Biochimica et Biophysica Acta, 1171 (1992) 133–140

BBAEXP 92437

Species and tissue specificity of mammalian GTP cyclohydrolase I messenger RNA

Markus Gütlich [a], Karin Schott [a], Thomas Werner [b], Adelbert Bacher [c] and Irmgard Ziegler [a]

[a] *GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Experimentelle Hämatologie, München (Germany),* [b] *GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Säugetiergenetik, Neuherberg (Germany)* and [c] *Technische Universität München, Lehrstuhl für Organische Chemie und Biochemie, Garching (Germany)*

(Received 18 May 1992)
(Revised manuscript received 7 August 1992)

Key words: Tetrahydrobiopterin; GTP cyclohydrolase I; Northern blot; Polymerase chain reaction

Northern blot analysis of rat RNA from cell lines and isolated organs with a specific rat cDNA probe detected two GTP cyclohydrolase I mRNA species of approx. 1.4 and 3.6 kb. The ratio between these two species varies between 0.6 and 2.4 in different rat organs. Using primers derived from highly conserved regions in the rat and *Escherichia coli* cDNA sequences a human GTP cyclohydrolase I probe was obtained by means of reverse transcription and PCR (polymerase chain reaction). The human PCR product consisting of 555 bp was cloned and sequenced. It shows a 92% identity with the published sequence of the rat gene. The analysis of various human cell lines with this specific probe shows only one species of GTP cyclohydrolase I mRNA with an approximate size of 3.6 kb.

Introduction

H_4biopterin[1] (**4**; Fig. 1) serves as electron donor for hydroxylation of the aromatic amino acids phenylalanine, tryptophan and tyrosine (for review see Ref. 1). Tissues involved in phenylalanine degradation or neurotransmitter biosynthesis such as liver, hypothalamus and adrenal medulla have high activities of enzymes involved in H_4biopterin synthesis [2]. More recently it was shown that H_4biopterin also serves as cofactor in the generation of nitric oxide from arginine in endothelial cells, Kupffer cells, neutrophilic granulocyts and macrophage [3,4].

There is accumulating evidence that H_4biopterin is also synthesized in cells which do not depend on H_4biopterin cofactor but undergo cytokine directed proliferation (for review see Ref. 5). In this case, H_4biopterin has an additional function which is apparently unrelated to its cofactor role described above. It modulates the clonal expansion of T cells [6–8] and the proliferation of erythroid cells [9,10]. Closer analysis has shown that it enhances the affinity of the IL-2 receptor complex to its ligand and subsequently affects various aspects of signal transmission [11].

A more detailed understanding of H_4biopterin levels in tissues and cells depends on analysis of the biosynthetic enzymes and the regulatory mechanisms involved. The first commited step in the de novo biosynthesis is the formation of dihydroneopterin triphosphate (**2**; Fig. 1) from GTP (**1**; Fig. 1) which is catalyzed by GTP cyclohydrolase I (EC 3.5.4.16) (for review, see Refs. 12, 13). The dihydropterin (**2**; Fig. 1) is subsequently converted to 6-pyruvoyl-H_4pterin (**3**; Fig. 1) by elimination of triphosphate which is accompanied by tautomerization. The complex reaction is catalyzed by 6-pyruvoyl-H_4pterin synthase. Reduction of the side chain carbonyl groups of **3** by sepiapterin reductase (EC 1.1.1.153) yields H_4biopterin (**4**; Fig. 1).

Gradually increasing activities of GTP cyclohydrolase I and sepiapterin reductase during lectin stimulation of resting human T lymphocytes satisfactorily explained the accumulation of H_4biopterin during blast

Correspondence to: M. Gütlich, GSF Institut für Klinische Molekularbiologie und Tumorgenetik, Marchioninistr. 25, D-8000 München, 70 Germany.

Abbreviations: H_4biopterin, (6*R*)-5,6,7,8-tetrahydrobiopterin; biopterin, 6-(L-erythro-1′,2′-dihydroxypropylpterin); neopterin, 6-(D-erythro-1′,2′,3′-trihydroxypropylpterin); 6-pyruvoyl-H_4pterin, (6*R*)-(1′,2′-dioxopropyl)-5,6,7,8-tetrahydropterin; FCS, fetal calf serum; PHA, phytohemagglutinin; RPMI 1640, Roswell Park Memorial Institute tissue-culture medium type 1640; PCR, polymerase chain reaction; HPLC, high performance liquid chromatography.

