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Cysteine Auxotrophy of Human Leukemic Lymphoblasts Is Associated with Decreased Amounts of Intracellular Cystathionase Protein†

L. Michael Glode,‡ Michael P. Kriegler,§ and David M. Livingston*

ABSTRACT: A series of human lymphoblastoid cell lines derived from nonleukemic donors are known to be cysteine prototrophs (*cys*$^+$), while several lymphoblastoid lines derived from leukemic donors are cysteine auxotrophs (*cys*$^-$). We have tested representative cell lines of each type for their content of cystathionase enzyme activity by a specific catalytic assay and their total cystathionase protein content by immunoprecipitation of in vivo labeled protein. There was a close correlation between the cellular content of the enzyme as determined in the two assays. Specifically, those *cys*$^+$ lines having readily measurable enzyme by catalytic assay were found to contain significantly higher levels of immunoprecipitable M_r 43 000 cystathionase subunit than those *cys*$^-$ lines tested which were depleted in active enzyme. Thus, the absolute cysteine requirement of the leukemic, *cys*$^-$ cell lines tested is likely due to an intracellular reduction of cystathionase protein.

During the acquisition of a leukemic phenotype, numerous changes are presumed to occur in the membrane composition and metabolic chemistry of a hematopoietic cell. For example, it has been shown that human peripheral blood leukemic cells have markedly different requirements for specific amino acids when compared to their nonleukemic counterparts (Ohnuma et al., 1971). In one case, certain human and rodent hematopoietic tumor cell lines are auxotrophic for L-cysteine (*cys*$^-$) when compared to nontumored cell lines of similar origin (Livingston et al., 1976; Iglehart et al., 1977). Furthermore, extracts of these leukemic (*cys*$^-$) cell lines contain markedly reduced levels of detectable cystathionase catalytic activity when compared to their nonleukemic (*cys*$^+$) counterparts. As a result, *cys*$^+$ cell lines are capable of normal growth in the absence of preformed cysteine when cystathionine is present in the medium, while *cys*$^-$ cell lines are growth-arrested under these conditions. These in vitro studies complement the observation that a reduction in murine thymocyte cystathionase activity occurs during thymic leukemogenesis whether induced by exogenous administration of a type C virus or arising spontaneously, as is the case in AKR mice (Livingston et al., 1976). Additional evidence suggests that in the majority of fresh human lymphoid and myeloid leukemic cells, obtained by bone marrow aspiration, there is a reduction in the concentration of cystathionase enzyme activity compared to that present in extracts of normal human bone marrow mononuclear cells (Glode et al., 1979). Thus, there is a strong correlation between the presence of the leukemic phenotype and depletion of cystathionase enzyme activity in cells of both lymphoid and myeloid origin. In none of these cases could the reduction in measurable cystathionase activity be accounted for by the presence of a soluble inhibitor of this enzyme.

† From the Laboratory of Neoplastic Disease Mechanisms, Sidney Farber Cancer Institute, and the Departments of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Massachusetts 02115 (L.M.G. and D.M.L.), and The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138 (M.P.K.). *Received April 21, 1980; revised manuscript received August 26, 1980.* This work was supported by Grant CA 21920 from the National Cancer Institute.

‡ Present address: Department of Medical Oncology, University of Colorado Medical Center, Denver, CO 80220.
§ Present address: Department of Molecular Biology, University of California, Berkeley, CA 94720.

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However, despite the results of cystathionase radioimmunoassays in murine leukemia cells which suggested a depletion in intact enzyme protein (Bikel et al., 1978), definitive results indicating whether or not intact enzyme molecules were present in *cys*$^-$ cells of various types and in what concentration were lacking. We address these questions in this paper.

Experimental Procedures

Cells. The established cell lines studied here were all of human origin. The history of their development and general properties of some (CEM, SB2, SB, and LAZ 221) have been reported (Foley et al., 1965, 1969; Lazarus et al., 1974, 1978). The remainder were established by identical procedures.

Cystathionase Assay. L-[1-^{14}C]Cystathionine was synthesized from L-[1-^{14}C]methionine (60 mCi/mol) (Amersham/Searle) by a modification of the method of Mudd et al. (1965) as previously described (Iglehart et al., 1977) and had a final specific activity of 1 mCi/mol. Cystathionase assays were carried out as previously described (Livingston et al., 1976; Iglehart et al., 1977).

