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ISOLATION OF HUMAN LIVER ARGINASE cDNA AND DEMONSTRATION OF NONHOMOLOGY BETWEEN THE TWO HUMAN ARGINASE GENES

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A human liver cDNA library was screened by colony hybridization with a rat liver arginase cDNA. The number of positive clones detected was in agreement with the estimated abundance of arginase message in liver, and the identities of several of these clones were verified by hybrid-select translation, immunoprecipitation, and competition by purified arginase. The largest of these human liver arginase cDNAs was then used to detect arginase message on northern blots at levels consistent with the activities of liver arginase in the tissues and cells studied. The absence of a hybridization signal with mRNA from a cell line expressing only human kidney arginase demonstrated the lack of homology between the two human arginase genes and indicated considerable evolutionary divergence between these two loci.

Arginase (L-arginine-urea hydrolase; EC 3.5.3.1) is the final enzyme of the mammalian urea cycle and catalyzes the hydrolysis of arginine to urea and ornithine. Studies in rats and normal man have demonstrated that the predominant enzyme species found in liver and kidney (AI and AII, respectively) are two distinct, but kinetically identical, proteins that seem to be coded by separate genes (1,2,3,4). It is the product of the AI locus which is lacking in patients with clinical hyperargininemia, while in these cases the AII protein is present at normal or augmented levels (4).

As a first step in studying the regulation of expression of these two arginase loci, we cloned the liver arginase gene from rat and showed that it cross-hybridizes, albeit to a limited degree, with the corresponding message from human liver (5). In order to expand this work to both normal human and arginase-deficient patient studies, we proceeded to clone the human gene, and report here the isolation of near full-length cDNA clones for human liver ar-

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ginase. In addition, we found no homology between the coding regions of the two forms of human arginase.

MATERIALS AND METHODS

Colony Hybridization. A human liver-derived cDNA library, obtained from Stuart Orkin (6), was screened for human liver arginase clones by colony hybridization (7). This library was probed with the rat liver arginase cDNA insert from our recombinant clone 3B1 (5), ^{32}P-labeled by random priming (8). Hybridizing clones were picked, subcloned, and subjected to a second round of screening.

Verification of Human Liver Arginase cDNA-Containing Clones. The sizes of cDNA inserts from clones selected by colony hybridization were determined by agarose gel electrophoresis of the PstI-digested recombinant plasmids (9). The shared identity of all these inserts was then determined by probing a Southern blot of the sizing gel with the largest of these inserts labeled with ^{32}P (10). The ability of this same cDNA to hybrid-select human liver arginase-specific mRNA was determined by previously described methods for RNA isolation, hybridization, in vitro translation, antibody production and immunoprecipitation, and SDS-polyacrylamide gel electrophoresis and fluorography (5).

Cell Culture. The continuous rat hepatoma line H4 (11) was cultured and induced with hydrocortisone, and arginase-specific activities were determined as previously described (12). Human embryonic kidney (HEK) cells transformed with adenovirus 5 were also maintained as described before (12,13).

Northern Blot Analysis. RNA was electrophoresed, blotted, and hybridized as previously described (5), except that the human liver arginase cDNA described herein and labeled with ^{32}P was used as probe.

RESULTS AND DISCUSSION

Approximately 60,000 colonies out of a 141,000-member human liver cDNA library in plasmid pKT218 (6) were screened by colony hybridization using a ^{32}P-labeled rat liver arginase cDNA probe. Sixty-eight positive colonies were identified, which is in agreement with the 0.1 percent abundance of arginase in liver, inferred from both purification and in vitro protein synthesis studies (unpublished observations). A second round of screening was performed on 40 of these colonies, and 37 again proved to be positive. Fig. 1 is an agarose gel demonstrating that the cDNA inserts from 22 of these clones approach a size limit of about 1550 bp, which corresponds to a near full-length representation of the 1700 base mRNA for arginase detected in human liver (5). This gel was then blotted to nitrocellulose, and one of the largest of the inserts (from recombinant plasmid p6B shown in lane 5) was labeled with ^{32}P and used to probe the filter. All the inserts hybridized to the probe (not shown), which indicates that they have a shared identity and that the rat

