ABSTRACT

Cytidine (CR) deaminase was purified 47,000-fold to homogeneity from human placenta. The molecular mass of CR deaminase was estimated to be 48.7 kDa by gel filtration and 16.1 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis, suggesting that it contains three or four identical subunits. We determined the amino acid sequence of several peptide fragments and designed 5'-primers to amplify, by the polymerase chain reaction, a specific 364-base pair DNA fragment using human liver complementary DNA (cDNA) as the template. This DNA fragment, which contains the codons of one peptide, was used as a probe to screen a cDNA library from human liver. We isolated and sequenced a cDNA clone of 910 base pairs that contained a 5' nontranslated region, a 438-base pair coding region, and a 3' nontranslated region with a polyadenylated tail. The translated region of the clone contained a deduced sequence of 146 amino acids, with a predicted molecular mass of 16.2 kDa and the sequences of our peptides. The cDNA was ligated in pGEX vector and expressed in Escherichia coli. The expressed protein had a high CR deaminase activity and molecular mass of 16.3 kDa. These data demonstrate clearly that the open reading frame of our cDNA clone codes for a functional human CR deaminase. Polymerase chain reaction amplifications of gene-specific DNA fragments from human/rodent hybrid cells indicate the localization of CR deaminase gene to human chromosome 1. The cDNA for CR deaminase will be a useful molecular probe to investigate the importance of this enzyme in chemotherapy.

INTRODUCTION

CR3 deaminase (EC 3.5.4.5) catalyzes the deamination of CR or CdR to UR or UdR, respectively (1). This enzyme can also catalyze the deamination of cytosine nucleoside analogues used as antileukemic drugs, such as ARA-C or 5-AZA-CdR (2–4). The deamination of ARA-C results in a significant loss of antineoplastic activity (5). The presence of high levels of CR deaminase in liver is responsible for the short half-life of CdR analogues in humans (6, 7). Several specific inhibitors of CR deaminase have been synthesized, and the kinetics have been investigated using deoxycytidine analogues as the substrate (8, 9). The CR deaminase inhibitor, THU, reduced the in vitro concentration of ARA-C required to produce 50% growth inhibition of KB cells (10). THU also increased ARA-CTP levels in leukemic cells from patients incubated in vitro with ARA-C (11). The CR deaminase inhibitors have the potential for clinical use since THU was shown to increase the concentration and half-life of ARA-C in patients with cancer (12).

CR deaminase may also be implicated in drug resistance to deoxycytidine analogues since leukemic cells with high levels of this enzyme have been detected at the time of relapse following therapy with these agents (13, 14). Treatment of myeloid cells with certain agents that induce differentiation of leukemic cells such as 5-AZA-CdR, vitamin D₃, or dimethyl sulfoxide produce an induction of CR deaminase activity (15, 16, 17). However, the genetic alterations responsible for the induction of the CR deaminase activity are unknown. Mature human granulocytes contain much higher levels of CR deaminase than leukemic myeloblasts (18). In order to investigate the genetic mechanisms that control the expression of CR deaminase, the gene for this enzyme should be isolated.

We purified the human CR deaminase from placenta and used the amino acid sequence to design primers for PCR cloning using the 3'-RACE protocol (19). We isolated and sequenced a cDNA clone for CR deaminase from a human liver library. Expression of this cDNA in Escherichia coli produced a protein with very high CR deaminase activity and molecular mass of predicted size. We have also localized the gene for human CR deaminase to chromosome 1 by PCR analysis of DNA from human/rodent cells.

