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Genetics

Structure and expression of human dihydropteridine reductase

(aromatic amino acid hydroxylase/biopterin/dihydrofolate reductase/dihydropteridine reductase deficiency/molecular cloning)

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ABSTRACT **Dihydropteridine reductase (DHPR; EC 1.6.99.7) catalyzes the NADH-mediated reduction of quinonoid dihydrobiopterin and is an essential component of the pterin-dependent aromatic amino acid hydroxylating systems. A cDNA for human DHPR was isolated from a human liver cDNA library in the vector λgt11 using a monospecific antibody against sheep DHPR. The nucleic acid sequence and amino acid sequence of human DHPR were determined from a full-length clone. A 112 amino acid sequence of sheep DHPR was obtained by sequencing purified sheep DHPR. This sequence is highly homologous to the predicted amino acid sequence of the human protein. Gene transfer of the recombinant human DHPR into COS cells leads to expression of DHPR enzymatic activity. These results indicate that the cDNA clone identified by antibody screening is an authentic and full-length cDNA for human DHPR.**

Tetrahydrobiopterin (BH_4) is the essential coenzyme for three aromatic amino acid hydroxylases (1, 2) that have important biochemical and physiological functions in higher eukaryotes (2). Tyrosine hydroxylase catalyzes the conversion of tyrosine to dihydroxyphenylalanine, which is a rate-limiting step in the synthesis of catecholamines. Tryptophan hydroxylase catalyzes the conversion of tryptophan to 5-hydroxytryptophan, which is a rate-limiting step in the synthesis of serotonin. Phenylalanine hydroxylase catalyzes the conversion of phenylalanine to tyrosine, which is an obligatory step in the degradation of phenylalanine. Deficiency of phenylalanine hydroxylase causes the disease phenylketonuria in which phenylalanine accumulation leads to a syndrome of mental retardation (3, 4). All three pterin-dependent hydroxylases catalyze coupled reactions in which the aromatic amino acid is hydroxylated and the tetrahydropterin is oxidized to the corresponding quinonoid dihydropterin (2–5).

The naturally occurring biopterin cofactor BH_4 (1) is synthesized from GTP (6) and is maintained in its reduced form by the enzyme dihydropteridine reductase (DHPR; EC 1.6.99.7) (5, 7). DHPR catalyzes the reduction of quinonoid dihydrobiopterin to BH_4 (5) utilizing a reduced pyridine nucleotide as the electron donor. Both NADPH and NADH are active (7), though NADH is the preferred cofactor (8–10).

Genetic deficiency of DHPR has been described in several individuals who were found to have hyperphenylalaninemia by routine newborn screening for phenylketonuria but suffered from severe mental retardation that was refractory to dietary therapy (11–13). These individuals had deficiencies of phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase secondary to the absence of DHPR (3, 4, 11).

DHPR is a ubiquitous enzyme in higher eukaryotes and has been purified to homogeneity from many sources (9, 10, 14–16). The enzyme is a dimeric protein consisting of two identical peptides whose molecular mass is variously reported between 21,000 and 27,000 Da (2, 3). The amino acid compositions of sheep, bovine, and human DHPR have been reported (9, 10, 14–16).

In this report we describe cloning and sequencing of a full-length cDNA for human DHPR, partial sequencing of purified sheep DHPR, and constitution of human DHPR enzymatic activity by genetic transfer of the cDNA clone.

MATERIALS AND METHODS

Purification and Sequencing of Sheep DHPR. The purification of DHPR from sheep liver and the preparation of a monospecific anti-sheep DHPR antiserum has been described (9, 17). Protein was cleaved with CNBr and fragments were isolated and sequenced on an Applied Biosystems (Foster City, CA) 470A gas-phase protein sequencer as described (18).