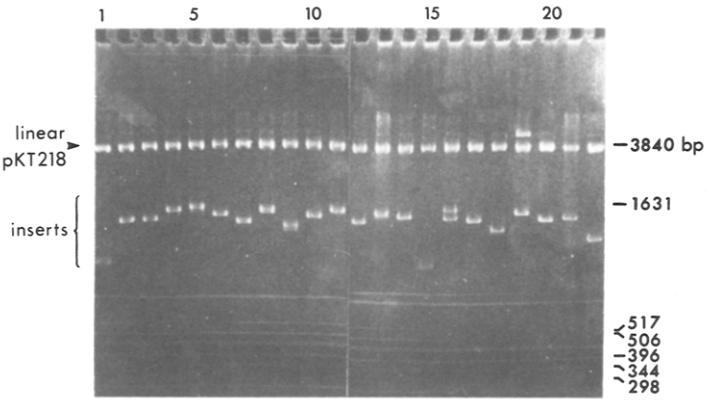

Fig. 1. Agarose gel to determine the sizes of cDNA inserts. A human liver-derived cDNA library was subjected to two rounds of screening by colony hybridization with a rat liver arginase cDNA probe. To determine the sizes of the cDNA inserts, the isolated recombinant plasmids from 22 of the positive clones were digested with PstI, electrophoresed through a 1.5% agarose gel, and visualized with ethidium bromide.

liver arginase probe used in the colony hybridization selected a unique population of human cDNAs.

Confirmation that these clones code for human liver arginase was achieved by hybrid-select translation assays. Fig. 2 shows the results of one such experiment involving clone 6B. The products of an in vitro translation of poly(A)$^+$ RNA from human liver performed in a rabbit reticulocyte lysate with added L-[^{35}S]methionine are shown in lane 1. A portion of this same translation lysate was then immunoprecipitated with rabbit anti-rat liver arginase serum, which cross-reacts with the human enzyme. The arrow at the left indicates the position of human liver arginase, which is seen in lane 2 among the immunoprecipitation products. This band is specifically absent from lane 3 where purified, unlabeled arginase was added as an immunological competitor. Lane 4 shows the translation products of a reaction primed with mRNA eluted from a filter to which p6B had been bound. A prominent band is present at the position of the arginase subunit. Lanes 5 and 6 show that this band is also precipitable with anti-arginase serum, and lane 7 confirms the specific inhibition of this precipitation by cold arginase. Comparable results were obtained for several of the putative human arginase clones using several anti-arginase sera. Some of these same antisera have also been used to immu-

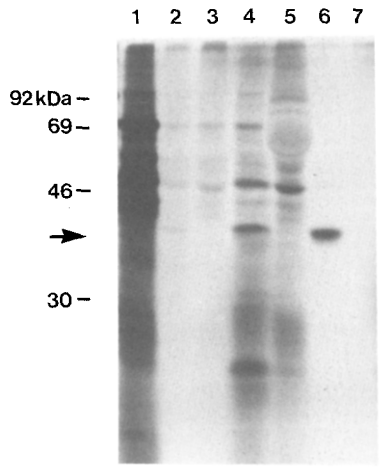

Fig. 2. Translation of human liver mRNA hybrid-selected with plasmid DNA from recombinant clone 6B. Plasmid DNA from clone 6B (p6B) was isolated, denatured, and bound to a nitrocellulose filter. This filter was incubated at 50°C for 3 h with 150 µg/ml of human liver poly(A)$^+$ RNA under hybridization conditions and then washed at 65°C. The bound RNA was eluted by boiling for 1 min. After phenol extraction the RNA was translated in 10 µl of reticulocyte lysate translation mixture in the presence of L-[^{35}S]methionine. One-third of the hybrid-select translation was electrophoresed on a 10% polyacrylamide gel to demonstrate total immunoprecipitation products. Of the remaining two-thirds, half was immunoprecipitated with anti-arginase serum, and the immunoprecipitate and its supernatant were electrophoresed. The remainder of the lysate (a volume equal to that just immunoprecipitated) was immunoprecipitated with anti-arginase serum in the presence of 3.6 µg of added purified rat liver arginase. Such a competition was also performed to compare anti-arginase immunoprecipitations from the translation products of unprocessed (i.e., total) human liver mRNA. Lane 1 contained the translation products of 100 ng of human liver poly(A)$^+$ RNA. The anti-arginase immunoprecipitate of the translation products directed by 200 ng of this same poly(A)$^+$ RNA was run in lane 2. Lane 3 showed the competition between 3.6 µg of pure rat arginase and the arginase produced in the translation of another 200 ng of poly(A)$^+$ RNA from human liver for anti-arginase immunoprecipitation. Lane 4 contained one-third of the translation lysate directed by the RNA hybrid-selected by recombinant 6B. Of the remaining two-thirds of this lysate, half was immunoprecipitated with anti-arginase serum (lane 6); and the other half was similarly immunoprecipitated, but with 3.6 µg of cold arginase added as competition (lane 7). The supernatant from the immunoprecipitation for lane 6 was run in lane 5. Distortion seen around 69 kDa in this lane was due to albumin in the antiserum.