MATERIALS AND METHODS

Materials. 5-[3H]CR and 5-AZA-CdR were obtained from Moravek Biochemicals, Inc. and Mack Co., respectively. Taq DNA polymerase and buffer, restriction enzymes, and random primed labeling kit were obtained from Boehringer Mannheim. α-[32P]dCTP was obtained from ICN Canada. Moloney murine leukemia virus reverse transcriptase was obtained from GIBCO/BRL. Oligonucleotides were synthesized at the Institut Armand-Frappier using Pharmacia Gene Assembler Plus instrument. The human liver cDNA library (ADR2) and human placenta and liver cDNA were obtained from Clontech. Molecular size markers (1000, 700, 500, 400, 300, 200, 100, and 50 base pairs) for PCR were obtained from Research Genetics. All other chemicals were ultrapure grade.

Enzyme Assay. CR deaminase activity was determined by either radiochemical or spectrophotometric assay, as described previously with slight modifications (9, 15). For fractions IV to VII, the assay was performed in the presence of 1 mg/mL of BSA to stabilize the enzyme. The spectrophotometric assay was performed in 50 mM Tris-HCl (pH 7.4) and 100 μM CR with diluted enzyme. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the deamination of 1 nmol cytidine/min at 37°C under the above conditions.

CR Deaminase Purification. All initial purification steps were performed at 4°C, unless indicated otherwise. Human placenta (700 g) was homogenized in 20 mM Tris-HCl (pH 7.5), 5 mM KCl, and 1 mM DTT. Streptomycin sulfate and phenylmethylsulfonyl fluoride were added to the homogenate to a final concentration of 0.5% and 0.5 mM, respectively, before centrifugation at 20,000 × g for 30 min (fraction I). CR deaminase was precipitated by ammonium sulfate between 40 and 55% of saturation. The precipitate was resuspended in 20 mM Tris-HCl (pH 7.5), 5 mM DTT, and 1 mM phenylmethylsulfonyl fluoride (fraction II). Aliquots (50 mL) of fraction II were incubated in a 75°C water bath for 12 min and rapidly cooled. Supernatant was recovered after centrifugation at 25,000 × g for 30 min and concentrated (Amicon Centriprep-30) to give fraction III. Fractions III to VI were purified by column chromatography using the fast protein liquid chromatography system (Pharmacia) and performed at room temperature. Column chromatography consisted of Sephacryl S-200 gel filtration, PAE-1000 ion exchange, and Mono-Q ion exchange chromatography at pH 6.2 and 7.5 (Fig. 1). The final step used in column chromatography yielded a single peak of CR deaminase activity to
pmol) was diluted and concentrated several times in 20 mM KH2PO4-20 mM (Amersham). Proteins were stained with Coomassie blue or silver.

determined by SDS-PAGE as described by Laemmli (21) using 12 or 15% gels containing ethidium bromide. The purity of the enzyme and the molecular mass of its subunits were determined by SDS-PAGE as described by Laemmli (21) using 12 or 15% acrylamide and the Mini Protean II apparatus (Bio-Rad; gel size, 7 x 8 x 0.75 cm). Markers were GIBCO low molecular mass and Rainbow standards markers (Amersham). Proteins were stained with Coomassie blue or silver.

**Amino Acid Composition and Peptide Sequencing.** Fraction VII (250 pmol) was diluted and concentrated several times in 20 mM KH2PO4-20 mM KCl using Centrifree to exchange the buffer. The amino acid composition was determined using an Alpha Plus amino acid analyzer. For determination of the cysteine content, the protein was oxidized with performic acid. Peptides were generated by Cbostripain digestion or by mild acid cleavage and were either directly purified or reduced and alkylated before purification by reverse phase high performance liquid chromatography (Applied Biosystems; 130A separation system) using a Brownlee C8-RP300, 7 µm column (2.1 x 30 mm) with a linear gradient of 0—70% acetonitrile in 0.085% trifluoroacetic acid. The purified peptides were submitted to automated Edman degradation in a Porton Macia A.L.F. automatic sequencer.

**cDNA Synthesis.** HL-60 human myeloid leukemic cells were treated with 5-AZA-CdR (1 µM for 72 h), and total RNA was isolated by the method of Chomczynski and Sacchi (22) using a kit from Promega. The 5-AZA-CdR treatment produced induction of CR deaminase activity in these leukemic cells (15). cDNA synthesis (1 h at 42°C) was performed on 2 µg of total RNA using oligo(dT)15 anchored primer (Boehringer Mannheim).

