Purification and Characterization of a Heavy-Metal-Modulated Nuclear Protein from SV40-Transformed Cells†

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ABSTRACT: Sodium arsenite was found to stimulate an SV40-transformed BALB/c cell line (SVT2) to synthesize a 31-kDa protein within 2.5 h. This SVT2 protein was purified to homogeneity. It is a nuclear protein which appears to be associated with membranes because it is not extractable from nuclear membrane preparations by 2 M salt. It is highly hydrophobic, eluting from a reverse-phase HPLC column at a similar acetonitrile concentration as a previously described 31-kDa BALB/c-3T3 cell nuclear protein. However, digestion of highly purified BALB/c-3T3 and SVT2 cell proteins with V8 protease revealed nonidentical fragmentation patterns. Moreover, amino acid analysis of the two proteins was also dissimilar, indicating different primary structures. Thus, these two nuclear membrane associated proteins appear to be distinct species.

Heavy-metal salts stimulate tissue culture cells to synthesize certain proteins. The synthesis of these proteins appears to be a part of the stress response. Such "stress" proteins are induced by treatment of murine BALB/c-3T3 cells, human melanoma cells (Caltabiano et al., 1986), or rat fibroblasts (Shelton et al., 1986) with sodium arsenite. One of the proteins induced in BALB/c-3T3 cells in heme oxygenase, a microsomal enzyme which plays an important role in heme catabolism (Yoshida & Kikuchi, 1979; Ishizawa et al., 1983). Another arsenite-induced stress protein of these cells is similar, if not identical, to a growth factor and phorbol ester induced protein of BALB/c-3T3 cells (Disa et al., 1989).

We have been studying the stress proteins of viral-transformed mouse fibroblasts. We now show that SV40-transformed mouse cells rapidly synthesize a 31-kDa protein in response to sodium arsenite. This protein was found to be localized to a nuclear membrane fraction. It has been purified to homogeneity and found to be highly hydrophobic. Its localization and marked hydrophobicity suggest that it is associated with nuclear membranes.

EXPERIMENTAL PROCEDURES

Reagents. Platelet-poor plasma was prepared as described (Scher et al., 1986). *Staphylococcus aureus* V8 protease was purchased from Boehringer Mannheim, [^{35}S]methionine (1122 Ci/mmol) from ICN, and twice-recrystallized sodium dodecyl sulfate (SDS)[1] from Polyscience. Other chemicals (reagent grade) were purchased from Fisher or Sigma.

Cells and Tissue Culture. BALB/c-3T3 (clone A31)-(Aaronson & Todaro, 1968) and SVT2 (Aaronson & Todaro, 1968) cells were grown in the high-glucose formulation of Dulbecco's modified Eagle's medium (DMEM)[1] supplemented with 10% calf serum (Hyclone). The medium was replenished every 3–4 days. Stock cultures of BALB/c-3T3 cells were passed every 7 days, and were replaced from frozen stores every 6–8 weeks. Cells were grown on either P60 or P150 plates in bicarbonate-buffered DMEM for growth in a 10% CO_2 atmosphere. The medium was replenished when the cultures became confluent. For experiments with growth-arrested BALB/c-3T3 cells, the cultures were used 3–4 days later. Confluent exponentially replicating SVT2 cells were used for protein preparation. The medium was replenished 1 day before experimentation. This medium change stimulated an approximate doubling in cell number.

Treatment with Arsenite. The tissue culture medium was replaced with DMEM containing 1.25% (0.38 mg/L) of the usual methionine concentration (Sigma). This medium was supplemented with [^{35}S]methionine (1122 Ci/mmol) and with sodium arsenite (20 μM), which were applied in either 5% platelet-poor plasma or 10% serum. Control cultures were treated with plasma or serum alone. (Addition of serum to the SVT2 cells did not prevent or enhance arsenite-induced stimulation of the arsenite-induced protein.) The cultures were incubated for 2.5 h at 37 °C, washed with cold serum-free DMEM containing the usual concentration of methionine (30 mg/L) and once with cold PBS, and scraped into PBS with a rubber policeman. The pellets were washed twice with cold reticulocyte saline buffer (RSB: 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 1.5 mM $MgCl_2$). In some experiments, the cell pellets were lysed in 100 °C Laemmli buffer.