Identification of cDNA Clones for Human DHPR. A human liver cDNA library in the expression vector λgt11 (19) was screened with monospecific antibody against sheep DHPR using methods previously described (20, 21). The library was rescreened with ^{32}P-labeled fragments of the DHPR cDNA (19). Two *Eco*RI fragments from clone hDHPR1 were used to hybrid-select mRNA from liver poly(A)$^+$ RNA (22). Hybrid-selected mRNA was translated in the presence of [^{35}S]methionine using a rabbit reticulocyte lyase system (New England Nuclear). Proteins were immunoprecipitated using anti-sheep DHPR antibody and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

DNA-Mediated Gene Transfer and Assay of DHPR. The full-length cDNA insert from clone hDHPR13 was isolated by partial *Eco*RI digestion and subcloned into the expression vector p91023B provided by Randy Kaufman (Genetics Institute, Boston, MA) (23). Clones were obtained with the DHPR sequence in the sense orientation [91023B-DHPR(+)] or in the antisense orientation [91023B-DHPR(−)]. These clones were transfected into COS cells (24) by calcium phosphate precipitation (25, 26) to achieve transient expression of the recombinant gene (27). Cytoplasmic extracts were prepared 72 hr after transfection and assayed for DHPR activity as described (17).

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Abbreviations: DHPR, dihydropteridine reductase; BH_4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; MTFR, methylenetetrahydrofolate reductase; ORF, open reading frame; PIR, Protein Identification Resource; EMBL, European Molecular Biology Laboratory.

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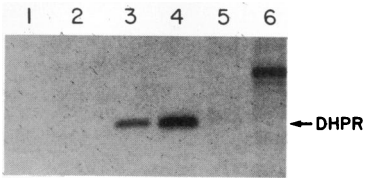


FIG. 1. Hybrid-select translation of human DHPR. Human liver mRNA was hybrid-selected with cloned DNA, translated *in vitro*, immunoprecipitated, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lane 1, no mRNA; lane 2, mRNA selected with pBR322; lane 3, mRNA selected with 5′ 573-base-pair (bp) *Eco*RI fragment immunoprecipitated with anti-sheep DHPR; lane 4, mRNA selected with 3′ 710-bp *Eco*RI fragment of hDHPR1 immunoprecipitated with anti-sheep DHPR; lane 5, mRNA selected with 710-bp fragment of hDHPR1, total translation products; lane 6, mRNA selected with a human α_1-antitrypsin cDNA and immunoprecipitated with anti-α_1-antitrypsin. The material that was immunoprecipitated with anti-sheep DHPR comigrated with purified sheep DHPR protein.

DNA Sequencing and Sequence Analysis. DNA sequence was determined by using the dideoxy chain-termination method of Sanger *et al.* (28). Computer analysis of the cDNA and predicted protein sequences were performed using the on-line facilities of the Protein Identification Resource (PIR), the PIR, GENBANK, and European Molecular Biology Laboratory (EMBL) data bases, and the FASTP, SEARCH, and ALIGN programs (29).

RESULTS

Identification of Human DHPR Clones. One million plaques from a human liver cDNA library in the vector λgt11 were screened for DHPR-immunoreactive protein using rabbit anti-sheep DHPR antibody (17). One positive clone was identified. This clone, hDHPR1, contained two internal *Eco*RI fragments of 710 and 573 bases.

Hybrid selection was performed to purify mRNA corresponding to each of the *Eco*RI fragments. *In vitro* translation of the hybrid-selected mRNA demonstrated that both fragments selected mRNA coding for a 25,000-Da protein that immunoprecipitated with anti-sheep DHPR antisera and comigrated with purified sheep DHPR (Fig. 1). No corresponding band was present in the translation products in the absence of RNA or in mRNA selected with pBR322 or an α_1-antitrypsin cDNA.

The cDNA library was rescreened with the 710-bp *Eco*RI fragment of hDHPR1 to identify longer cDNA clones. Forty hybridizing colonies were identified and analyzed by Southern blotting (30). The longest clone, hDHPR13, contained two *Eco*RI fragments of 646 and 872 bases. Clones hDHPR1 and hDHPR13 were sequenced using the strategy illustrated in Fig. 2.