**DNA Sequencing.** The PCR product was purified using Magic PCR Preps DNA Purification System (Promega), following an agarose gel electrophoresis, and cloned in pCRII plasmid using the TA Cloning kit (Invitrogen). The insert was sequenced using Sequenase 2.0 kit (USB). pDR2-1 was sequenced in both orientations by the chain termination method using fluoro-dATP and a Pharmacia A.L.F. automatic sequencer.

**Synthesis of DNA Probe and Screening cDNA Library.** The DNA insert from pCRII plasmid containing the partial sequence for CR deaminase was used to design the exact sequence 5'- and 3'-primers (P4 and P5; Fig. 5). These primers were used with human liver cDNA to amplify a specific DNA probe, which was radioabeled using random oligonucleotide priming (24). The radioactive probe was purified through a 0-50 Sephadex spin column and cloned in pCRII plasmid containing the partial sequence for CR deaminase cDNA (Fig. 5) was performed at an annealing temperature of 58°C. Amplified products were separated electrophoretically on 2% agarose gels containing ethidium bromide.

**cDNA Amplification.** The PCR 5'-primers (P1 to P3) for CR deaminase were designed from the amino sequence of peptide E (Fig. 3) using optimum codon choice (23). The sequences of these primers were: P1, GGCACCAACT-GGCCTGTCTACATGAC (nucleotides 451-476; Fig. 5); P2, GTCTAAGG-GACAGACCTGATG (nucleotides 466-488; Fig. 5); and P3, GAGCTC-GATGCACCTACATTGATG (nucleotides 478-504, Fig. 5). The reaction mixture (50 µl) contained 5 µl of 10X PCR buffer, 200 µM of each deoxynucleotide triphosphate, 50 pmol of each primer, and 2 µl of cDNA from HL-60 cells or 50 µg of cDNA from human placenta or human liver. Amplification, using oligo(dT)15 containing a XbaI linker (5'-GGCTTAGAGGCTGAGCTA-3') as a 3'-primer, was initiated after a hot start by the addition of 1.25 units of Taq DNA polymerase to the reaction mixture. Amplification consisted of 20—35 cycles using the following protocol: denaturation for 30 s at 95°C, annealing for 30 s at 42°C or 45°C, elongation for 1 min at 72°C, and a final incubation for 7 min at 72°C. PCR amplification using the sense primer (P4) and antisense primer (P5) with exact sequence for the human CR deaminase (Fig. 5) was performed at an annealing temperature of 58°C. Amplified products were separated electrophoretically on 2% agarose gels containing ethidium bromide.

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A human liver cDNA library in ADR2 phage (Clontech) was screened with the radiolabeled DNA probe. Plaques (1 x 10^5) were blotted on nylon filters (Hybond-N; Amersham). The filters were prehybridized in 6X SSC, 2X Denhart's solution, and 1% SDS at 68°C for 2 h. The denatured 32P-labeled probe was added to the hybridization solution to give 2 x 10^6 cpm/ml. The hybridization was performed overnight at 68°C. The filters were washed once at room temperature for 15 min with 2X SSC/0.1% SDS and then twice with 0.2X SSC/0.1% SDS at 58°C for 15 min. The filters were wrapped and exposed to Fuji X-ray films with intensifying screen at -70°C. One positive clone was isolated and converted to pDR2 plasmid using the method of clone was isolated and converted to pDR2 plasmid using the method of

Expression of CR Deaminase in E. coli Using pGEX. The coding region (nucleotides 124–561; Fig. 5) of the CR deaminase cDNA was amplified by PCR technique using a 5'-primer (P0) containing BamHI restriction sites (ACGGGATCCATGGCCAGAACGCTCTTG) and a 3'-primer (P7) with a XhoI site (CCTGCTGAGTCTACTGAGTCCTTGGAAG). The amplified DNA was subcloned into vector pGEX-4T-1 (Pharmacia) and transformed into DH5a competent E. coli. The pGEX-4T-1 vector alone was used as a control for GST production. The fusion protein (GST-CR deaminase) was induced with isopropyl-β-D-thiogalactoside and purified using glutathione affinity chromatography column from the GST Purification Module (Pharmacia).