Preparation of Crude Extracts of Tissue Culture Grown Cells. Crude extracts of cells in the logarithmic phase of growth were prepared as previously described (Iglehart et al., 1977). Supernatants were either assayed immediately or stored at −70 °C for up to 1 month prior to assay. Protein determinations were carried out according to the method of Lowry et al. (1951) after protein precipitation with 6% trichloroacetic acid in the presence of 0.002% sodium deoxycholate.

Preparation of Highly Purified Human Liver Cystathionase. The techniques used for purification of human liver cystathionase have been previously described (Iglehart et al., 1977). Two modifications of that method have been utilized in these experiments. Human liver, obtained at autopsy, was stored at −70 °C prior to use. Tissue was allowed to thaw at 4 °C and was then minced in 1 volume of buffer A (0.05 M Tris[1]-HCl, pH 8.0, 0.05 M KCl, 5% glycerol, 5×10^{-5} M PLP, and 14 mM β-ME) equal to the wet weight (100 g) of the tissue. It was then homogenized for 5 min at 0 °C in a Sorvall OmniMixer. The homogenate was incubated at 25 °C for 40 min in the presence of 20 μg/mL each of pancreatic RNase and DNase (Calbiochem, San Diego, CA) and 10 mM MgCl. Ammonium sulfate precipitation was carried out at 0 °C, and the fraction precipitating between 45 and 65% saturation was redissolved in 0.01 M Tris-HCl, pH 7.0, 5×10^{-5} M PLP, 5% glycerol, and 14 mM β-ME (buffer B) in a volume equal to one-fourth of the original homogenate. This solution was dialyzed overnight against three changes of buffer B (50–100 volumes each). Insoluble material was then removed by centrifugation. The protein solution was then percolated through a DEAE-cellulose column (5 × 20 cm) equilibrated with buffer B. The column was washed with buffer B until protein could no longer be detected in the eluate by absorbance measurements at 280 nm, and the enzyme was eluted in a linear gradient of KCl between 0 and 0.1 M in buffer B. The enzyme eluted at ~40 mM KCl. Enzyme-containing fractions were pooled and concentrated by precipitation with 70% ammonium sulfate. The precipitate was dissolved in buffer B, and the ensuing solution was dialyzed against buffer B as noted above. This fraction was further subjected to rate–zonal sedimentation in 39-mL, 8–40% (v/v) glycerol gradients in buffer A. Gradients were overlaid with ~1 mL of the dialyzed DEAE-cellulose fraction and centrifuged at 4 °C for 45 h in an SW 27.1 rotor at 26 500 rpm. Fractions (1.5 mL) were collected. Peak cystathionase-containing fractions were pooled and concentrated 4-fold by ultracentrifugation using an Amicon XM-50 membrane (Amicon, Lexington, MA). The enzyme was then subjected to isoelectric focusing at 4 °C in an LKB ampholine column (type 8101) with an LKB 3371 power supply. Gradients (110 mL) were 5–40% in sucrose, and the final concentration of ampholines (pH 5–8) was 1%. Current was applied at 600 V, and electrophoresis was allowed to proceed for 48 to 65 h. The cystathionase peak fractions, identified at pH 5.9, were pooled and stored at −70 °C. In later experiments, a simpler approach, in which the isoelectric focusing and zonal sedimentation steps were eliminated, was employed. After DEAE-cellulose elution and ammonium sulfate precipitation, the enzyme was dissolved in buffer B and 1.5 mL applied to a 1.5 × 90 cm column (Pharmacia) of Ultrogel AcA 34 (LKB, Stockholm, Sweden). This procedure was repeated once, and the resultant enzyme was subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis (vide infra). It was found to be >95% pure after Coomassie blue staining (Figure 2).

Serum. Antiserum to highly purified cystathionase was prepared by injecting 5 μg of purified enzyme, emulsified in 2 mL of complete Freund's adjuvant, into a female New Zealand albino rabbit. Three injections were performed at 2 week intervals. Ten days after the last injection, blood was collected from a marginal ear vein. Preimmune rabbit serum (NRS) was obtained by prebleeding the same rabbit used for immunization. Normal lamb serum (NLS) was obtained commercially.

One-Dimensional Gel Electrophoresis. Gel electrophoresis was performed in 12% $NaDodSO_4$–polyacrylamide slab gels with 4% polyacrylamide stacking gels, according to Laemmli (1970). For quantitative immunoprecipitation experiments, both the protein A–Sepharose bead eluate and the beads themselves were loaded atop the gel to ensure quantitative recovery of immune complexes. This procedure did not affect the resolution of the proteins tested. Gels containing radioactive proteins were subjected to autofluorography (Bonner & Laskey, 1974).