The nucleic acid sequence and predicted amino acid sequence of human DHPR are shown in Fig. 3. Clone hDHPR1 contains 1283 bp and has a single long ORF from the beginning of the clone to base 812. The AUG sequence at position 81 is presumed to represent a start codon though it is preceded by an 80-base 5′ sequence that does not contain stop codons in the long ORF. Another AUG is present at position 11, which is followed by a TAA termination codon at position 35. Clone hDHPR13 has a shorter 5′ extent and the sequences are identical in the overlapping regions. Clone hDHPR1 contains a 452-base 3′ sequence following the TAG termination codon and a poly(A) tail beginning 28 bases after an AATTAAA sequence at residue 1241, which is presumably a poly(A) signal. Clone hDHPR13 contains 725 bases of 3′ untranslated sequence including a 17-base poly(A) tail beginning 12 bases after a consensus AATAAA poly(A) site at position 1535. Another AATAAA sequence that could function in polyadenylylation exists at base 1395.

The ORF comprises 732 bases and codes for a protein of 244 amino acids and a molecular mass of 25,774 Da. This is consistent with the 26,000 Da of human DHPR reported (14). The amino acid composition predicted by the ORF agrees with the amino acid compositions of human, sheep, and bovine DHPR reported (9, 10, 14–16) (Table 1).

Amino Acid Sequence of Purified Sheep DHPR. The partial amino acid sequence of purified sheep DHPR was determined. The 112 amino acids correspond to the carboxyl-terminal portion of the human molecule from amino acid 106 to 217. Only 8 of 112 overlapping residues differ and most are highly conservative: Ile_{111}-Val; Lys_{137}-Arg; Gly_{154}-Ala; Gln_{161}-Arg; Met_{170}-Leu; Ile_{177}-Val; Val_{179}-Leu; Met_{188}-Val. Attempts to sequence the amino terminus of sheep DHPR were unsuccessful due to blocking of the amino-terminal amino acid.

Gene Transfer and Expression of DHPR Enzymatic Activity. To establish that this cDNA coded for the complete DHPR enzyme, the cDNA insert from hDHPR13 was subcloned into the expression vector 91023B (Fig. 4 *Upper*) and introduced into COS cells by DNA-mediated gene transfer. Control transfections were performed with the hDHPR13 cDNA cloned into 91023B in the antisense orientation. Low levels of DHPR activity are present in untransformed COS cells and COS cells transformed with the expression vector containing hDHPR13 in the antisense orientation (Fig. 4 *Lower*). Substantially higher levels of DHPR activity were present in cells transformed with the expression vector containing the hDHPR13 cDNA in the sense orientation (Fig. 4 *Lower*).

DISCUSSION

This report describes the cloning of a cDNA for human DHPR, determination of its nucleic acid and amino acid sequence, and expression of DHPR enzymatic activity following DNA-mediated gene transfer of the recombinant clone. Three experiments establish the authenticity of this clone. (*i*) *In vitro* translation of mRNA purified by hybridization with each of the two internal *Eco*RI fragments of the DHPR clones produces a protein that immunoprecipitates with anti-sheep DHPR antibody and comigrates with purified sheep DHPR on $NaDodSO_4$/polyacrylamide gels. (*ii*) The amino acid sequence predicted by the ORF matches the amino acid sequence determined from purified sheep DHPR

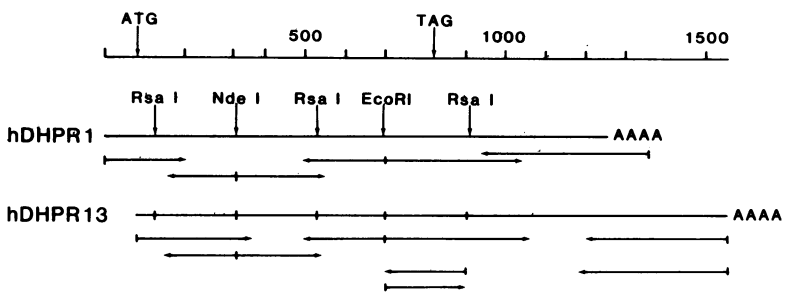


FIG. 2. Structure of clones hDHPR1 and hDHPR13. Sequenced fragments are indicated.