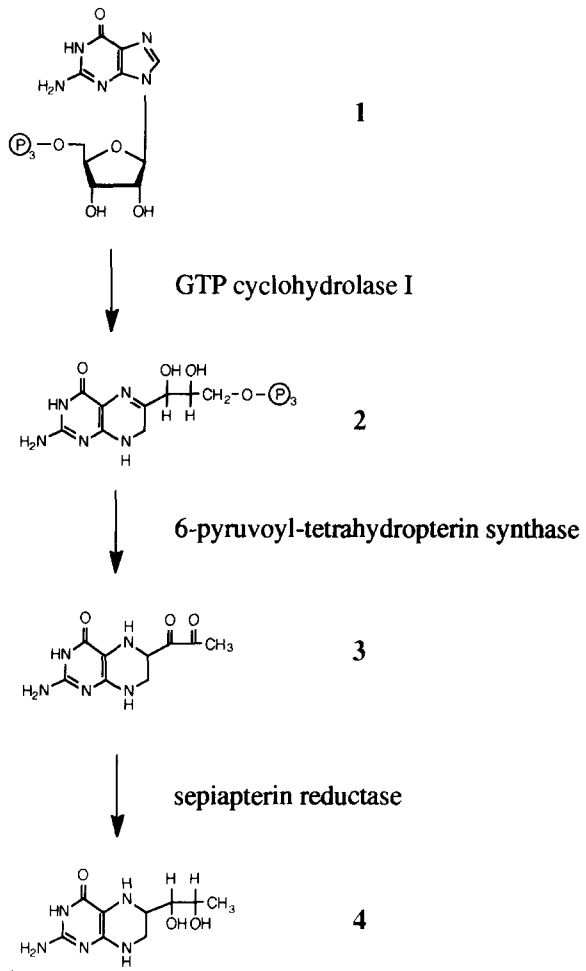

Fig. 1. Biosynthesis of tetrahydrobiopterin. **1**, GTP; **2**, dihydroneopterin triphosphate; **3**, 6-pyruvoyl-tetrahydropterin; **4**, tetrahydrobiopterin.

transformation [14]. In primed human T cells, an accelerated synthesis of H_4biopterin results from increased activities of GTP cyclohydrolase I, 6-pyruvoyl-H_4pterin synthase and sepiapterin reductase which are controlled by the synergistic action of IFN-γ and IL-2 [15].

In rat thymocytes, H_4biopterin synthesis is associated with the cell-cycle. It culminates at the time of S-phase entry, in parallel with the specific activities of both GTP cyclohydrolase I and sepiapterin reductase. These increases in enzyme activities correlate with the steady state mRNA levels for both enzymes, whereas the subsequent decrease in the activity of the rate limiting enzyme GTP cyclohydrolase I is due to post-translational modification [16]. These preliminary studies make it evident that a closer understanding of the regulatory steps involved in H_4biopterin synthesis of human cells critically depends on genetic analysis of the rate limiting enzyme GTP cyclohydrolase I.

The genes coding for GTP cyclohydrolase I from rat and *Escherichia coli* have been cloned and sequenced [17–19]. Hatakeyama et al. reported on a clone from a cDNA library from rat with an overall insert length of 1024 bp [17]. The sequence contained an open reading frame coding for a 25.8 kDa peptide consisting of 230 amino acids. The predicted amino acid sequence was in agreement with the partial sequences of various peptide fragments obtained from rat GTP cyclohydrolase I.

Katzenmeier et al. determined the sequence for GTP cyclohydrolase I from *Escherichia coli* which had a remarkably high level of sequence homology to the rat enzyme [19].

This study reports the cloning of a 555 nucleotide fragment of the human cDNA coding for GTP cyclohydrolase I using a PCR strategy based on the sequence homology between the bacterial and the rat gene. PCR generated probes were used for characterization of species and tissue specificity of the GTP cyclohydrolase I mRNA.