Radioiodination. Purified cystathionase protein was iodinated as described by Fraker & Speck (1978). Where indicated, iodinated proteins were subjected to electrophoresis through a 12% $NaDodSO_4$–polyacrylamide gel (Laemmli, 1970).

Immunoprecipitation of in Vivo Labeled Cystathionase. Cellular proteins were labeled in vivo by seeding 5×10^6 cells, already in the logarithmic phase of growth, in 5 mL of Hank's balanced salt solution, to which 50 μL (500 μCi) of [^{35}S]-methionine (450 Ci/mmol) was added. Such cultures were incubated at 37 °C for 4 h. The cells were harvested by centrifugation and resuspended in 1 mL of lysing buffer (20 mM Tris-HCl, pH 9.0, 137 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10% glycerol, and 1% NP40). Lysates were freed of cellular debris by centrifugation. Cellular incorporation of [^{35}S]methionine was measured by spotting 3 μL of lysate supernatant on Whatman 3 MM paper filters. These filters were washed once with ice-cold 10% trichloroacetic acid (Cl_3CCOOH), once with hot Cl_3CCOOH (90 °C), rinsed twice with ice-cold acetone, dried, and subjected to scintillation counting in liquifluor–toluene. In experiments designed to quantitate labeled cystathionase in different cell lines, aliquots

[1] Abbreviations used: Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; β-ME, β-mercaptoethanol; RNase, ribonuclease; DNase, deoxyribonuclease; DEAE, diethylaminoethyl; $NaDodSO_4$, sodium dodecyl sulfate; mRNA, messenger ribonucleic acid; DNA, deoxyribonucleic acid; TPCK, tosylphenylalanine chloromethyl ketone; PLP, pyridoxal phosphate.

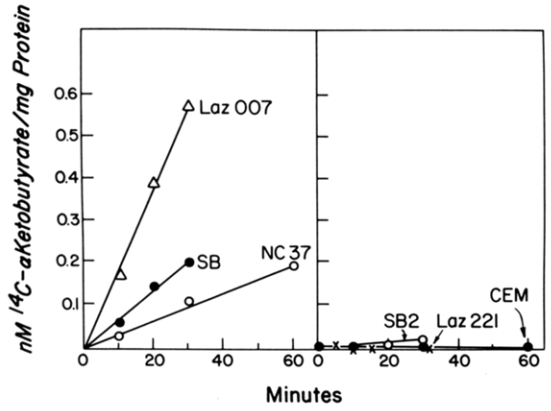

FIGURE 1: Cystathionase activity in crude extracts of human lymphoid cell lines. Crude extracts of cell lines harvested in the logarithmic phase of growth were prepared and enzymatic activity was determined as described under Experimental Procedures. 0.4 mg of protein from each extract was tested. (Left panel) *cys*$^+$ cell lines. (Right panel) *cys*$^-$ cell lines.

of cell lysates containing equivalent quantities of incorporated radioactivity were brought to a final volume of 1 mL with lysing buffer and immunoprecipitated. To each 1 mL of supernatant, 10 μL of normal lamb serum and 20 μL of preimmune rabbit serum were added together with 50 μL of a 50% (v/v) suspension of protein A–Sepharose beads (Pharmacia Fine Chemicals, Sweden). The tubes were agitated for 1 h at 4 °C, and the immune complexes were bound to the bead pellet isolated by centrifugation at 6000*g*. The procedure of adding the above-noted sera and beads, incubating, and centrifuging was repeated twice and, then, either 3 μL of normal rabbit serum (control) or 3 μL of rabbit anti-human cystathionase serum was added to the multiply adsorbed lysate together with another aliquot (50 μL) of protein A–Sepharose beads. After 12–14 h of agitation at 4 °C, the bead–immune complexes were pelleted and the supernatant was discarded. Following five washes with 1 mL of washing buffer (100 mM Tris-HCl, pH 9.0, 0.5 M LiCl, and 1% β-ME), protein was eluted from the beads by heating at 95 °C for 5 min in 30 μL of $NaDodSO_4$ elution buffer [20 mM Tris-HCl, pH 9.0, 5% sodium dodecyl sulfate ($NaDodSO_4$; w/v), 5% β-ME, and 20% (v/v) glycerol]. After electrophoresis, stained gels were subjected to autofluorography (Bonner & Laskey, 1974) and then exposed to Kodak XR-1 film at −70 °C.

