift of the cells from 42°C to 35°C (lane 4), was more strongly labeled 4 hr after shift (lane 5), was little labeled in MC29-transformed cells (lane 6), and was unlabeled in normal chicken embryo fibroblasts (lane 7). By Coomassie blue staining (data not shown),

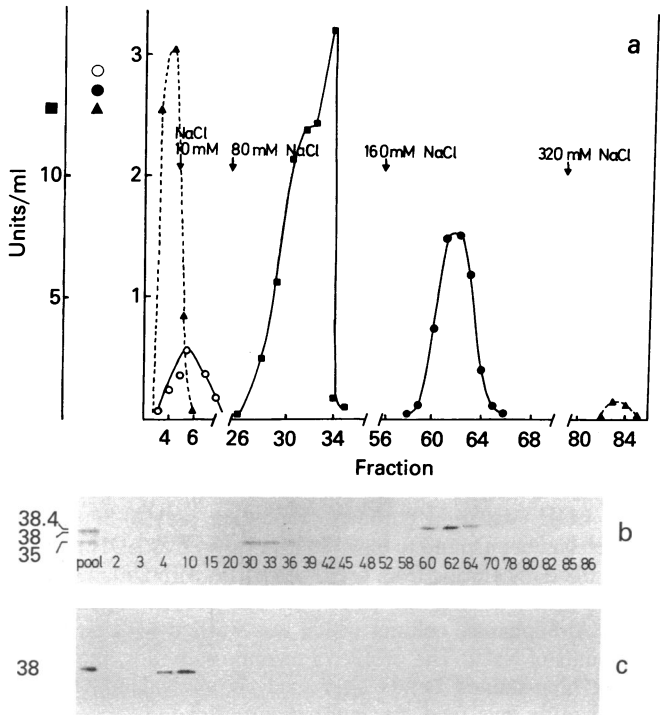

FIG. 2. Chromatography of pooled fractions from the hydroxylapatite column chromatography of RSV-transformed cells on poly(A)-Sepharose. Fractions 42–49 of the hydroxylapatite column run as described in the legend of Fig. 1 were pooled, dialyzed for 4 hr against 10 vol of buffer II (10 mM Tris·HCl/1 mM EDTA/1 mM 2-mercaptoethanol/5% glycerol, pH 7.8), and applied to a 1 × 10 cm column of poly(A)-Sepharose. At a flow rate of 60 ml/hr, 6.5-ml fractions were collected. After the dialyzed pool (20 ml) had entered the gel, the column was washed with 100 ml of 10 mM NaCl, 150 ml of 80 mM NaCl, 100 ml of 160 mM NaCl, and 100 ml of 320 mM NaCl, all in buffer II, adjusted to pH 7.4. (*a*) Analysis of the fractions from the poly(A) column for MDH (○), LDH (■), GAPDH (●), and PK (▲). (*b*) Analysis of column fractions by $NaDodSO_4$/polyacrylamide electrophoresis and staining with Coomassie brilliant blue for protein. M_r shown × 10^{-3}. (*c*) Autoradiogram of gel shown in *b*. The specific activities of the enzymes in their peak fractions were: MDH, 100 units/mg; LDH, 450 units/mg; GAPDH, 80 units/mg; and PK, 300 units/mg.