Treatment of Nuclei. The RSB-washed cells from 100 P150 plates of SVT2 cells were suspended in RSB containing PMSF (170 μg/mL), aprotinin (0.24 trypsin inhibiting unit/mL), leupeptin (20 μg/mL), pepstatin (5 μg/mL), and antipen (10 μg/mL), disrupted by 30 pulls of a Dounce homogenizer, and pelleted by centrifugation at 700*g* for 10 min at 4 °C to prepare a crude nuclear fraction. The nuclei were washed 3 times with RSB plus protease inhibitors and stored for 18 h at 4 °C in the presence of protease inhibitors to prevent destruction of the arsenite-induced protein. These preparations were suspended in 10 mL of cold 2 M NaCl with protease inhibitors, sonicated, and incubated at 4 °C for 30 min. The nuclear

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[1] Abbreviations: Ars, sodium arsenite; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; HPLC, high-pressure liquid chromatography; NP40, Nonidet P40; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; PITC, phenyl isothiocyanate; PPO, 2,5-diphenyloxazole; PPP, platelet-poor plasma; RSB, reticuloycte saline buffer; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

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remnants were pelleted by centrifugation at 110000*g* at 4 °C on an SW41 rotor, resuspended in 10 mL of RSB with protease inhibitors, sonicated, and pelleted again. The RSB wash was repeated once more with the final pelleting being done at 110000*g* at 4 °C in an SW50.1 rotor. The final pellet was lysed in 3.5 mL of 4 °C RSB containing protease inhibitors and 1% NP40. The remnants were pelleted, and the solution was brought to 1 × Laemmli buffer (final volume, 5–10 mL), heated at 100 °C for 3 min, and used for SDS–PAGE or HPLC.

Sucrose Gradient Purification of Nuclei. Centrifugation through sucrose was used to prepare highly enriched populations of nuclei. SVT2 cells were treated for 2.5 h with 10% serum alone or serum supplemented with sodium arsenite (20 μM) in [^{35}S]methionine-supplemented DMEM (20 μM). One portion of whole cells was directly harvested in 100 °C Laemmli (1970) buffer. The remainder was dounced 30 times, and crude nuclear and cytoplasmic fractions were isolated by centrifugation at 700*g*. The nuclear fractions could be stained by trypan blue. For further purification, the nuclear pellets were suspended in 2 mL of 1 M sucrose, carefully overlaid on a 1.8 M sucrose cushion, and spun at 115000*g* for 30 min at 4 °C in an SW50.1 rotor. The nuclear pellet was washed 3 times with cold RSB by successive cycles of suspension and low-speed (700*g*) centrifugation. The cytoplasmic fractions as well as the crude and sucrose-purified nuclear fractions were dissolved in Laemmli buffer, and equal amounts of acid-insoluble counts (25 000 cpm) were analyzed on 5–20% SDS–PAGE.

SDS–PAGE. Preparative SDS–gel electrophoresis was run on 1.5-mm slab gels and analytical SDS–PAGE on 0.75-mm slab gels using the buffer system described by Laemmli (Laemmli et al., 1970) with 1% SDS. Before being loaded, the samples were reduced with 10% β-mercaptoethanol. The stacking gels contained 3% acrylamide, and the separating gels were 5–20% linear gradients of acrylamide. After being stained with Coomassie brilliant blue, the gels were destained in 5% acetic acid and 25% methanol in water for 18 h and vacuum-dried. A modification of this method was used for preparative electroelution of the arsenite-induced protein: the unstained gels were fixed for 10–15 min with 7.5% acetic acid and 25% methanol in water and washed with 25% methanol in water for 15 min and then vacuum-dried. In some cases, the gels were stained with Coomassie brilliant blue and autoradiographed using Kodak XAR-5 film. In others, the gels were fixed with 15% trichloroacetic acid for 18 h, treated with dimethyl sulfoxide with one change, and impregnated with PPO for fluorography. Prestained molecular mass standards were obtained from Sigma and Amersham, and ^{14}C-labeled markers were obtained from Amersham. Prestained markers were lysozyme (14 kDa), trypsin inhibitor (22 kDa), triosephosphate isomerase (27 kDa), carbonic anhydrase (30 kDa), and lactic dehydrogenase (37 kDa). Radiolabeled standards were lysozyme (14 kDa), carbonic anhydrase (30 kDa), and ovalbumin (46 kDa).

Electroelution. For the purpose of purification, the location of the [^{35}S]methionine-labeled arsenite-induced band was identified by autoradiography, the dried gel marked, and the appropriate gel piece cut out and soaked in water, with several changes, at 4 °C for 3–4 h. The protein was electroeluted according to the methodology of Hunkapiller (Hunkapiller et al., 1983) using an Elutrap (Schleicher & Schuell) according to the methodology of the manufacturer. The electroeluted proteins were collected in 20 mM ammonium carbonate containing 0.1% SDS. The volume (3–4 mL for 10 preparative gels) was reduced to 0.75–0.90 mL using a Centricon-10 concentrator. The eluted proteins were reduced with 10% β-mercaptoethanol, heated at 100 °C for 5 min, and centrifuged for 10 min at 14 000 rpm on a microcentrifuge (Eppendorf, Model 5415) prior to further purification by HPLC.