Materials and Methods

The following materials were obtained from the suppliers indicated: [α-^{32}P]dCTP (3000 Ci/mmol) and the multiprime labelling kit from Amersham Buchler, Braunschweig, Germany; T7 sequencing kit, *Escherichia coli* JM 105, pUC 19 DNA, and Nick columns from Pharmacia, Freiburg, Germany; *Taq* DNA polymerase, restriction endonucleases, alkaline phosphatase, DNA molecular weight standards III (λ/*Eco*RI *Hin*dIII) and V (pBR 322/*Hae*III) and primer $(dT)_{15}$ from Boehringer-Mannheim, Germany; PHA and Ficoll ($\rho = 1.077$) from Biochrom, Berlin, Germany; mRNA isolation kit PolyATtract, and RNasin from Promega, Serva, Heidelberg, Germany; ^{35}S-Sequetide from New England Nuclear, Dreieich, Germany; Moloney murine leukemia virus reverse transcriptase from Gibco-BRL, Eggenstein, Germany; Micrograde 12% homogeneous polyacrylamide gel HST-012 from Gradipore, Pyrmont, Australia; Biodyne A nylon membrane from Pall, Dreieich, Germany; HPLC column 4000–5 DEAE 125 × 6 mm from Macherey & Nagel, Düren, Germany; DEAE membrane NA-45 from Schleicher & Schüll, Dassel, Germany; Ultrafree 10 ultrafiltration units from Millipore, Eschborn, Germany; Kodak X-OMAT AR X-ray film from Eastman Kodak, Rochester, USA; Agfa X-Ray 90, 20 × 40 cm X-ray film from Agfa, München, Germany. Other reagents were of high-quality commercial grade.

Tissues

Rats (*Rattus norvegicus*) were killed, and liver, spleen, kidney, brain (whole organ) and bone marrow from femur were dissected immediately and frozen in liquid nitrogen.

Cell culture

The rat liver cell line HTC, the human liver cell line HuH7 and the human T cell line HUT 102 were grown in RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 2 mM glutamine, streptomycin (100 μg ml^{-1}) and penicillin (100 U ml^{-1}). The adherent liver cell lines were seeded at a density of $4 \cdot 10^4$ cells/cm^2 and grown to confluence. The T cell line Hut 102 was cultured as described previously [15].

Human peripheral blood lymphocytes were obtained from buffy coat and were purified by Ficoll ($\rho = 1.077$) density gradient centrifugation. They were seeded at a density of $7 \cdot 10^5$ cells ml^{-1}, activated by addition of PHA (1 μg ml^{-1}), and maintained as described above. After 18 h, the monocytes became adherent, whereas the T cells remained in suspension. The T cells were transferred to new culture dishes and harvested for RNA isolation after 48 h.

RNA purification

Frozen tissue (1–2 g) was triturated under liquid nitrogen in a mortar. Lysis buffer (10 ml) containing 5 M guanidinium isothiocyanate, 5 mM β-mercaptoethanol and 25 mM sodium citrate (pH 7.0), was added, and triturating under liquid nitrogen was repeated. The powder was allowed to thaw, and the resulting suspension was passed repeatedly through a 20-G needle for homogenization. Further purification of total RNA as well as isolation of RNA from cultured cells was performed by the modified procedure of Chirgwin [20].

Poly(A)$^+$ RNA was obtained from 0.7–2.0 mg of total RNA with the magnet bead method of the PolyATtract kit.

Oligonucleotide synthesis

Oligonucleotides were synthesized and purified using an Applied Biosystems DNA synthesizer (model 381 A). The purification of the primers was performed by precipitation with 1-butanol according to Sawadogo and van Dyke [21].