Chromosomal Localization. Genomic DNA from 24 human/rodent cell lines (Coriell; mapping panel no. 2) and from human, mouse, and hamster cell lines was screened for the presence of CR deaminase gene using PCR. The reaction mixture (50 μl) contained 150 ng DNA, 0.2 μM of sense primer AATGCCCAGCTGCTGAAAGCCAC (nucleotides 574–598; Fig. 5), and antisense primer P5 (nucleotides 842–865; Fig. 5) of CR deaminase cDNA 3'-nontranslated region, PCR reaction mixture, and 1.25 units of Taq DNA polymerase (Promega). The first three cycles consisted of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. This was followed by 27 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The reaction products were analyzed on 2% agarose gel and stained with ethidium bromide. Based on the primers chosen for PCR, the predicted size of amplified DNA was 292 base pairs (nucleotides 574–865; Fig. 5).

Northern Blotting. Northern blot of poly(A) RNA from different human and rodent cell lines (Clontech) was hybridized with the cDNA for human liver CR deaminase. The cDNA was radiolabeled using a reaction mixture containing 20 pmol of antisense primer CTCCGCTGCTCAGCTGGAAGCTTCTCTTG (nucleotides 550–573; Fig. 5), denatured 2.4 μg pBluescript containing CR deaminase cDNA, dATP, dGTP, and α-32P-dCTP, and 2.5 units Klenow DNA polymerase (Promega). The probe was added to the hybridization solution to give 2 X 106 cpm/ml. The filters were wrapped and exposed to Fuji X-ray films with intensifying screen at -70°C. One positive clone was isolated and converted to pDR2 plasmid using the method of...
cDNA CLONING AND EXPRESSION OF HUMAN CR DEAMINASE

A few disagreements were found mostly at the positions of the cysteine (C) and the lysine (K) residues.

PCR Amplification of a Nucleic Sequence Specific to the Human CR Deaminase. The amino acid sequence of peptide E (Fig. 3) was used to design nested primers for PCR amplification of specific sequences of CR deaminase using the method of Lathe (23) for the selection of the optimal codons. The 5'-primers P1, P2, and P3 were used in combination with the oligo(dT)19 3'-primer, according to a modified 3'-RACE protocol (19). We used DNA template from human placenta or liver. In addition, we used cDNA isolated from human HL-60 leukemic cells after treatment with 5-AZA-CdR, an agent which induces CR deaminase in these cells (15).

The first PCR amplification using primers P1 and oligo(dT)19 did not produce specific DNA bands (Fig. 4, A-C). An aliquot of this reaction mixture was used in a second PCR amplification with nested primer P2 and oligo(dT)19 and produced a DNA product of about 470 base pairs in each group. A third PCR was performed using an aliquot of the reaction mixture from the second amplification with the nested primer P3 and oligo(dT)19 and showed specific DNA product of about 460 base pairs. The difference in size of the amplified DNA, 10 base pairs, was predicted by the position of the codons for these primers in peptide E. HL-60 cells that were not treated with 5-AZA-CdR did not show a specific DNA band after the third PCR.