HPLC. Chromatography was performed on an LKB—Pharmacia apparatus equipped with a gradient marker. Samples were run on a analytical reverse-phase Bondapack C_{18} column (Supelco). Solution A contained 5% acetonitrile in 0.1% trifluoroacetic acid and solution B 95% acetonitrile in 0.1% trifluoroacetic acid. The gradient used was from 45% to 95% acetonitrile. The flow rate was 1 mL/min.

Protease Digestion. The arsenite-induced proteins were purified from confluent [^{35}S]methionine-labeled BALB/c-3T3 cells and exponentially replicating SVT2 cells which had been treated with sodium arsenite. Purification was performed by SDS–PAGE and electroelution followed by C_{18} reverse-phase HPLC with an acetonitrile gradient. Both the BALB/c-3T3 and SVT2 cell proteins eluted from the C_{18} column at the same acetonitrile concentration (62%). The purified proteins were dissolved in digestion buffer (0.125 M Tris-HCl, pH 6.8, 0.5% SDS, 10% glycerol, and 0.1 M EDTA), and equal amounts of acid-insoluble counts (30 000 cpm) were incubated with 6 or 12 μg of V8 protease and BSA (0.67 mg/mL) in a final volume of 30 μL at 37 °C for 60 min. The reaction was terminated by addition of 2 × Laemmli buffer containing 10% β-mercaptoethanol and heated at 100 °C for 5 min. The samples were analyzed by SDS–PAGE and visualized by fluorography.

Amino Acid Analysis. The highly purified arsenite-induced protein of SVT2 cells was hydrolyzed in 6 M HCl/1% phenol in the vapor phase for 1 h at 160 °C. The amino acids were derivatized with PITC and the derivatized amino acids separated and quantified using HPLC.

RESULTS

Synthesis by SV40-Transformed Cells. The induction of stress proteins by treatment with sodium arsenite was compared in nontransformed BALB/c-3T3 cells and SVT2 (Aaronson & Todaro, 1968), an SV40-transformed BALB/c cell line. Density-arrested cultures of BALB/c-3T3 cells and exponentially replicating cultures of SVT2 cells were treated with arsenite for 2.5 h in the presence of [^{35}S]methionine. The cultures were harvested in 100 °C Laemmli buffer, and equal quantities of acid-precipitable counts were analyzed by SDS–PAGE and fluorography. Arsenite modulated the production of a protein of similar size, approximately 31 kDa, in both cell lines (Figure 1). For the SVT2 cells, it made no difference whether the arsenite was added in serum, which contains PDGF [a growth factor which stimulates synthesis of a 31-kDa protein in BALB/c-3T3 cells (Pledger et al., 1982; Disa et al., 1989)], or plasma, which lacks it (data not shown). The SVT2 cell protein was characterized further.

Nuclear Localization. Subcellular fractionation was performed in order to determine the subcellular localization of the arsenite-modulated SVT2 cell protein. To determine whether the arsenite-induced protein of SVT2 cells is a nuclear protein, cultures of SVT2 cells were treated with arsenite or left untreated. After the labeling period, one group of cultures was harvested in Laemmli buffer containing SDS. Another group was dounced, and a crude nuclear preparation was obtained by low-speed centrifugation. Highly purified nuclei were obtained by centrifuging the crude nuclear fractions through 1.8 M sucrose. The arsenite-stimulated protein of

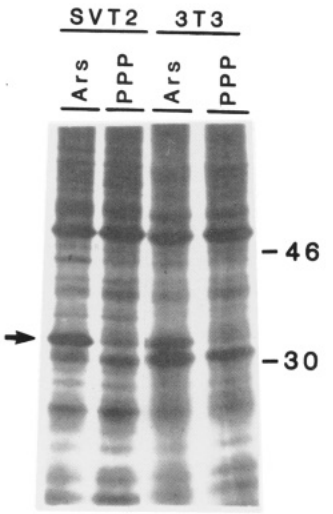

FIGURE 1: Arsenite stimulates exponentially replicating SVT2 cells and density-arrested BALB/c-3T3 cells to synthesize 31-kDa proteins. Duplicate cultures of exponentially replicating SVT2 cells and density-arrested BALB/c-3T3 cells were treated for 2.5 h with [^{35}S]-methionine and 5% platelet-poor plasma alone (PPP), or plasma supplemented with arsenite (Ars). The cultures were harvested in hot Laemmli buffer, and equal quantities of acid-insoluble counts were used for SDS–PAGE. The arrow marks the position of the arsenite-stimulated protein. The positions of molecular weight markers ($\times 10^{-3}$) are shown.