PCR

Reverse transcriptase-PCR was performed according to Kawasaki [22]. Reaction mixtures contained 1 μg of total RNA, 10 U of Moloney murine leukemia virus reverse transcriptase, 0.1 μg of primer-dT$_{15}$, 10 U of RNasin, 1 mM of each dATP, dGTP, dTTP and dCTP, 60 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.3% (v/v) Tween 20 and 15 mM Tris-HCl (pH 8.4), in a total volume of 10 μl. The mixture was incubated for 20 min at 42°C, heated to 95°C for 5 min, and cooled on ice. The resulting cDNA solution was directly used as template for PCR. Reaction mixtures contained 10 μl of reverse transcription mixture, 75 pmol of both forward and reverse primers (cyclo 1 to cyclo 6 see Table I), 1.5 U of *Taq* DNA polymerase, 0.2 mM of each dATP, dGTP, dTTP and dCTP, 60 mM KCl, 2.6 mM $MgCl_2$, 0.3% (v/v) Tween 20 and 15 mM Tris-HCl (pH 8.4), in a final volume of 50 μl. PCR was performed for 40 cycles in a thermocycler. Denaturation, annealing, and chain extension conditions were as follows: 95°C for 1 min, 59°C for 1 min, 72°C for 1 min, and additionally 5 min at 72°C after the last step. Reamplification of purified products was performed by 20 cycles of PCR using the same experimental conditions.

Purification of PCR products

Products of reverse transcription-PCR were separated by electrophoresis on a 2% agarose gel and visualized by staining with ethidium bromide. Bands of DNA were collected by electrophoretical transfer to anion exchange membranes NA-45 [23]. After elution from the membrane, the DNA was precipitated by ethanol and used for reamplification. PCR products were purified by anion exchange HPLC on a Nucleogen 4000–7 DEAE column using a gradient of 0–1.2

TABLE I

Primers used in PCR experiments

Positions are indicated according to the published sequence of rat GTP cyclohydrolase I [17]. The primers cyclo 2 * and cyclo 6 * contain *Sal*I restriction sites at their 5'-ends.

Primer	Direction	Position	Sequence
cyclo 1	forward	142–161	GGTGTAAGGTGCACCAA(T/C)GG
cyclo 2	forward	275–294	GCAGCGAGGAGGATAACGAG
cyclo 3	forward	480–499	ACGAGATGGTGATTGTGAA(G/A)G
cyclo 4	reverse	638–658	GGTAAGGCGTTCTTGAAC(G/T)TG
cyclo 5	reverse	668–687	GCTTCTGTGATGGCCACN [a]GG
cyclo 6	reverse	812–831	AACTCCTCCCGAGTCTTNGG
cyclo 2 *	forward	275–294	TTATGTCGACGCAGCGAGGAGGATAACGAG
cyclo 6 *	reverse	812–831	TTATGTCGACAACTCCTCCCGAGTCTTNGG

[a] N = A, C, G and T.

M NaCl containing 30 mM phosphate buffer (pH 6.7), and 6 M urea. The flow rate was 1 ml min^{-1}. As a standard, 1 mg of pBR322/*Hae*III DNA was separated under the same conditions. Fractions containing DNA were concentrated and dialysed by ultrafiltration using Ultrafree 10 filter units.

Cloning of the PCR fragment

The PCR fragment generated by primers cyclo 2 and cyclo 6 was reamplified using primers cyclo 2* and cyclo 6* containing *Sal*I restriction sites (see Table I). After reamplification, the product was purified by agarose gel electrophoresis and extracted from the gel. Digestion of the insert and the vector (pUC 19) with *Sal*I, dephosphorylation of the vector by alkaline phosphatase, ligation by T4-DNA ligase, and transformation of *E. coli* JM 105 was performed according to standard protocols [23].

DNA sequencing

The chain termination reaction of Sanger et al. [24], as modified by Bachmann et al. [25], was used for direct sequencing of PCR products. The T7 sequencing kit was used according to the manufacturers' instructions with the following modifications. The annealing reaction contained 100 ng (10 μl) of HPLC-purified DNA, 20 pmol of the specific primer, and 1 μl of Tween 20/Nonidet P40 (1 : 1, v/v). For labelling of the reaction products with ^{35}S, 4 μl of ^{35}S-Sequetide were added instead of a ^{32}P-labelled nucleotide and the labelling mix supplied with the kit. The labelling solution was mixed immediately with each of the four different 'short mix' aliquots. The extension reaction was stopped after 5 min at 37°C by adding 5 μl of the formamide/dye solution. The reaction mixtures (4 μl) were separated by polyacrylamide gel electrophoresis. Autoradiography was performed for 2 days at room temperature (AGFA X-Ray 90 film).