Tryptic Peptide Maps. Tryptic peptide mapping of in vivo labeled polypeptides isolated by $NaDodSO_4$–polyacrylamide gel electrophoresis was performed as described (Elder et al., 1977).

Results

Cystathionase Activity of Cys$^+$ *and Cys*$^-$ *Human Lymphoid Cell Lines.* Representative human lymphoid cell lines established from peripheral and/or bone marrow mononuclear cell pellets of patients with leukemia as well as of healthy donors were selected for analysis of enzyme activity. One group (SB, NC37, and LAZ 007) was composed of cells which grew as well in cysteine-depleted media containing 2×10^{-4} M L-cystathionine as in cysteine-containing medium. These cells were designated *cys*$^+$. The second group (SB2, CEM, and LAZ *221*) grew only in the latter medium and were designated *cys*$^-$.

Crude extracts of these lines were assayed for cystathionase activity under conditions in which product formation was linear with respect to time (Figure 1). The specific activity of the enzyme in three of the six cell lines was markedly reduced

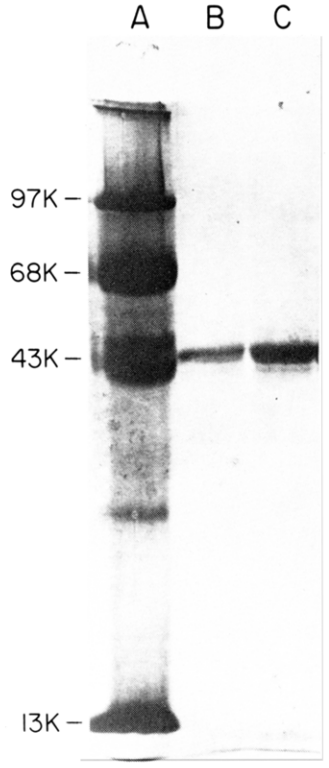

FIGURE 2: Polyacrylamide gel electrophoresis of purified human cystathionase. Human liver cystathionase was purified and subjected to electrophoresis in a 12% $NaDodSO_4$–polyacrylamide gel as described under Experimental Procedures. Slot A contains molecular weight standards, slot B, 15 μg of purified human cystathionase, and slot C, 25 μg of purified human cystathionase.

compared to that of the other three. Each of the cystathionase-depleted lines was shown, previously, to be a cysteine auxotroph (Iglehart et al., 1977; H. Lazarus, personal communication).

Characteristics of Rabbit Anti-Human Cystathionase Serum. The results of previous experiments demonstrated that, except for the marked differential in enzyme content, a number of properties of partially purified cystathionase from *cys*$^-$ or *cys*$^+$ cell lines were indistinguishable. Furthermore, no evidence for a dissociable inhibitor of the enzyme in *cys*$^-$ cells was found (Iglehart et al., 1977). We, therefore, wished to determine whether the reduction in cystathionase activity in the *cys*$^-$ cells which were cystathionase depleted could be attributed to a reduction in immunochemically detectable enzyme. To approach this question, we purified human cystathionase to ~95% purity as determined by gel electrophoresis (Figure 2). Anti-human cystathionase serum was raised against this fraction in a New Zealand white rabbit. This serum was used to precipitate purified, radioiodinated, human cystathionase. When the radioactive, immunoprecipitated protein present in the immune complex was subjected to gel electrophoresis and autoradiography, a single band of M_r 43 000 was detected (Figure 3). It comigrated with the purified enzyme. No band was observed when the iodinated human cystathionase was treated with rabbit preimmune (NRS) serum. Although these experiments were performed under conditions of antibody excess, we were unable to precipitate all of the radioiodinated, M_r 43 000 cystathionase present in the sample. We suggest that this loss of immunoreactivity was due to denaturation of the antigen during the radioiodination procedure. In keeping with this hypothesis, when a similar sample of unlabeled, purified human cystathionase was subjected to two-dimensional gel electrophoresis,