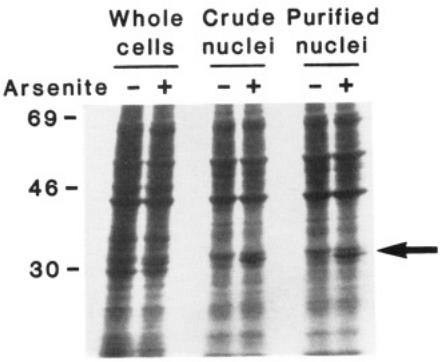

FIGURE 2: Nuclear association of the SVT2 protein. Cultures of SVT2 cells were treated for 2.5 h with [^{35}S]methionine and 10% serum alone (–) or serum supplemented with arsenite (+). One set of cultures was harvested in hot Laemmli buffer. The other was dounced, and crude nuclear fractions were isolated by centrifugation at 700g. A portion of each nuclear fraction was centrifuged at 150000g through 1.8 M sucrose and the purified nuclear pellet isolated. Equal quantities of acid-insoluble counts from all fractions were used for SDS–PAGE. The position of the arsenite-induced protein is marked by the arrow.

SVT2 cells was found in the sucrose-purified nuclear fraction (Figure 2), demonstrating its nuclear localization.

To learn whether the arsenite-induced protein of SVT2 cells is associated with nuclear membranes, a crude nuclear membrane preparation (Figure 3, lane C) was separated from the cytoplasm (lane B) of disrupted cells by low-speed centrifugation and analyzed by SDS–PAGE. The nuclear fraction contained the 31-kDa protein. (The cytoplasm was relatively lightly labeled because individual SVT2 cells are small with a large nuclear:cytoplasmic volume ratio.) The nuclei were sonicated and washed with 2 M sodium chloride. This salt extract did not contain the 31-kDa protein (lane D), but treatment of the salt-extracted nuclear membrane pellet with 1% NP40 solubilized it (lane E).

Taken together, the data show that the arsenite-induced nuclear protein of SVT2 cells appears to be associated with nuclear membranes because it is found in the membrane pellet

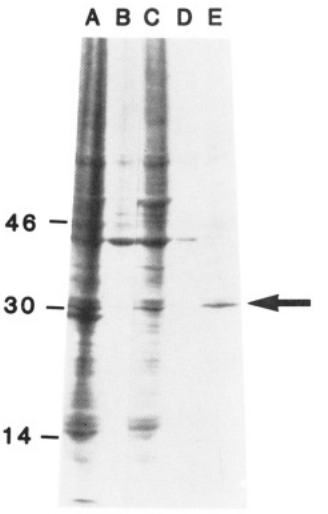

FIGURE 3: Washing crude nuclear membranes and extraction with NP40 allow considerable purification. SDS–PAGE was performed on 50-μL aliquots of 10-mL fractions. One hundred P150 plates of SVT2 cells were treated for 2.5 h with [^{35}S]methionine and 10% serum supplemented with arsenite and suspended in RSB (lane A). The cells were dounced; cytoplasmic (lane B) and nuclear (lane C) fractions were isolated by low-speed centrifugation. The nuclear fraction was sonicated and extracted with 2 M salt, and the nuclear pellet from the 2 M salt supernatant (lane D) was isolated after ultracentrifugation. The nuclear pellet was then washed extensively with RSB and extracted with 1% NP40 (lane E). The position of the 31-kDa protein is indicated by the arrow.

and is not solubilized by 2 M salt. Furthermore, the extensive washing together with NP40 extraction allowed extensive purification. This degree of purification was not achieved when the washed nuclear pellets were extracted with SDS-containing Laemmli buffer because NP40-insoluble proteins were extracted also (data not shown). The high-salt wash and NP40 extraction technique was used for preliminary purification in all other experiments.

HPLC. To allow purification by HPLC, it was necessary to define the acetonitrile concentration at which the arsenite-induced protein elutes when the extracts are applied to a reverse-phase HPLC column. SVT2 cells were treated with arsenite or left untreated. After salt extraction, the sonicated nuclear fragments were solubilized in 1% NP40 and denatured in 100 °C Laemmli buffer, and equal quantities of acid-insoluble counts from the arsenite-treated and untreated specimens were analyzed on a C_{18} reverse-phase HPLC column using a 45–95% acetonitrile gradient. Elution of proteins was followed by monitoring the A_{280}. The elution patterns of the arsenite-treated and untreated cells were the same (data not shown). In both cases, the vast majority of proteins eluted before 60% solution B, approximately 57% acetonitrile (Figure 4, top). Selected fractions that eluted from the C_{18} column were applied to SDS–PAGE, and the 31-kDa protein was identified by autoradiography. In the arsenite-treated samples, a major 31-kDa protein was found to elute at 62% acetonitrile (Figure 4B, fractions 27 and 28). The arsenite-induced protein of BALB/c-3T3 cells also eluted at a 62% concentration (Disa et al., 1989). In the untreated control cultures, the amount of the 31-kDa protein was greatly diminished (Figure 4A). An arsenite-induced protein was not observed in the other fractions (data not shown). This result demonstrates that the arsenite-induced nuclear protein of SVT2 cells is highly hydrophobic because it elutes at a high acetonitrile concentration well after the majority of the other proteins.