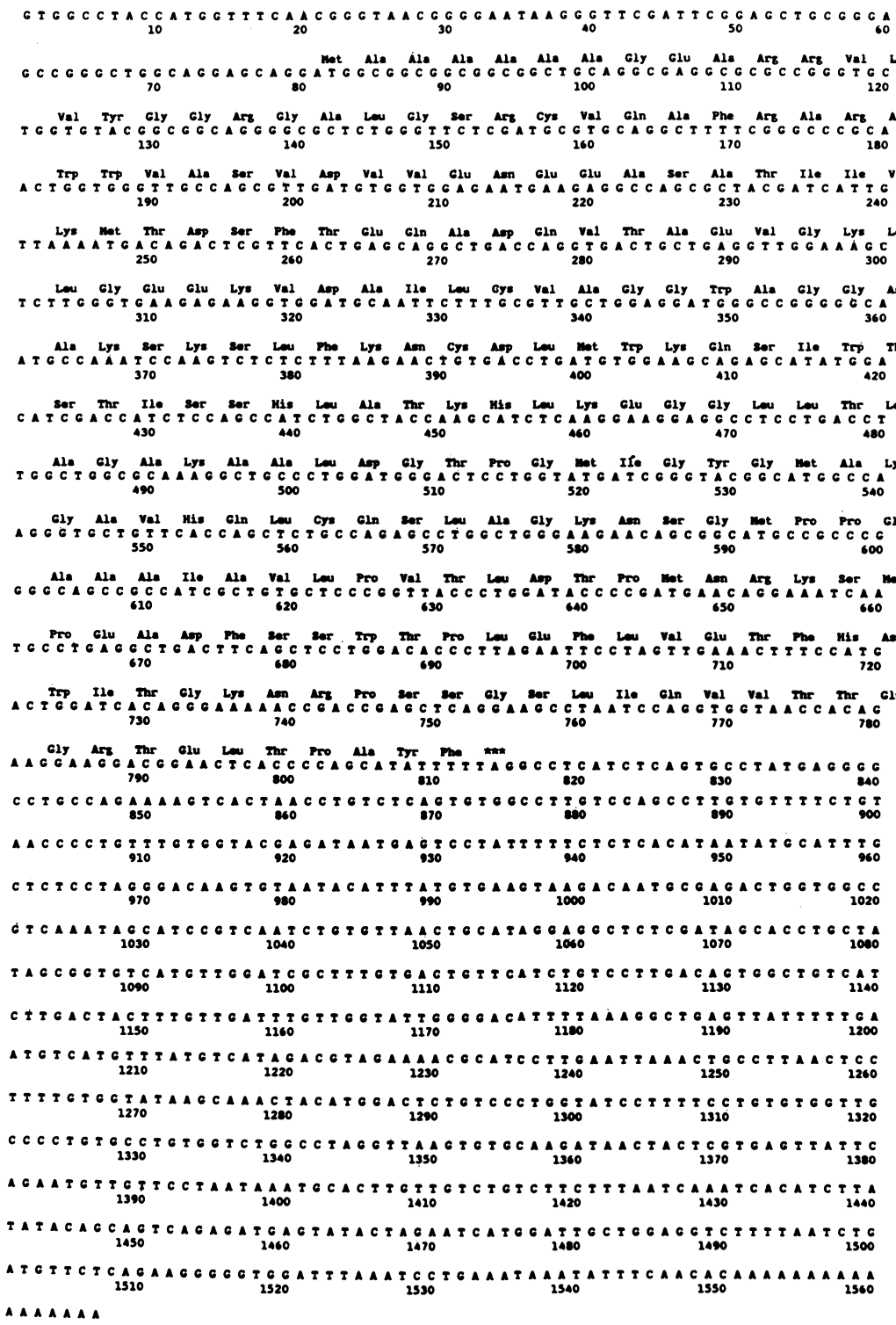


FIG. 3. Sequence of human hDHPR. The amino acid sequence is predicted from the nucleic acid sequence of the longest open reading frame (ORF).