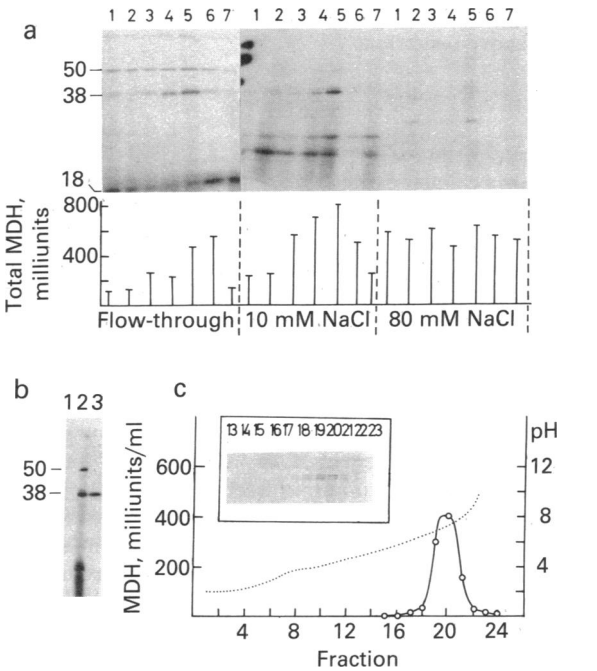

FIG. 3. Poly(A)-Sepharose chromatography of extracts from temperature-sensitive NY68-infected cells at different times after shift to the permissive temperature. The lysates from NY68-infected cells, normal chicken embryo fibroblasts, and MC29-transformed fibroblasts (one 10-cm plate of each in 1.2 ml) were passed in parallel through DEAE-Sephacel columns (0.5 × 6 cm). The dialyzed effluent from these columns (buffer II, see legend of Fig. 2) was applied to poly(A)-Sepharose columns (1 × 2 cm). After the columns were washed with 2 ml of buffer II, they were eluted stepwise with 8 ml of 10 mM NaCl and 14 ml of 80 mM NaCl. (*a*) Autoradiogram of 20-μl samples from the different eluates, analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lanes: 1, NY68-infected cells at 42°C; 2, 20 min at 35°C; 3, 40 min at 35°C; 4, 60 min at 35°C; 5, 240 min at 35°C; 6, MC29-transformed cells; 7, normal chicken embryo fibroblasts. MDH activity is shown in a bar graph under each polyacrylamide gel track. M_r shown × 10^{-3}. (*b*) Autoradiogram of sample 5 of the poly(A)-Sepharose flow-through (*a*) immunoprecipitated with normal rabbit serum (lane 1) or with antiserum against purified M_r 34,000–36,000 protein (lane 3) or without immunoprecipitation (lane 2). (*c*) Isoelectric focusing of pp38 and MDH: 3 ml of sample 5 from the poly(A)-Sepharose 10 mM NaCl wash (*a*) as focused in an LKB-4801 column for 48 hr at 120 V; 80% of the MDH activity peaked at pH 7.5 and a minor fraction peaked at pH 9.6. The fractions at pH 7.5 were pooled and subjected to a second isoelectric focusing as shown with a less steep gradient ·····, pH; ○, MDH activity. (*Inset*) Autogradiogram of the fractions, analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

about equal amounts of protein were present in the pp38 region of all the flow-through and 10 mM NaCl fractions.

To prove its identity further, sample 5 of the flow-through from the poly(A)-Sepharose column was immunoprecipitated with antiserum against M_r 34,000–36,000 protein (27) (Fig. 3*b*). The antiserum clearly recognized pp38. The immunoprecipitated phosphoprotein (lane 3) migrated identically to the unprecipitated one (lane 2), proving that, although we determined a slightly different apparent M_r in our gel system, we were dealing with the putative target of $pp60^{src}$. In agreement with the observations by others (14, 15), pp38 was found to contain phosphotyrosine as well as some phosphoserine (data not shown).

About 30% of the MDH activity in the DEAE-Sephacel flow-through was recovered in the flow-through fractions from the poly(A) columns and another 30% was in the 10 mM NaCl wash (Fig. 3*a*). The remaining MDH activity eluted with 80 mM NaCl. This fraction also contained LDH (see Fig. 2). Because some LDH isoenzymes are known to be able to convert oxalacetate as well and thus mimic MDH activity (24), the MDH activity found in the 80 mM NaCl wash could be due either to a true MDH isoenzyme or to an abnormal LDH. Because these fractions did not contain any pp38, we have not studied this further.

The pI of the M_r 34,000–36,000 putative substrate of $pp60^{src}$ has been determined to be 7.5 (13–15). In order to demonstrate further the identity of pp38 with this protein and to test if MDH activity and pp38 were not separable, we subjected sample 5 of the 10 mM NaCl wash (Fig. 3*a*) to isoelectric focusing. MDH activity was detected in two peaks, a major one at pH 7.5 and a minor one at pH 9.6. The MDH with pI 7.5 was pooled and subjected to a second isoelectric focusing (Fig. 3*c*) and found to comigrate with pp38. Splitting of the pp38 band as seen in Fig. 3*c Inset* has occasionally been observed. We do not know if it is due to partial dephosphorylation or proteolytic degradation. The specific activity of MDH after isoelectric focusing was 130 units/mg of protein, corresponding to a purification by a factor of about 100 compared with the extract.