The 470 base pairs PCR product obtained with primer P2, oligo(dT)19, and cDNA from HL-60 cells (Fig. 4C) was purified and cloned in pCRII plasmid. The DNA insert was partially sequenced from the 5'-end and contained the codons for peptide E, additional codons for amino acids up to the stop codon, and 3'-nontranslated sequence (nucleotides 489—600; Fig. 5). The partial sequence obtained from the 3'-end contained part of the 3'-nontranslated region and the poly(A)+ tail (nucleotides 827—916; Fig. 5). These data indicated that Peptide E was at the COOH-terminal end of CR deaminase. The DNA sequence of the insert was used to design 5' and 3'-primers (P4 and P5) with the exact nucleotide sequence (Fig. 5). These primers were used to amplify by PCR a specific DNA probe (364 base pairs) from liver cDNA.

Isolation of a Human Cytidine Deaminase cDNA Clone. The DNA probe obtained with primer P2, oligo(dT)19, and cDNA from HL-60 cells (Fig. 4C) was purified and cloned in pCRII plasmid. The DNA insert was partially sequenced from the 5'-end and contained the codons for peptide E, additional codons for amino acids up to the stop codon, and 3'-nontranslated sequence (nucleotides 489—600; Fig. 5). The partial sequence obtained from the 3'-end contained part of the 3'-nontranslated region and the poly(A)+ tail (nucleotides 827—916; Fig. 5). These data indicated that Peptide E was at the COOH-terminal end of CR deaminase. The DNA sequence of the insert was used to design 5' and 3'-primers (P4 and P5) with the exact nucleotide sequence (Fig. 5). These primers were used to amplify by PCR a specific DNA probe (364 base pairs) from liver cDNA.

Table 2 Amino acid composition of human placental CR deaminase

<table>
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<th>Amino Acid</th>
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<tr>
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</tr>
<tr>
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<td>9</td>
</tr>
<tr>
<td>Cys</td>
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<tr>
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<tr>
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<td>Tyr</td>
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</tr>
<tr>
<td>16.1 kDa</td>
<td></td>
<td>16.2 kDa</td>
</tr>
</tbody>
</table>

a All calculations based on a molecular mass of 16.1 kDa.
b Determined as cysteic acid after performic acid oxidation.
c Values were extrapolated to zero time of hydrolysis.

Table 2 Amino acid composition of human placental CR deaminase

CR deaminase. A few disagreements were found mostly at the positions of the cysteine (C) and the lysine (K) residues.

Fig. 3. Deduced amino acid sequence of cDNA clone pDR2—1 for human liver CR deaminase. The open reading frame of pDR2—1 cDNA encodes for a 146-amino acid protein. In boxes, determined amino acid sequence of purified peptide fragments from human placenta CR deaminase. Letters under boxes, amino acids in disagreement with deduced sequence or due to weak signals during experimental analysis. X, unknown residue.

Fig. 4. Analysis of PCR products from 3'-RACE protocol by agarose gel electrophoresis. Sense nested primers P1, P2, and P3 designed from the codons of peptide E were used for the first, second, and third PCR, respectively, in combination with antisense oligo(dT). The template for the first PCR was cDNA from liver (A), placenta (B), and HL-60 cells treated with 5-AZA-CdR (C). Aliquots from first and second PCR were used as templates for second and third PCR, respectively. M, DNA size markers.
plaque. After conversion of the clone to a plasmid (pDR2-1), the insert size was estimated to be approximately 950 base pairs and was sequenced in both orientations (Fig. 5). The 910-base pair clone ending with a poly(A) tail. The translated region coded 146 amino acids, predicting a molecular mass of 16.3 kDa as estimated by SDS-PAGE (Fig. 6, Lane 3).

Expression of CR Deaminase in E. coli Using pGEX Vector. The cDNA of the clone pDR2-1 was ligated into pGEX-4T-1 vector. The nucleotide sequence of pDR2-1 (upper line) and deduced 146 amino acid sequence (lower line). Nucleotides in the open reading frame are capitalized. The 5'-terminal BamHI site and 3'-terminal XbaI site of pDR2—1 are in italic letters. The DNA probe to screen the cDNA library was synthesized by PCR using sense P4 primer (nucleotides 842-865) with human liver cDNA.