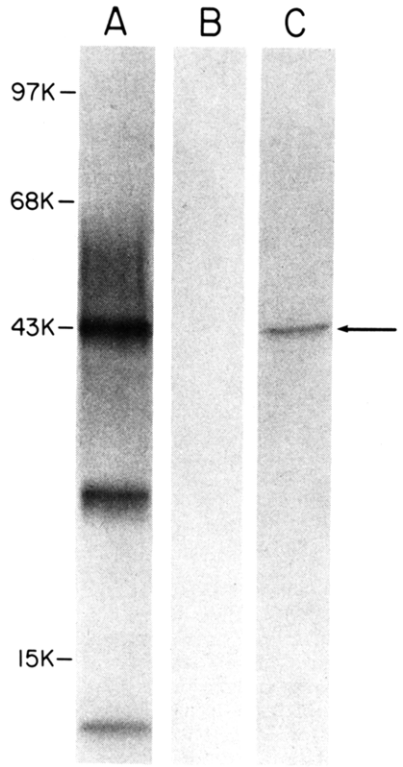

FIGURE 3: Immunoprecipitation of purified radioiodinated human cystathionase. An aliquot of purified human liver cystathionase, isolated as described under Experimental Procedures and represented in Figure 2, was radioiodinated, and identical aliquots were subsequently incubated with either rabbit anti-human cystathionase serum or preimmune rabbit serum and protein A–Sepharose beads, as described under Experimental Procedures. The washed, immune complexes were eluted, and the eluates were subjected to gel electrophoresis. The autoradiogram of the gel is shown above. (Slot A) Purified radioiodinated human cystathionase (5×10^4 cpm). (Slot B) An eluate of immune complexes formed with ^{125}I-labeled human cystathionase (5×10^4 cpm) and preimmune serum (15 μL). (Slot C) An eluate of immune complexes formed with ^{125}I-labeled human cystathionase (5×10^4 cpm) and anti-human cystathionase serum (15 μL). An excess (at least 15-fold) of anti-enzyme antibody was employed here. An arrow indicates the migration position of M_r 43 000 (43K) human liver cystathionase.

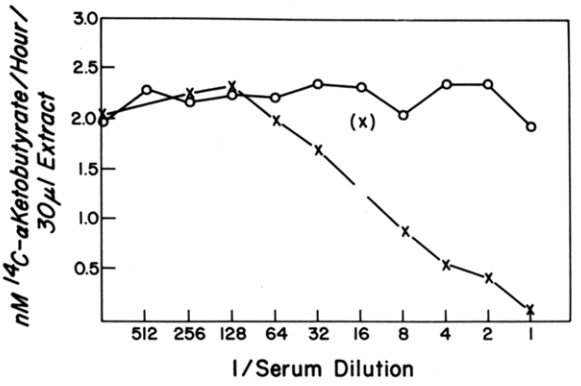

FIGURE 4: Inactivation of purified human liver cystathionase with anti-human liver cystathionase serum. Human liver cystathionase was purified as described under Experimental Procedures. Standard reaction mixtures (100 μL) were constituted, and 1.5 μg of enzyme were present. Increasing dilutions of serum (10 μL) were then added. The reaction mixtures were incubated for 1 h at 37 °C and then 48 h at 4 °C after which immune complexes were pelleted at 15000*g* and the supernatants (30 μL) removed and tested for cystathionase activity. The reaction was initiated by the addition of [1-^{14}C]cystathionine and allowed to proceed for 1 h at 37 °C. The α-[1-^{14}C]ketobutyrate formed was assessed by the method described under Experimental Procedures. Activity of residual human cystathionase incubated with anti-human cystathionase serum (X) and of human cystathionase treated with nonimmune serum (O).

a single spot of M_r 43 000 protein was observed (data not shown), suggesting that all of the M_r 43 000 protein in this fraction is cystathionase. When the radioiodinated protein was electrophoresed in parallel, two bands were detected, one of M_r 43 000 and a second of M_r 27 000. The significance of the latter band is discussed in a subsequent section.

The ability of rabbit immune serum to bind catalytically active human liver cystathionase was also examined. Figure 4 shows that the immune serum bound increasingly to the enzyme over a 128-fold range of concentrations. Comparable volumes of preimmune serum were inactive.