noprecipitate liver arginase from normal humans and to demonstrate the lack of detectable arginase protein in hyperargininemic patients (4,14).

The cDNA insert from clone 6B was then used to detect homologous mRNA derived from different sources and separated by electrophoresis. Fig. 3a shows that a unique species of approximately 1700 bases is present in human liver. This band corresponds to the message previously detected in human and rat liver by northern analysis using rat liver arginase probes (5,15). In contrast, very little if any signal is detected in lane 7, which contained 20

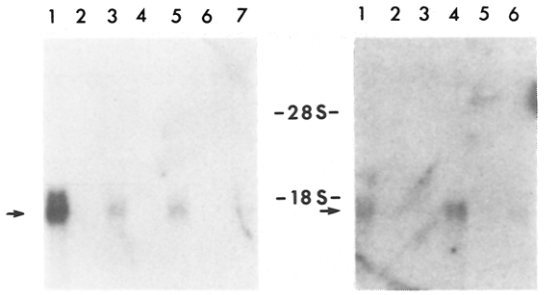

Fig. 3. Size and relative abundance of liver arginase mRNA. Poly(A)$^+$ RNA from human and rat liver and cell lines were denatured in a formaldehyde/formamide-containing buffer, electrophoresed on a 1.25% formaldehyde agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled cDNA from recombinant 6B. Fig. 3a was autoradiographed for 3 h with a single Du Pont Cronex Quanta II intensifying screen, and Fig. 3b was similarly autoradiographed, but for 6 h. 3a) Lane 1 contained 3 µg of poly(A)$^+$ RNA from human liver, and lanes 3,4 and 5 contained, respectively, 1 µg, 0.1 µg, and 1 µg of this same RNA. Lane 7 contained 20 µg of poly(A)$^+$ RNA from HEK cells. Lanes 2 and 6 were empty. 3b) Lane 1 contained 3 µg of poly(A)$^+$ RNA from rat liver, and lanes 2 and 3 each contained 1 µg of this same RNA. Lanes 4 and 5 contained, respectively, 3 µg and 1 µg of poly(A)$^+$ RNA from H4 rat hepatoma cells induced 72 h with hydrocortisone. Lane 6 contained 5 µg of poly(A)$^+$ RNA from uninduced H4 cells.

µg of poly(A)$^+$ RNA from HEK cells. This cell line expresses high levels of the second, or kidney, form of arginase (approximately equivalent to uninduced H4, see Fig. 3b), while producing little or no liver isozyme (13). The absence of significant hybridization in this lane demonstrates the lack of cross-hybridization between the two genes for arginase and confirms the inferrences drawn from immunological and electrophoretic data (3,4).

The ability of this probe to detect message for liver arginase from rat is shown in Fig. 3b. Taking into account the fact that this figure was from the same northern blot as shown in Fig. 3a, but exposed about twice as long, the signal seen in lane 1 may be as little as one-tenth that observed in the first lane of Fig. 3a, although equivalent amounts of poly(A)$^+$ RNA were present in these two lanes. As expected, the analogous situation had been observed when human RNA was probed with a rat liver arginase cDNA (5). This previous northern blot also revealed an increase in the message for liver arginase paralleling the increase in arginase activity following 72 h hydrocortisone induction of H4 cells. When corrected for the different amounts of poly(A)$^+$ RNA applied, lanes 4 and 6 of Fig. 3b show a relationship identical to that previously detected with the rat liver arginase probe (5).