Purification. To obtain the arsenite-induced protein in a highly purified state, 100 P150 plates of confluent SVT2 cells

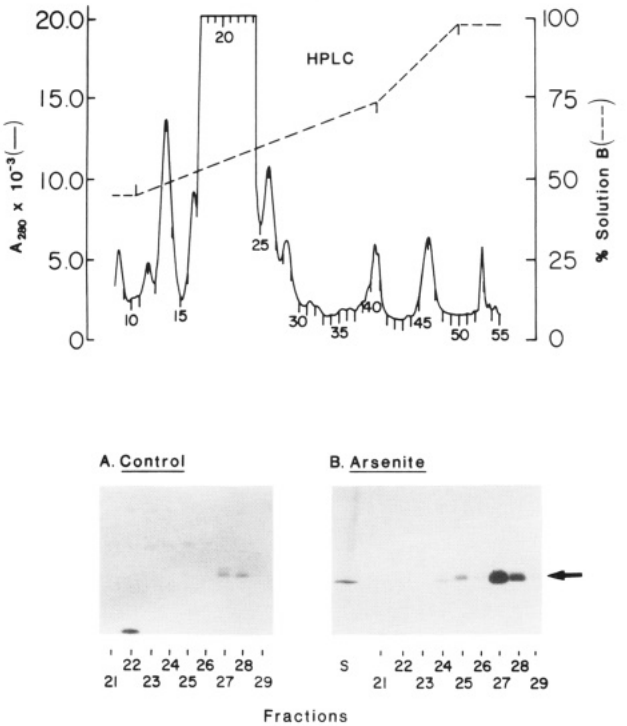

FIGURE 4: Reverse-phase HPLC. Twenty P150 plates of SVT2 cells were left untreated (A) or treated with arsenite (B). The sonicated nuclear fragments were washed, solubilized in 1% NP40, and denatured in 100 °C Laemmli buffer as described above, and equal quantities of acid-insoluble counts (350 000 cpm) from the arsenite-treated and untreated specimens were applied to a C_{18} column and eluted using a 45–95% acetonitrile gradient. The upper portion of the figure shows the protein elution profile which was identical in the arsenite-treated and untreated samples. Solution A contained 5% acetonitrile, and solution B contained 95% acetonitrile. Parts A and B are SDS–PAGE of selected HPLC fractions. One-milliliter fractions from HPLC were collected, and 500-μL aliquots of each fraction were lyophilized and used for SDS–PAGE and autoradiography. S is the start after extraction of nuclei with NP40 but before HPLC.

(approximately 8×10^9 cells) were treated with arsenite, the nuclei isolated, sonicated, and washed with 2 M salt, and the nuclear proteins solubilized with NP40. The solubilized proteins were applied to SDS–PAGE, and the arsenite-induced protein was identified by autoradiography. This membrane-associated nuclear protein was eluted from SDS gels and applied to HPLC. The elution of proteins from the HPLC column was monitored by the UV absorption at 280 nm, and the position of the arsenite-induced protein was determined by acid-insoluble counts (Figure 5A). A major A_{280} peak coincided with the [^{35}S]methionine-labeled peak. This peak was isolated and used for further studies. It was reapplied to a C_{18} column and eluted with an acetonitrile gradient. The eluate was monitored by the absorption at 214 nm, to monitor both tryptophan and the peptide bond, and at 280 nm, to measure aromatic amino acids. Acid-insoluble counts were also determined. The three methods of protein quantitation demonstrated the presence of the same single protein peak (Figure 5B). As an additional method of measuring purity, serial dilutions, approximately 15–1.5 of the HPLC-purified protein, were applied to SDS–PAGE. Coomassie blue staining showed that more than 95% of the protein was present in a single major band, with a molecular mass of 31 kDa (Figure 6). This band was also identified by autoradiography (Figure 6).