Immunoprecipitation of Radioactive Proteins from Cys$^+$ and Cys$^-$ Cell Lines with Anti-Cystathionase Antibody. An effort was made to determine whether the reduction in cystathionase activity in *cys*$^-$ cell lines was the result of a decreased cystathionase protein content. Once in the logarithmic phase of growth, replicate cultures of each line were labeled with [^{35}S]methionine, and equivalent amounts of newly synthesized protein from each cell line were tested for their content of cystathionase protein. By use of the immune serum described above, the presence of labeled cystathionase was assessed by immunoprecipitation and $NaDodSO_4$–polyacrylamide gel electrophoresis of the dissolved immunoprecipitates. Our results revealed the presence of a M_r 43 000 band comgrating with authentic, purified human liver cystathionase in eluates of anti-cystathionase immunoprecipitates from each of three *cys*$^+$ cell lines (SB, NC37, and LAZ 007) (Figures 5I–III, slot B of each). The total amount of cystathionase detected in these cells is quite small. For example, in a typical experiment with any of the lines, cystathionase constituted 0.002–0.004% of the radioactivity incorporated into newly synthesized protein during the incubation period. This was determined by comparative densitometric analysis of the immunoprecipitable M_r 43 000 cystathionase subunit bands with standard [^{35}S]methionine-labeled protein bands of known radioactive content. For these experiments, it was shown that the intensity of each radioautofluorographic image was directly proportional to its radioactive content.

Three *cys*$^-$ cell lines were also tested in parallel for their content of immunoreactive M_r 43 000 cystathionase. Aliquots of radioactive extracts of these lines were subjected to anti-cystathionase antibody or preimmune serum precipitation, and the autofluorograms of these experiments are also shown in Figure 5. As noted above, SB, NC37, and LAZ 007 (*cys*$^+$) cell extracts each contained a M_r 43 000 band. In contrast, SB2 (derived from the same individual as SB), CEM, and LAZ 221 (*cys*$^-$) extracts did not. A minimal estimate of the relative differences in cystathionase concentration in the *cys*$^+$ and *cys*$^-$ cell lines tested was obtained by densitometric analysis of the autofluorograms. In all experiments, the *cys*$^+$ cell lines contained at least 16–20 times more M_r 43 000 cystathionase than their *cys*$^-$ counterparts.

In all experiments, anti-cystathionase immunoprecipitates of those *cys*$^-$ and *cys*$^+$ cell lines tested were both found to contain a second minor band of M_r 27 000. This molecule was not precipitated by preimmune serum. In some experiments with both *cys*$^+$ and *cys*$^-$ cells, a M_r 22 000 band was observed in anti-cystathionase precipitates. In others, with the same reagents, it was absent while the M_r 43 000 and M_r 27 000 proteins were constantly present in unvarying amounts. In order to assess whether the constantly observed M_r 27 000 band might be structurally related to the M_r 43 000 cystathionase protein, we subjected these two polypeptides from NC37 cells to two-dimensional tryptic fingerprinting. Proteins were iso-