Fig. 5. Nucleic acid and deduced protein sequence of human liver CR deaminase. The nucleotide sequence reported in this paper has been submitted to the GenBank with accession number L27943.

Chromosomal Localization of Human CR Deaminase. Genomic DNA from human, mouse, and hamster cell lines and from human/rodent hybrid cell lines was screened for the presence of the CR deaminase gene by PCR. A single DNA band of 292 base pairs as predicted by the location of the primers in the 3'-nontranslated region of CR deaminase cDNA (Fig. 5) was detected in the human cell line and in rodent/human hybrid cell line GM/NA07299 (Fig. 7). This rodent/human cell line contains human chromosomes 1 and X. No bands were detected in the mouse and hamster cell lines and in rodent/human hybrid cell lines that do not contain human chromosome 1. The CR deaminase gene, therefore, appears to be localized on human chromosome 1. This assignment was confirmed with a second human chromosome mapping panel (Bios Laboratories) using the PCR method.5

Expression of CR Deaminase in Human Tissues. A Northern blot of poly(A)+ RNA from different human tissues when hybridized with radiolabeled CR deaminase cDNA showed a single band at about 1 kilobase for both placenta and liver (Fig. 8). The mRNA species detected are similar in size to the cDNA of CR deaminase (Fig. 5). Human liver was reported to contain high levels of CR deaminase activity (3). We choose human placenta to purify CR deaminase, since it contained significant levels of this enzyme. We did not detect the presence of mRNA for CR deaminase in heart, brain, and muscle, which correlated with the very low activity of this enzyme in these tissues as reported by Ho (3).

DISCUSSION

CR deaminase catalyzes the deamination of cytosine nucleosides and their respective analogues. This enzyme plays a major role in the metabolism of cytosine nucleoside analogues used in the therapy of leukemia since their deamination results in a loss of activity (2, 4, 5).
cDNA CLONING AND EXPRESSION OF HUMAN CR DEAMINASE

Fig. 7. Assignment of human CR deaminase gene to chromosome 1. Genomic DNA from human, mouse, and hamster cell lines and rodent/human hybrid cell lines was screened by PCR for the presence of CR deaminase gene using specific primers to amplify a 292-base pair fragment from the 3'-nontranslated region of its cDNA. The products of the reaction were subjected to agarose gel electrophoresis and stained with ethidium bromide. DNA from cell lines and from rodent/human hybrid cell lines are presented with their assigned human chromosome. M, DNA size markers; numbers and letters, specific human chromosomes. Cell hybrid 1X contains both chromosomes 1 and X.

Steuart and Burke (13) demonstrated that leukemic cells from patients that responded to ARA-C therapy contained six times less CR deaminase than found in leukemic cells from nonresponders. These results suggest that drug resistance to ARA-C in some patients may be related to the activity of CR deaminase. Our laboratory observed that in a patient with acute myeloid leukemia that relapsed after therapy with ARA-C, following treatment with 5-AZA-CdR there was a 9-fold increase in CR deaminase in the leukemic cells as compared to the analysis before therapy (14). The level of the CR deaminase in leukemic cells before treatment may be one of the parameters that determines the response to the therapy. In order to understand the genetic mechanisms that control the expression of CR deaminase, we have cloned its cDNA. In addition, a method for detection of the mRNA for this enzyme may be a useful tool to evaluate the response in leukemic patients to cytosine analogues.

We have purified 47,000-fold the human CR deaminase from placenta (Table 1; Fig. 1). SDS-PAGE analysis of the enzyme after the final step of purification showed that it was homogeneous and had a molecular mass of 16.1 kDa (Fig. 2). Since gel filtration of the placenta enzyme showed a molecular mass of 48.7 kDa, the enzyme is composed of three or four identical subunits. Molecular masses of 51 kDa and 49 kDa, estimated by gel filtration, were reported for CR deaminase from human granulocytes and from human acute myeloblastic leukemia cells, respectively (10, 18). Cacciamani et al. (25) also purified the human CR deaminase from human placenta and reported a molecular mass of 52 kDa. However, the amino acid composition of their enzyme is different from our values obtained by chemical analysis and from the codons of our cDNA sequence. These differences could be due to a genetic variant form of the enzyme or due to the presence of trace proteins in their enzyme preparation.