In all organisms examined so far, two forms of MDH could be distinguished and were found to be coded for by separate genes: one MDH is localized in the cytoplasm (c-MDH), and the other is a mitochondrial enzyme (mt-MDH). Except for shrimp muscle, tuna heart, and sea urchins, the mitochondrial enzyme is more alkaline than the cytosolic one (28). Therefore,

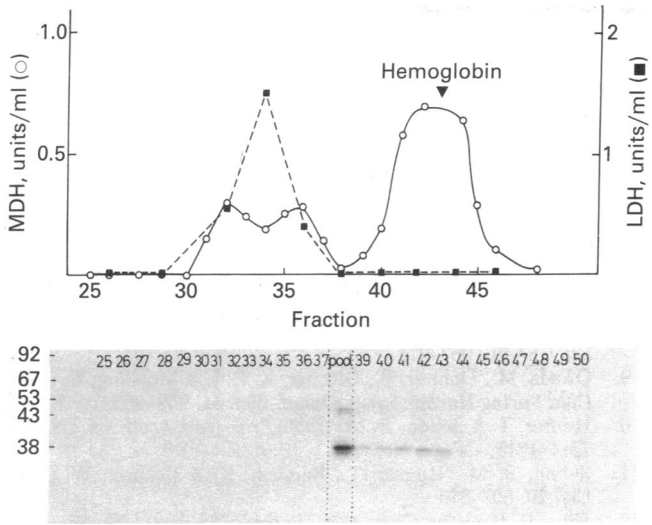

FIG. 4. Gel filtration of pp38 and MDH on Sephacryl S-200. Extract from 17 10-cm dishes of NY68-infected CEF, labeled with $^{32}PO_4^{3-}$ for 4 hr at 42°C and for an additional 4 hr after shift to 35°C, was passed through DEAE-Sephacel (1.5 × 8 cm) and poly(A)-Sepharose (see Fig. 2), and then applied to a hydroxylapatite column (1 × 6 cm). MDH and pp38 eluted at 250 mM phosphate. Pooled fractions (4.5 ml) were chromatographed on a Sephacryl S-200 column (2.5 × 48 cm) in buffer I containing 50 mM phosphate at a flow rate of 30 ml/hr. Fractions (2.5 ml) were analyzed for MDH, GAPDH, and LDH (GAPDH was undetectable) and for radioactivity after NaDodSO$_4$/polyacrylamide gel electrophoresis. Pool, sample of the final preparation just prior to Sephacryl S-200 chromatography.

it was likely that the MDH activity associated with pp38 was c-MDH. Proof for this came from chromatography on blue Sepharose. When pp38 was purified by DEAE-Sephacel and poly(A) chromatography and applied to blue Sepharose, it was found in the flow-through and 1 mM NADH wash. Most MDH activity was found to elute identically; a small fraction bound more tightly and could only be recovered by washing the column with 5.5 mM NADH and 5.5 mM malate (data not shown). Because under these conditions mt-MDH binds more tightly than c-MDH (29), we concluded that the MDH copurifying with pp38 is the cytosolic form.

MDHs generally are composed of two identical subunits of about 38,000 daltons; the M_r of the native enzyme is about 70,000 (22). When a partially purified preparation of pp38 was subjected to gel filtration on a Sephacryl S-200 column under nondenaturing conditions (Fig. 4), pp38 eluted with the hemoglobin marker, appropriate for a dimer of M_r 68,000, just like the native form of the M_r 34,000–36,000 protein (15). MDH activity was found in two peaks, one coincident with the peak of pp38 and the other eluting at a higher native M_r together with LDH. It is likely that the latter activity represents an unusual reaction of the LDH isoenzyme because, when the oxidation of malate by NAD^+ (i.e., the reverse reaction) was monitored, no activity could be detected between fractions 31 and 37 (Fig. 4). The MDH eluting with pp38 had a specific activity of 80 units/mg of protein, corresponding to purification by a factor of about 100.

DISCUSSION

The discovery of the protein product of the transforming gene of RSV and of its associated protein kinase activity has made available an excellent tool for the study of biochemical events during the transformation process. The *src* gene is generally viewed as coding for a pleotropic function (see ref. 12). Hence, several targets may be anticipated for pp60^{src}. The M_r 34,000–36,000 protein which has been identified as a physiological substrate of pp60^{src}, appears to be a good candidate for one such target. Because it is abundant, is conserved in evolution, is unglycosylated, and is cytosolic, we have investigated the possibility that it could be the subunit of one of the cytosolic enzymes LDH, GAPDH, or MDH, which are known to be of similar size.