That 6B and the other clones just described are all specific for human liver arginase has been established in several ways: Colony hybridization between a human liver cDNA library and a rat liver arginase cDNA detected a number of clones in accordance with the relative abundance of arginase in that tissue, and the shared identity of the 22 clones studied was subsequently confirmed by their mutual hybridization to the cDNA insert from one of the largest of these, 6B. The largest of the 22 homologous inserts approached a size limit slightly smaller than liver arginase message, which is consistent with their identity as near full-length cDNAs for human liver arginase. The plasmids from several of these clones, including p6B, were then shown to specifically hybridize to human liver arginase message, as shown by hybrid-select translation assays, and, finally, the cDNA from clone 6B was able to detect arginase message on northern blots at levels consistent with the activities of liver arginase in the tissues and cells studied, while failing to hybridize to message for kidney arginase.

The availability of a liver arginase-specific cDNA probe has already permitted the definition of the corresponding genomic locus from rat and mouse (5), the mode of induction of liver arginase in H4 hepatoma cells (5), the nature of arginase (and other urea cycle enzyme) induction in vivo in rats by hormones and diet (S.M. Morris, Jr., et al., submitted), the mapping of the human liver arginase gene to chromosome band 6q23 (16), and the definition of a restriction fragment length polymorphism at this site (J.R. Kidd, et al., submitted). In addition, we have now demonstrated a lack of significant homology between the liver and kidney arginases in man, supporting the notion of two distinct arginase genes. The human liver arginase cDNA that we have identified will continue to be vital to investigations into the evolution of the arginases (4), the nature of the common and disparate regulatory signals affecting them (and the other urea cycle enzymes), and the nature of their tissue-specific expression differences (17).

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REFERENCES

1. Herzfeld, A., and Raper, S.M. (1976) Biochem. J. 153, 469-478.
2. Reddi, P.K., Knox, W.E., and Herzfeld, A. (1975) Enzyme 20, 305-310.
3. Spector, E.B., Rice, S.C.H., Moedjono, S., Bernard, B., and Cederbaum, S.D. (1982) Biochem. Med. 28, 165-175.
4. Spector, E.B., Rice, S.C.H., and Cederbaum, S.D. (1983) Pediatr. Res. 17, 941-944.
5. Dizikes, G.J., Spector, E.B., and Cederbaum, S.D. (1986) Somat. Cell Mol. Genet. 12, 375-384.
6. Michelson, A.M., Markham, A.F., and Orkin, S.H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 472-476.
7. Grunstein, M., and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3961-3965.
8. Feinberg, A.P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13.
9. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
10. Southern. E.M. (1975) J. Mol. Biol. 98, 503-517.
11. Pitot, H.C., Peraino, C., Morse, P.A., and Potter, V.R. (1964) Natl. Cancer Inst. Monogr. 13, 229-242.,
12. Haggerty, D.F., Spector, E.B., Lynch, M., Kern, R., Frank, L.B., and Cederbaum, S.D. (1982) J. Biol. Chem. 257, 2246-2253.
13. Spector, E.B., Kern, R.M., Haggerty, D.F., and Cederbaum, S.D. (1985) Mol. Cell Biochem. 66, 45-53.
14. Bernar, J., Hanson, R.A., Kern, R., Phoenix, B., Shaw, K.N.F., and Cederbaum, S.D. (1986) J. Pediatr. 108, 432-435.
15. Kawamoto, S., Amaya, Y., Oda, T., Kuzumi, T., Saheki, T., Kimura, S., and Mori, M. (1986) Biochem. Biophys. Res. Commun. 136, 955-961.
16. Sparkes, R.S., Dizikes, G.J., Klisak, I., Grody, W.W., Mohandas, T., Heinzmann, C., Zollman, S., Lusis, A.J., and Cederbaum, S.D. (1986) Am. J. Hum. Genet. 39, 186-193.
17. Grody, W.W, Dizikes, G.J., and Cederbaum, S.D. (1986) Human Arginase Isozymes. In: Isozymes -- Current Topics in Biological and Medical Research, Alan R. Liss, New York, (In press).