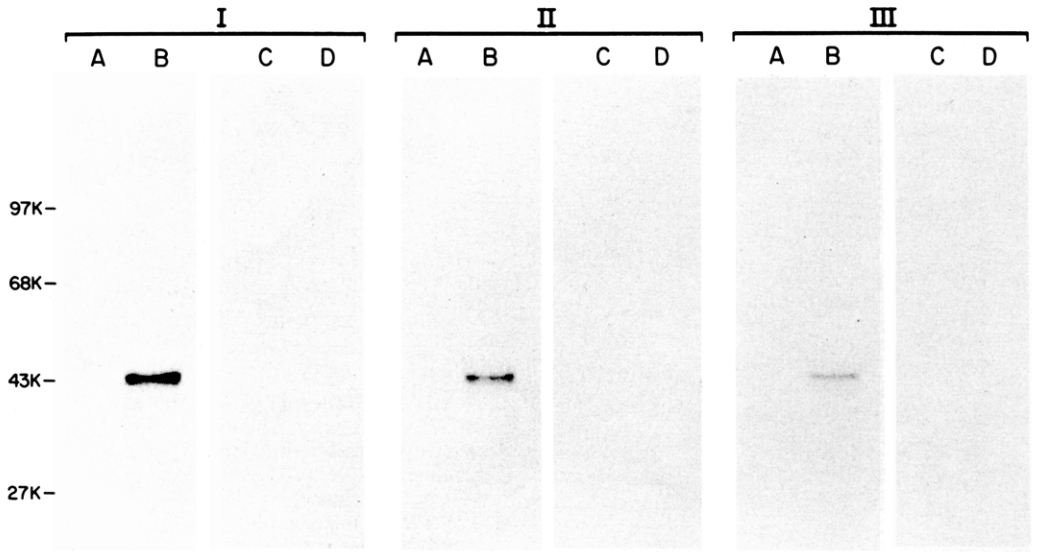

FIGURE 5: Immunoprecipitation analysis of intracellular cystathionase (M_r 43 000) content of whole-cell lysates derived from nonleukemic (*cys*$^+$) and leukemic (*cys*$^-$) cell lines. Pairs of human lymphoblast cell lines were seeded at 1×10^6 cells/mL and labeled for 4 h at 37 °C in the presence of [^{35}S]methionine (450 Ci/mmol, concentration 500 μCi/mL), and whole-cell lysates (1 mL) were prepared, as described under Experimental Procedures. Aliquots (3 μL) of these lysates were precipitated with trichloroacetic acid and counted, and equivalent quantities of radioactivity were treated with either anti-human cystathionase serum or preimmune serum also as described under Experimental Procedures. The following amounts of newly synthesized protein were incubated with serum: (I) SB and SB2, 2.4×10^7 cpm; (II) NC37 and CEM, 2.5×10^7 cpm; (III) LAZ 007 and LAZ 221, 1.7×10^7 cpm. Solubilized immune complexes were subjected to electrophoresis in a 12% $NaDodSO_4$–polyacrylamide gel. Labeled protein bands were detected by autofluorography. (I) Slots A and B, *SB* (*cys*$^+$) (slot A, preimmune serum precipitate; slot B, anti-cystathionase serum precipitate). Slots C and D, *SB2* (*cys*$^-$) (slot C, preimmune serum precipitate; slot D, anti-cystathionase serum precipitate). (II) Slots A and B, *NC37* (*cys*$^+$) (slot A, preimmune serum precipitate; slot B, anti-cystathionase serum precipitate). Slots C and D, *CEM* (*cys*$^-$) (slot C, preimmune serum precipitate; slot D, anti-cystathionase serum precipitate). (III) Slots A and B, *LAZ 007* (*cys*$^+$) (slot A, preimmune serum precipitate; slot B, anti-cystathionase serum precipitate). Slots C and D, *LAZ 221* (*cys*$^-$) (slot C, preimmune serum precipitate; slot D, anti-cystathionase serum precipitate).

lated by preparative immunoprecipitation from [^{35}S]-methionine-labeled cells. The peptide maps resulting from the digests of each protein alone and a 1:1 mixture of the two proteins are shown in Figure 6. The two maps are wholly dissimilar, suggesting totally different and unrelated original structures for the two proteins. The map of the digest of a mixture of the two proteins reveals a complete mixture of the two sets of peptides, indicating that trivial differences in the conditions of the tryptic digestions and/or electrophoresis and chromatography do not account for the differences in the maps. These results strongly suggest that the 27 000 molecular weight protein seen in these cell lines is not structurally related to cystathionase. Therefore, it is likely that this protein either cross-reacts immunologically with cystathionase or that the existing sera also contain antibodies against a minor contaminant present in the highly purified cystathionase fraction used for immunization of the rabbit. As mentioned earlier, a band of M_r 27 000 was observed in gels of radioiodinated preparations of purified human liver cystathionase (Figure 3). This band, however, was not observed in Coomassie blue stained gels of the same preparation, even when heavily loaded with M_r 43 000 cystathionase. Thus, it is possible that this M_r 27 000 species is the contaminant mentioned above. If this is the case, it is curious that the radioiodinated M_r 27 000 is not precipitated with immune serum. Perhaps the antigenicity is lost during the radioiodination procedure.

Discussion

In an attempt to understand the basis for the coordinate appearance of the leukemic phenotype and cysteine auxotrophy, we have performed a comparative analysis of a group of continuous human lymphoid, *cys*$^-$ cell lines with a leukemic phenotype and a series of *cys*$^+$ lines lacking this property. The available evidence indicates that the *cys*$^-$ lines are depleted in enzymatically active cystathionase relative to the *cys*$^+$ group and that this reduction in cystathionase enzyme activity may underlie the *cys*$^-$ phenotype (Iglehart et al., 1977). Thus, a reasonable hypothesis would suggest that *cys*$^+$ lines contain more intact cystathionase protein than their *cys*$^-$ counterparts. A test of this hypothesis was afforded by performing semiquantitative immunologic analyses of cystathionase protein in these various cell lines. The results of the experiments described here indicate that those *cys*$^-$ lines which are depleted in enzymatically active cystathionase are also depleted in intact M_r 43 000 cystathionase protein compared to their *cys*$^+$ counterparts. Densitometric analysis of these immunoprecipitation experiments reveals that there is at least 16–20 times more intact M_r 43 000 cystathionase in the *cys*$^+$ cell lines than in their *cys*$^-$ counterparts.