The methods used to analyze the purity of the arsenite-induced SVT2 protein-separate proteins were based on distinctive physicochemical characteristics. Reverse-phase

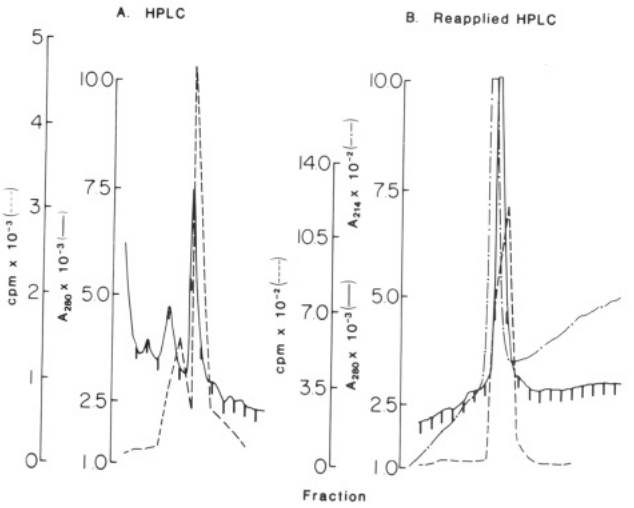

FIGURE 5: Purification of the arsenite-induced protein. One hundred P150 plates of SVT2 cells were treated with arsenite; their nuclei were sonicated, washed, and extracted with NP40. The arsenite-induced protein was purified on 10% SDS gels, identified by autoradiography, and electroeluted. (A) The electroeluted protein was applied to a C_{18} reverse-phase column and eluted with a gradient of 45–95% acetonitrile. Proteins eluting from the column were monitored by measuring the A_{280}. One-milliliter fractions were collected (as indicated by the tic marks), and 5 μL was precipitated with TCA to determine acid-insoluble counts. (B) The fraction containing the A_{280} and [^{35}S]methionine-labeled peak in (A) was lyophilized, dissolved in 0.1% SDS, and reapplied to the C_{18} reverse-phase column. The column was eluted with the same gradient of acetonitrile. Proteins eluting from the column were monitored by measuring the A_{214} and A_{280}. One-milliliter fractions were collected, and 10 μL was precipitated with TCA to determine acid-insoluble counts. The peaks are offset for the purpose of visualization.

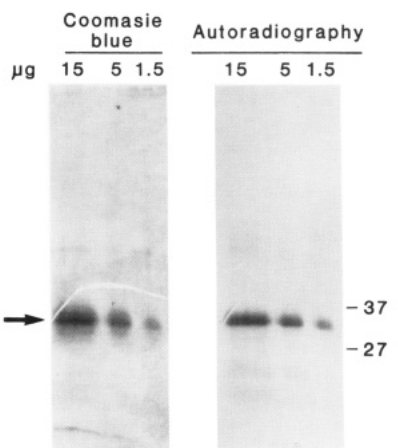

FIGURE 6: SDS–PAGE of the highly purified arsenite-induced protein of SVT2 cells. Serial dilutions of the [^{35}S]methionine-labeled arsenite-stimulated nuclear protein (which had been purified by extraction of nuclei with NP40, SDS–PAGE, and electroelution followed by HPLC) were applied to SDS–PAGE. (Left panel) Coomassie blue staining of the gel. (Right panel) Autoradiography.

HPLC isolates proteins by virtue of their hydrophobicity. SDS–PAGE segregates proteins on the basis of size. Analysis by each of these methods demonstrates that the arsenite-induced protein has been obtained in a highly purified near-homogeneous state.

Comparison with the BALB/c-3T3 Protein. Both BALB/c-3T3 (Disa et al., 1989) and SVT2 cells synthesize highly hydrophobic 31-kDa nuclear membrane associated proteins in response to sodium arsenite. The arsenite-induced nuclear membrane associated proteins of SVT2 cells and BALB/c-3T3 cells have very similar physicochemical characteristics: they have the same mobility on SDS–PAGE and the same

Table I: Amino Acid Composition of the Arsenite-Induced SVT2 Cell Nuclear Protein: Comparison to the BALB/c-3T3 Cell Nuclear Protein and Heme Oxygenase[a]

	SVT2 nuclear protein			BALB/c-3T3	
				nuclear protein	heme oxygenase
amino acid	mol %	calcd residues	no. of residues	no. of residues	no. of residues
Asx	8.24	20.7	21	23	14
Glx	17.01	42.8	43	33	48
Ser	5.84	14.7	15	25	17
Gly	2.86	7.2	7	34	11
His	3.31	8.3	8	6	10
Arg	4.71	11.8	12	15	14
Thr	5.47	13.7	14	16	19
Ala	8.49	21.3	21	25	27
Pro	8.09	20.3	20	13	20
Tyr	3.52	8.8	9	8	10
Val	4.89	13.5	14	15	15
Met	3.75	9.4	9	3	11
Ile	3.89	9.8	10	13	10
Leu	9.89	24.9	25	26	31
Phe	3.84	9.7	10	11	12
Lys	6.21	15.6	16	17	16
Cys	ND[b]	ND[b]	ND[b]	ND[b]	1
Trp	ND[b]	ND[b]	ND[b]	ND[b]	3
total	100.01	252.5	254	283	289