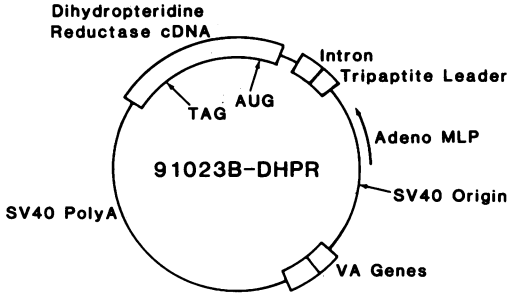
protein. (*iii*) Genetic transfer of the recombinant DHPR clone into monkey COS cells in a eukaryotic expression vector results in expression of DHPR enzymatic activity.

The fact that genetic transfer of the DHPR clone results in expression of DHPR enzymatic activity suggests that this clone is a "full-length" cDNA and contains the complete ORF. The ORF was established at the 3′ end of the clone by the identity between the sheep DHPR protein sequence and predicted sequence of human DHPR. The integrity of the ORF through the 5′ end of the clone was tested by sequencing two independent clones that had identical sequences. This reading frame extends 5′ to the beginning of the clone without a stop codon. The first AUG is at position 81, and the amino acid composition of the reading frame from position 81 to 812 corresponds to the reported amino acid compositions of purified human, sheep, and bovine DHPR. Another AUG is present in a different reading frame at position 11; however, this frame is interrupted by a TAA stop codon after only 7

Table 1. Amino acid composition of DHPR

Amino acid	Human DHPR Predicted		Human DHPR Ref. 14*	Sheep DHPR Ref. 9*	Sheep DHPR Ref. 10*	Bovine DHPR Ref. 16*	Bovine DHPR Ref. 15*
Ala	33		31	30	32	26	32
Arg	9		9	8	10	8	9
Asn	7	16	16	18	19	17	15
Asp	9						
Cys	4		3	2	3	9	1
Gln	7	21	22	26	26	23	21
Glu	14						
Gly	25		26	26	24	20	22
His	4		5	5	5	5	4
Ile	9		8	6	7	7	6
Leu	21		21	20	25	21	22
Lys	14		14	15	14	14	14
Met	8		4	4	5	5	5
Phe	7		7	6	7	6	6
Pro	9		9	10	9	10	9
Ser	18		18	21	20	18	22
Thr	18		17	15	18	15	18
Trp	7		5	2	9	3	8
Tyr	3		3	2	3	3	2
Val	18		17	17	17	16	17

The predicted amino acid composition was deduced from the nucleic acid sequence of DHPR cDNA. Values are normalized for a molecular mass of 25,000 Da after Firgaira *et al.* (14).
*Liver DHPR.



Sample	Activity nmol NADH oxidized/mg/min
Human liver	59.4
Human liver (boiled)	1.0
COS	8.1
COS (boiled)	1.2
Experiment 1	
COS + 91023B-DHPR(+)	14.0
COS + 91023B	4.8
Experiment 2	
COS + 91023B-DHPR(+)	53.0
COS + 91023B-DHPR(-)	9.1

FIG. 4. (*Upper*) Structure of expression vector containing human DHPR. The full-length cDNA hDHPR1 was cloned into the *Eco*RI site of the vector 91023B in the sense orientation (shown) and antisense orientation. SV40, simian virus 40; MLP, major late promoter. (*Lower*) DHPR activity of COS cells transformed with recombinant DHPR and controls. Results of two separate transfection experiments with the sense and antisense DHPR constructs are given.

amino acids. None of the 40 clones examined extended further at the 5′ end than hDHPR1; therefore, we believe that the AUG at position 81 represents the authentic initiation codon. The absence of termination codons 5′ to this AUG codon was fortuitous in that the full length of the DHPR protein was apparently produced as a fusion product with β-galactosidase by clone DHPR1. This fusion protein was the only protein detected by screening one million plaques with the anti-sheep DHPR antibody.