The pp38 protein reported here is identical to the M_r 34,000–36,000 protein (13–15) because it was phosphorylated in temperature-sensitive transformation-defective mutant-infected cells 1 hr after shift from the nonpermissive to the permissive temperature, contained phosphoserine and phosphotyrosine, and displayed a native M_r of 68,000 and an isoelectric point of 7.5. Furthermore, it was precipitated by antiserum against the M_r 34,000–36,000 protein (27).

pp38 could be separated from LDH and GAPDH activity but it was not separable from c-MDH by six different chromatographic procedures. According to biochemical experience, this result is a strong argument that pp38 could be c-MDH. Comparison of the yield and purification factors of c-MDH in our pp38 preparations with the results from purification of c-MDH from other sources (24, 28, 30–37) demonstrates that c-MDH must be the major constituent in the pp38 preparations (Table 1). Finally, the antiserum against the M_r 34,000–36,000 d pro-

Table 1. Purification of c-MDH

Starting material	pI of pure enzyme	Purification factor*	Yield, %†	Method
RSV-transformed chicken embryo fibroblasts	7.5	1	100	Extracts (see *Materials and Methods*)
		3.9	93	DEAE flow-through
		8.5	80	Poly(A)-Sepharose flow-through
		12.5	18	Hydroxylapatite chromatography‡
		100	6	Sephacryl S-200 (this paper)
Chicken liver, 2 isoenzymes	A: 6.3	100	2	Ca phosphate adsorption, $(NH_4)_2SO_4$ precipitation,
	B: 5.1	97	10	DEAE-cellulose flow-through, chromatography on hydroxylapatite and Sephadex G-100 (24)
Pig heart		50	8.3	CM-cellulose flow-through, DEAE-cellulose flow-through, and hydroxylapatite chromatography (31–33)
Saccharomyces cerevisiae (yeast)	6.8	560	14.5	Blue dextran, $(NH_4)_2SO_4$ precipitation, Celite
	7.1			adsorption, DEAE-cellulose chromatography (36)

* Because different authors used different definitions of enzyme units, the purification factors are compared. Our preparations had specific activities between 80 and 130 units/mg of protein, depending on how fast the first three steps of purification were performed. With the same assay, MDH from chicken liver (24) had a specific activity of 230 units/mg of protein.

† Percentage of extracted enzyme.

‡ The separation from mt-MDH occurs at this step.

tein inhibited the c-MDH activity, although this result is not real proof that pp38 is the subunit of c-MDH because the antigen used for immunization (27) was prepared by procedures similar to those for c-MDH (see Table 1).

If c-MDH is pp38, then it must occur as a phosphoprotein. Indeed, charge differences in two forms of c-MDH which were identical in amino acid composition have been observed (36). Cassman and Vetterlein (38) isolated two c-MDH isoenzymes from beef heart; they contained 0.3–0.6 and 1.3–1.8 mol of phosphate per mol of enzyme, respectively. They showed no difference in amino acid composition, and both were dimeric. Interestingly, the highly phosphorylated form was more active and was no longer regulated by the glycolytic intermediate fructose 1,6-bisphosphate. These striking differences led the authors to suggest that phosphorylation of c-MDH could represent a metabolic control mechanism similar to that known for other regulatory enzymes. In this context, the increase in MDH activity observed in the temperature-shift experiment in Fig. 3 should be pointed out. However, more detailed kinetic analysis will be necessary to establish a functional significance for c-MDH phosphorylation.

Transformed cells are known to have a higher rate of lactate production (39) and a higher ratio of total NADH/NAD^+ than do normal cells (20). Hence, a defect in mitochondria (40) and an increased rate of glycolysis were ascribed to transformed cells, though it is now recognized that the mitochondria are working more or less normally. Alternative mechanisms, however, could equally well lead to increased lactate production. HeLa cells have been found to derive more than half of their energy from glutamine, even when high amounts of glucose are present, and more than 98% from amino acid metabolism when the carbohydrate in the medium is fructose or galactose (41). Thus, the primary function of sugar may be to provide precursors for biosynthesis (16, 21, 42, 43). Treatments that stimulate cell multiplication also activate those pathways that convert amino acids through glutamate into pyruvic acid and hence to lactic acid (19).

The presence of 2-oxycarboxylic acids in the medium becomes essential for cultured human fibroblasts when serum is made rate-limiting for cell growth, suggesting that 2-oxycarboxylic acid metabolism may be related to the mechanism by which serum growth factors regulate cell multiplication (41).

MDH is involved in the transport of reducing equivalents (NADH) from the cytoplasm to the mitochondria via the malate/aspartate shuttle (18). Furthermore, the production of malate is the branch point at which amino acid metabolism in the citric acid cycle is connected to the glycolytic pathway via the production of pyruvate from malate. It is intriguing to imagine that MDH, the enzyme that exerts the control at these branch points, could be modified by the protein product of the transforming gene of a tumor virus.

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