[a] The amino acid composition of the SVT2 nuclear protein was determined from the amino acid analysis. The amino acid composition of the BALB/c-3T3 cell nuclear protein and BALB/c-3T3 cell heme oxygenase has been given previously (Disa et al., 1989). [b] ND, not determined.

hydrophobicity on HPLC. To learn whether the SVT2 protein is identical to that of BALB/c-3T3 cells, the proteins of each cell line were purified to homogeneity. Confluent SVT2 cells and confluent BALB/c-3T3 cells were treated with sodium arsenite (20 μM) and labeled with [^{35}S]methionine. The purification methodologies for isolating the proteins from each cell line were identical and involved washing sonicated nuclei with 2 M salt and extraction with NP40 followed by sequential SDS–PAGE and electroelution. The proteins were then each applied to a C_{18} HPLC column, and the labeled peaks from each cell line eluting at 62% acetonitrile were concentrated and studied.

Initial experiments showed that [^{35}S]methionine-labeled SVT2 and BALB/c-3T3 cell proteins had different fluorographic patterns on SDS–PAGE after digestion with V8 protease (data not shown). Amino acid analysis was used to determine whether the difference in V8 protease digestion patterns reflected a difference in the amino acid content. Amino acid analysis based on a molecular mass of 30 kDa demonstrated the presence of 254 amino acid residues in the SVT2 protein (Table I), whereas the BALB/c-3T3 protein, which is shown for comparison (Table I), had 283 amino acids (Disa et al., 1989). Although only one digestion was performed for each protein, the amino acid composition of the SVT2 protein was found to differ substantially from that of the BALB/c-3T3 nuclear protein. Specifically, the SVT2 protein had far more proline and methionine residues, whereas the BALB/c-3T3 protein had many more serines and glycines. The SVT2 protein also differed from heme oxygenase, an arsenite-inducible gene product of BALB/c-3T3 cells (Kageyama et al., 1988).

DISCUSSION

The present data show that sodium arsenite stimulates both quiescent nontransformed BALB/c-3T3 cells and replicating SVT2 cells to rapidly synthesize 30–31-kDa nuclear proteins. Each of these proteins appears to be associated with nuclear membranes because washing such membrane preparations with both high and low concentrations of salt does not cause dissociation. In addition, the BALB/c-3T3 and SVT2 proteins have similar physicochemical characteristics. Both are highly hydrophobic, requiring detergents or organic solvents for solubilization. They both elute at a 62% acetonitrile concentration when chromatographed on a C_{18} reverse-phase HPLC column, demonstrating that they have a similar high hydrophobicity. Furthermore, both the SVT2- and BALB/c-3T3-derived proteins have similar mobilities on SDS–PAGE. However, protease digestion of electrophoretically homogeneous preparations has shown that these proteins are not identical, and amino acid analysis confirms that they are distinct species.

It is important to be sure that the protein preparations are pure when making comparisons of protein species. The p31 nuclear protein of BALB/c-3T3 cells has been shown to be pure by three criteria, SDS–PAGE, HPLC, and isoelectric focusing (Disa et al., 1989). Similarly, the nuclear protein of SVT2 cells is homogeneous by SDS–PAGE and HPLC; capillary electrophoresis also suggests purity (data not shown). Thus, the difference in amino acid analysis provides strong evidence for nonidentity.

The arsenite-inducible SVT2 cell protein does not appear to arise from proteolytic processing of an SVT2 cell homologue of the BALB/c-3T3 nuclear protein. Such posttranslational modification is ruled out because the number of proline and methionine residues in the SVT2 protein exceeds that of the BALB/c-3T3 cell nuclear protein. Similarly, the BALB/c-3T3 cell protein could not have arisen from modification of an SVT2-type precursor because there are more serines and glycines in the BALB/c-3T3 protein. The divergence in the content of the amino acids of these two proteins appears to reflect differences in primary protein structure.

The arsenite-inducible SVT2 protein does not appear to be encoded by the SV40 genome. Only two SV40 proteins are produced by SV40-transformed mouse cells, the large T and small t antigens. The T antigen is a nuclear protein but is much larger than the arsenite-inducible protein and migrates like a 94-kDa species on SDS gels (Griffin et al., 1978). The t antigen is found in the cytoplasm and is 20 kDa (Bikel et al., 1983). Clearly, the arsenite-inducible protein of SVT2 cells is encoded by cellular genes. One possibility is that the arsenite-inducible SVT2 protein is transformation-specific. It is found in the SV40-transformed cells, but not in their nontransformed cell counterparts. Further work is needed to confirm this possibility.