The amino acid sequence predicted from the ORF of the cDNA clones is highly homologous to the amino acid sequence of sheep DHPR. The differences between these sequences are consistent with the evolutionary distance between sheep and human (31). We searched the PIR, GENBANK, and EMBL data bases using the FASTP and SEARCH programs of PIR and were unable to identify any sequence homologies to the predicted sequence of DHPR. This result was somewhat surprising since two other enzymes, dihydrofolate reductase (DHFR) and methylenetetrahydrofolate reductase (MTFR), have analogous activities. All three enzymes have substituted pteridine substrates; all three enzymes utilize a pyridine nucleotide cofactor (though DHFR preferentially utilizes NADPH) (8–10); DHPR and DHFR are inhibited by methotrexate; DHFR and MTFR will catalyze the reduction of quinonoid dihydrobiopterin (32); and DHPR will catalyze the reduction of quinonoid dihydrofolate (33).

We were unable to identify statistically significant sequence homology between DHPR, DHFR, or MTFR using the Needleman and Wunsch algorithm (34) or analysis of homology matrices. Thus, the analogous activities of DHPR and DHFR may represent vestigial homology between loci that have diverged to the point that sequence similarity cannot be demonstrated by statistical methods. Alternative-

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                1       10        20        30        40        50        60        70
                '       '         '         '         '         '         '         '
ECDHFR          MISL--IAALAVDRVIGMENAMPWN-LPADLAWFKRN-TL------NK--PVIMGRHTWESI---GRPLP
LCDHFR          TAFLW--AQWRDGL-IGKDGHLPWH-LPDDLHYFRAQ-TV------GK--IMVVGRRTYESFP--KRPLP
HDHFR           VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM-TTT-SSVEGKQNLVIMGKKTWFSIPEKNRPLK
                  o  oooo o ooo  o          oo o o   ooo oo o      ooo o o ooo   o o
HDHPR            1-AAAAAAGEARRVLVYGG-17                    45-EEASATIIV-KMT-DSFTEQADQV-76
                                    103-CDLMWKQSIWTSTISS-118

                71        80        90        100       110       120       130       140
                '         '         '         '         '         '         '         '
ECDHFR          GRKNIILSSQ-PGTDDRVTWV-KSVD-EAIAACGD-      -----VPEIMVIGGGRVYEQFL--PKAQ---KL
LCDHFR          ERTNVVLTHQEDYQAQGAV-VVHDVA-AVFAYAKQ-HLDQ----ELVIAGGAQIFTAFKDDVD-----TL
HDHFR           GRINLVLSRELKEPPQGAHFLSRSLD-DALKLTEQPELANKVDMVW-IVGGSSVYKEAMNHPGHL---KL
                 o     o ooo oo  oo oo o   ooo oo        ooo oo oooo  o  ooo
HDHPR                                                    78-KVDAILCVAGGWAGG-WAKSK-95
          54-KMTDSFTEQADQTVAEVGKLLGEEKVDAI-LC-84