We used a modified 3'-RACE protocol of Frohman (19) to isolate the cDNA for the human CR deaminase. The amino acid sequence of peptide E from the enzyme was used to design 5'-oligonucleotide primers by selection of the preferred codons as described by Lathe (23). Using cDNA from human liver, placenta, and HL-60 leukemic cells (treated with 5-AZA-CdR to induce CR deaminase) and a series of nested 5'-primers, we amplified a DNA band of identical size for the three cell types, indicating specificity of the PCR. Determination of the sequence of this DNA showed that it contained the codons of peptide E from CR deaminase and additional sequence data. This correct nucleotide sequence was used to design specific primers for PCR amplification using human liver cDNA as the template of a specific DNA probe (364 base pairs) to be used to screen a cDNA library.

Over 10^6 plaque-forming units from human liver ADR2 cDNA library were screened with the specific 364 base pair DNA probe to obtain one positive clone, pDR2-1. We obtained the DNA sequence for this enzyme may be a useful tool to evaluate the response in leukemic patients to cytosine analogues.
of the clone and demonstrated that it contained a 5'-nontranslated region, an open reading frame of 438 base pair with an initiation Met, and 3'-nontranslated region (Fig. 5). In the 5'-region, two Met codons at positions 23 and 124 can serve as the initiation codon. The first Met will translate a short peptide of 43 amino acids, and the corresponding sequence does not agree with the peptide sequence from the protein. The second Met has a consensus sequence for initiation proposed by Kozak (26) and the deduced sequence of 146 amino acids predicted a molecular mass of 16.2 kDa, which agrees with the mass of the subunit of the purified protein (Fig. 2, Lane 4). In the deduced 146-amino acid sequence of the CR deaminase cDNA, we identified all of the peptide fragments in which we determined the mRNA in liver and placenta, organs that contain significant levels of this enzyme, and a molecular size similar to our cDNA (Fig. 8).

By screening a subtractive library, Kühn et al. (27) isolated an incomplete cdNA sequence for CR deaminase from U937 monocyteoid leukemic cells. However, they are missing the 120 base pairs from the 5'-nontranslated region and the initial methionine of our protein sequence (nucleotides 7–126; Fig. 5). In addition, part of the 3'-nontranslated including the poly(A)' tail (nucleotides 862–916) were also absent in the U937 cdNA sequence. The homology of the coding region of their cdNA and their deduced amino acid sequence was 99% identical to our sequences.

The liver cdNA when cloned in pGEX expression vector produced, after thrombin cleavage, a protein with a molecular mass of 16.3 kDa, which is almost identical as deduced from the coding region and from the subunit of the purified protein (Fig. 2, Lane 4; Fig. 6, Lane 3). In addition, the 16.3 kDa protein had a high specific activity, similar to homogeneous enzyme from placenta (Table 1, fraction VII). This expression system in pGEX vector can be used to produce large quantities of the pure CR deaminase protein for antibody production. The purification of the pure enzyme from tissues gives a yield that is too low. The antibodies can be used as a diagnostic tool to detect the presence of CR deaminase in cells from patients. The cloning of the cdNA for the human CR deaminase will provide the molecular probes to investigate the importance of this enzyme in the therapy of leukemia with cytosine nucleoside analogues. Preliminary data indicate that there are differences in the rate of deamination of ARA-C in patients with leukemia (28).

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Human Cytidine Deaminase: Purification of Enzyme, Cloning, and Expression of Its Complementary DNA

Josée Laliberté and Richard L. Momparler


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