Sakiyama and co-workers (Kageyama et al., 1988) have found that sodium arsenite induces BALB/c-3T3 cells to express the transcript for heme oxygenase, an mRNA which encodes a 32-kDa protein. We have previously shown that the arsenite-induced nuclear protein of BALB/c-3T3 cells is not heme oxygenase (Disa et al., 1989). Amino acid analysis of the SVT2 protein demonstrates that it too is not heme oxygenase. Furthermore, the SVT2 protein is also unlikely to be a metallothionein, a protein which binds to heavy-metal salts and detoxifies them. Unlike the arsenite-induced protein of SVT2 cells, metallothioneins are cytoplasmic proteins which are typically less than 10 kDa (Hamer et al., 1986).

Few nuclear membrane associated proteins with properties similar to the proteins described herein have been characterized. For example, nuclear pore proteins have different solubility characteristics. Recently several nuclear envelope proteins have been identified by preparing monoclonal an-

tibodies against nuclear envelopes. These proteins (Senior & Gerace, 1988; Worman et al., 1988, 1990; Padan et al., 1990; Bailer et al., 1991) were localized to the inner nuclear membranes and shown to interact with the nuclear lamins. They range from 54 to 75 kDa, and thus do not appear to be identical to the arsenite-induced proteins of SVT2 and BALB/c-3T3 cells. A myosin heavy chain-like nuclear envelope protein with ATPase activity and a differentiation-modulated protein (termed AGF 2.3) have also been found to be associated with nuclear envelopes (Berrios & Fisher, 1986; Lord et al., 1988). However, both AGF 2.3 and the myosin-like proteins are 6–7-fold larger than the arsenite-modulated proteins of SVT2 and BALB/c-3T3 cells.

We have recently obtained amino acid sequence data of two peptides generated from the arsenite-induced nuclear protein of SVT2 cells after cyanogen bromide digestion. EMBL and GenBank data base searches revealed that the SVT2 protein's primary structure differs from other previously characterized proteins. Additional sequence data will require cDNA cloning and sequencing.

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REFERENCES

Aaronson, S. A., & Todaro, G. J. (1968) *Science 162*, 1024–1026.

Bailer, S. M., Eppenberger, H. M., Griffiths, G., & Nigg, E. A. (1991) *J. Cell Biol. 14*, 389–400.

Berrios, M., & Fisher, P. A. (1986) *J. Cell Biol. 103*, 711–724.

Bikel, I., Roberts, T. M., Bladon, M. T., Green, R., Amann, E., & Livingston, D. M. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 906–910.

Caltabiano, M. M., Koestler, T. P., Poste, G., & Grieg, R. G. (1986) *J. Biol. Chem. 261*, 13381–13386.

Disa, S., Manilla, A. C., & Scher, C. D. (1989) *J. Biol. Chem. 264*, 15993–15999.

Griffin, J. D., Light, S., & Livingston, D. M. (1978) *J. Virol. 27*, 218–226.

Hamer, D. H. (1986) *Annu. Rev. Biochem. 55*, 913–951.

Hunkapiller, M. W., Lujan, E., Oshander, F., & Hood, L. (1983) *Methods Enzymol. 19*, 227–235.

Ishizawa, S., Yoshida, R., & Kikuchi, G. (1983) *J. Biol. Chem. 258*, 4220–4225.

Kageyama, H., Hiwasa, T., Tokunaga, K., & Sakiyama, S. (1988) *Cancer Res. 48*, 4795–4798.

Laemmli, U. K. (1970) *Nature 227*, 680–685.

Lord, J. M., Thick, J. A., Bunce, C. M., Taylor, A. M. R., Gallimore, P. H., Mills, D., & Brown, G. (1988) *J. Cell Sci. 90*, 237–245.

Padan, R. S., Nainudel-Epszteyn, S., Goitein, R., Fainsod, A., & Gruenbaum, Y. (1990) *J. Biol. Chem. 265*, 7808–7813.

Pledger, W. J., Hart, C. A., Locatell, K. L., & Scher, C. D. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4358–4362.

Scher, C. D., Engle, L. J., Eberenz, W. M., & Ganguli, K. (1986) *J. Cell. Physiol. 126*, 333–340.

Senior, A., & Gerace, L. (1988) *J. Cell Biol. 107*, 2029–2036.

Shelton, K. R., Todd, J. M., & Egle, P. M. (1986) *J. Biol. Chem. 261*, 1935–1940.

Worman, H. J., Yuan, J., Blobel, G., & Georgatos, S. D. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8531–8534.

Worman, H. J., Yuan, J., & Blobel, G. (1990) *J. Cell Biol. 111*, 1535–1542.

Yoshida, T., & Kikuchi, G. (1979) *J. Biol. Chem. 254*, 4487–4491.