                141       150       160       170       180       190       200
                '         '         '         '         '         '         '
ECDHFR          YLTHIDAEVEGDTHFPDYEPDDWESVFSEFH----DADAQN-SHS----YCFEILERR-
LCDHFR          LVTRLAGSFEGDTKMIPLNWDDFTKVSSRTV----EDTNPALTHT----YEVWQKKA
HDHFR           FVTRIMQDFESDTFFPEIDLEKYK-LLPEYPGVLSDVQEEKGIKYKFEVYEKND
                     oo  oo o o    o o  oo ooo o   oo o o o   oo
HDHPR              205-EFLVET-FHDWITGKNRP-SSGSLIQVV-TTEGR-TLTP--AYF-243
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FIG. 5. Sequence similarity between methotrexate and pyridine nucleotide binding sites of DHFR and sequences within DHPR. Optimal homology between DHFR from *L. casei* (LCDHFR), *E. coli* (ECDHFR), and human (HDHFR) was determined using the program ALIGN. Residues within the *L. casei* and *E. coli* sequence that form molecular bonds to methotrexate or pyridine nucleotides (36–38) are underlined. Fragments of human DHPR are aligned to maximize similarity to these active sites. ●, Alignment of identical residues in DHPR and any of the three DHFR sequences shown. ○, Alignment of residues with analogous properties: (E,D); (N,Q); (T,S); (A,G,V,I,L); (K,R).

ly, the analogous activities may reflect convergent evolution of two distinct sequences that have evolved analogous functions from different origins (35).

We did observe similarities in the sequences of DHPR and DHFR in regions known to comprise the binding sites for methotrexate and the pyridine nucleotide cofactor in DHFR. These residues have been identified by crystallography of two prokaryotic DHFR proteins (*Escherichia coli* and *Lactobacillus casei*) (36–38). Fig. 5 shows an alignment of fragments of DHPR with residues of DHFR that contribute to methotrexate and pyridine nucleotide binding. Considerable variation is apparent among the DHFR sequences that are apparently compatible with activity of the enzyme. The aligned fragments of DHPR contain many residues that are identical or functionally analogous to residues in the aligned DHFR sequences and may represent determinants for analogous functions.

The demonstration that DHPR enzymatic activity can be constituted by DNA-mediated gene transfer of the DHPR cDNA will enable experiments aimed at exploring the structure activity relationships of DHPR. Genetic transfer of DHPR may also enable experiments in somatic gene therapy for DHPR deficiency and phenylketonuria. Somatic gene therapy of DHPR deficiency would entail genetic transfer of DHPR into various cells in the body to reconstitute pathways required for aromatic amino acid hydroxylation (39, 40). Such therapy may be complicated by the necessity for replacing this enzymatic function in cells of the central nervous system. DHPR may also be useful in the therapy of phenylketonuria as an adjuvant to reconstitution of the phenylalanine hydroxylase holoenzyme by gene replacement (41, 42).