These results, of themselves, do not indicate whether the depletion in the concentration of the M_r 43 000 polypeptide is a result of synthesis or degradation, or both. However, more recent studies (L. M. Glode and D. M. Livingston, unpublished observations) suggest that there is a small but detectable amount of M_r 43 000 cystathionase in some *cys*$^-$ lines and that in one *cys*$^+$/*cys*$^-$ pair (NC37 and CEM) the turnover rate of the enzyme in the two lines was identical (~24 h). Thus, it appears that the relative cystathionase deficit in CEM cells is a result of a reduced rate of synthesis rather than an increased rate of degradation. In keeping with this contention, it has been observed that CEM and SB2 are also greatly depleted in cystathionase-specific mRNA compared to their *cys*$^+$ counterparts (Kriegler et al., 1980).

Although our results indicate a reduced concentration of intact enzyme in *cys*$^-$ cells, they do not eliminate the possibility that such cells synthesize abnormal cystathionase gene products. However, we have not observed polypeptides which cross-react with the M_r 43 000 protein and share large segments of their amino acid sequence with this protein. A M_r 27 000 protein was recognized by the anti-cystathionase serum

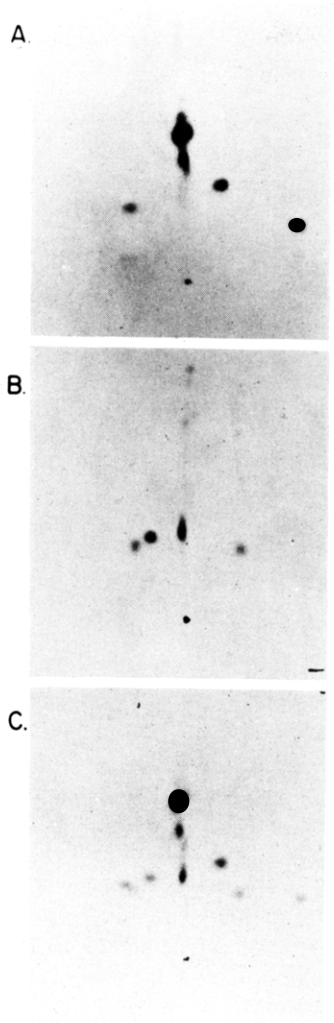

FIGURE 6: Tryptic peptide maps of ^{35}S-labeled, immunoprecipitated, M_r 43 000 and M_r 27 000 proteins from NC37 cells. Anti-human cystathionase precipitated M_r 43 000 and M_r 27 000 [^{35}S]-methionine-labeled bands from an extract of NC37 cells were individually excised, eluted, and digested with TPCK-trypsin, as noted under Experimental Procedures. (A) 5500 cpm of the M_r 43 000 polypeptide tryptic digest, (B) 5300 cpm of the M_r 27 000 polypeptide digest, and (C) 5500 cpm of a (1:1) mixture of the digests of the M_r 43 000 and M_r 27 000 polypeptides were analyzed as described under Experimental Procedures. Each film was exposed for 10 days.

in addition to the M_r 43 000 polypeptide. From the available tryptic peptide fingerprints, it appears that the two molecules are not products of the same DNA sequence read in the same frame. The appearance of a M_r 22 000 polypeptide in those samples treated with anti-cystathionase serum was an inconsistent finding.

A less well-defined but analogous situation to that observed from the *cys*$^+$ and *cys*$^-$ human cell lines has been observed by using a solution radioimmunoassay for murine cystathionase to measure the enzyme in nonleukemic and leukemic thymocytes from AKR mice and from mice inoculated with a thymotropic strain of murine type C virus (Livingston et al., 1976; Bikel et al., 1978). From those experiments, it was apparent that the antigenicity of cystathionase or a cystathionase cross-reactive protein was markedly altered after leukemogenesis. However, the results failed to discriminate between the antigenic modification of the normal complement of enzyme molecules, the synthesis of less antigenic but still cross-reactive cystathionase molecules instead of the normal enzyme, and a simple reduction in cystathionase content accompanied by synthesis of an unrelated but fortuitously cross-reacting molecule. The results presented here add to those observed in the mouse system by specifically assessing the relative intracellular concentration of the M_r 43 000 cystathionase subunit and the possibility of definable, cross-reactive proteins in well-characterized cell lines. They further point to a specific phenotypic alteration in cystathionase gene expression associated with the appearance of the leukemic phenotype in certain types of human leukemia cells.

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