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1. Kaufman, S. (1963) *Proc. Natl. Acad. Sci. USA* **50,** 1085–1093.
2. Kaufman, S. & Fisher, D. B. (1974) in *Molecular Mechanisms of Oxygen Activation*, ed. Hayaishi, O. (Academic, New York), pp. 285–368.
3. Kaufman, S. (1976) *Adv. Neurochem.* **2,** 1–132.
4. Kaufman, S. (1983) *Adv. Hum. Genet.* **13,** 217–297.
5. Kaufman, S. (1964) *J. Biol. Chem.* **239,** 332–338.
6. Nichol, C. A., Smith, G. K. & Duch, D. S. (1985) *Annu. Rev. Biochem.* **54,** 729–764.
7. Kaufman, S. (1957) *J. Biol. Chem.* **226,** 511–524.
8. Nielsen, K. M., Simonsen, V. & Lind, K. E. (1969) *Eur. J. Biochem.* **9,** 497–502.
9. Craine, J. E., Hall, E. S. & Kaufman, S. (1972) *J. Biol. Chem.* **247,** 6082–6091.
10. Cheema, S., Soldin, S. J., Knapp, A., Hofmann, T. & Scrimgeour, K. G. (1973) *Can. J. Biochem.* **55,** 1229–1239.
11. Kaufman, S., Holtzman, N. A. Milstien, S., Butler, L. J. & Krumholz, A. (1975) *N. Engl. J. Med.* **293,** 785–790.
12. Brewster, T. G., Moskowitz, M. A., Kaufman, S., Breslow, J. L., Milstien, S. & Abroms, I. F. (1979) *Pediatrics* **63,** 94–99.
13. Firgaira, F. A., Choo, K. H., Cotton, R. G. H. & Danks, D. M. (1981) *Biochem. J.* **198,** 677–682.
14. Firgaira, F. A., Cotton, R. G. H. & Danks, D. M. (1981) *Biochem. J.* **197,** 31–43.
15. Hasegawa, H. (1977) *J. Biochem. (Tokyo)* **81,** 169–177.
16. Korri, K., Chippel, D., Chauvin, M. M., Tirpak, A. & Scrimgeour, K. G. (1977) *Can. J. Biochem.* **55,** 1145–1152.
17. Milstien, S. & Kaufman, S. (1980) *Methods Enzymol.* **66,** 723–725.
18. Lischwe, M. A., Ochs, R. L., Reddy, R., Cook, R. G., Yeoman, L. C., Tan, E. M., Reichlin, M. & Busch, H. (1985) *J. Biol. Chem.* **260,** 14304–14310.
19. Kwok, S. C. M., Ledley, F. D., DiLella, A. G., Robson, K. J. H. & Woo, S. L. C. (1985) *Biochemistry* **24,** 556–561.
20. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1194–1198.
21. Johnson, D. A., Gautsch, J. W., Spatsman, J. R. & Elder, J. H. (1984) *Gene Anal. Tech.* **1,** 3–8.
22. Miller, J. S., Paterson, B. M., Ricciardi, R. P., Cohen, L. & Roberts, B. E. (1983) *Methods Enzymol.* **101,** 650–674.
23. Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A. & Clark, S. C. (1985) *Science* **228,** 810–815.
24. Mellon, P., Parker, V., Gluzman, Y. & Maniatis, T. (1981) *Cell* **27,** 279–288.
25. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52,** 456–460.
26. Ledley, F. D., Grenett, H. E., DiLella, A. G., Kwok, S. C. M. & Woo, S. L. C. (1985) *Science* **228,** 77–79.
27. Chang, L. J., Gamble, C. L., Izaguirre, C. A., Minden, M. D., Mak, T. W. & McCulloch, E. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 146–150.
28. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
29. Barker, W. S. & Dayhoff, M. O. (1972) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Natl. Biomed. Res. Found., Washington, DC), Vol. 5, pp. 101–110.
30. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
31. Dayhoff, M. O., ed. (1978) in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, DC), Vol. 5, pp. 89–99.
32. Matthews, R. G. & Kaufman, S. (1980) *J. Biol. Chem.* **255,** 6014–6017.
33. Pollock, R. J. & Kaufman, S. (1978) *J. Neurochem.* **31,** 115–123.
34. Needleman, S. B. & Wunsch, C. D. (1970) *J. Mol. Biol.* **48,** 443–453.
35. Doolittle, R. F. (1981) *Science* **214,** 149–159.
36. Matthews, D. A., Alden, R. A., Bolin, J. T., Freer, S. T., Hamlin, R., Xuong, N., Kraut, J., Poe, M., Williams, M. & Hoogsteen, K. (1977) *Science* **197,** 452–455.
37. Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C. & Kraut, J. (1982) *J. Biol. Chem.* **257,** 13650–13662.
38. Filman, D. J., Bolin, J. T., Matthews, D. A. & Kraut, J. (1982) *J. Biol. Chem.* **257,** 13663–13672.
39. Ledley, F. D. (1987) *J. Pediatr.* **110,** 1–8.
40. Ledley, F. D. (1987) *J. Pediatr.* **110,** 167–174.
41. Ledley, F. D., Grenett, H. E., McGinnis-Shelnutt, M. & Woo, S. L. C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 409–413.
42. Ledley, F. D., Hahn, T. & Woo, S. L. C. (1987) *Som. Cell. Mol. Gen.*, in press.