Sequence alignment

The alignments were carried out with the program package provided by Doolittle [26].

Probe labelling by PCR

Reaction mixtures contained 1 ng of the purified plasmid DNA, 75 pmol of both forward and reverse primers, 1.5 U of *Taq* DNA polymerase, 20 μM of each dATP, dGTP, dTTP, 50 μCi [α-^{32}P]dCTP (3000 Ci/mmol), 60 mM KCl, 2.6 mM $MgCl_2$, 0.3% (v/v) Tween 20, and 15 mM Tris-HCl (pH 8.4), in a total volume of 50 μl. Denaturation, annealing and chain termination were performed in a thermocycler by preheating for 5 min to 95°C followed by 9 cycles of 1 min at 95°C, 1 min at 59°C and 1 min at 72°C with extension of the last cycle for 10 min at 72°C. The probe was purified by gel filtration chromatography with Sephadex G-50 in Nick columns. After heat denaturation for 5 min at 95°C, an aliquot of $5 \cdot 10^6$ cpm was added to 10 ml of hybridization solution.

Northern blot analysis

Messenger RNA (3.5 μg) or total RNA (20 μg) was dissolved in 10 μl of a solution containing 40% formamide, 6% formaldehyde, 40 mM MOPS, 1 mM

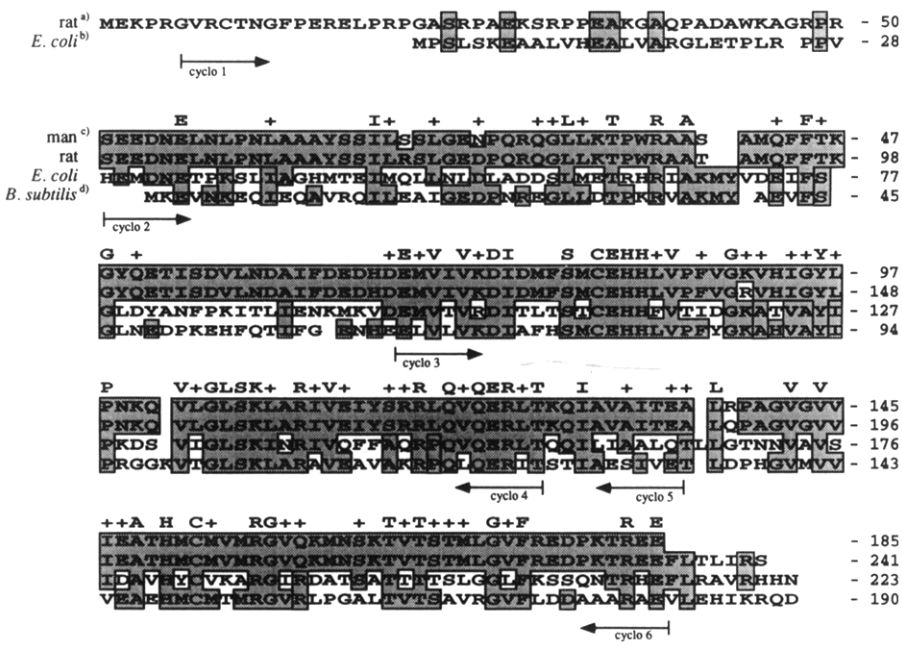

Fig. 2. Alignment of predicted amino acid sequences of GTP cyclohydrolase I. Positions of PCR primers are indicated. Absolute consensus amino acids and conservative exchanges are shown in the top line. [a] Data from Ref. 17; [b] data from Ref. 19; [c] partial amino acid sequence deduced from cDNA segment; [d] deduced amino acid sequence of the mtr A gene product of *B. subtilis* [27].

EDTA and 10 mM sodium acetate (pH 7.0), in a final volume of 10 μl [23]. The sample was heated at 65°C and subsequently size fractionated on a denaturing formaldehyde/Mops gel [23]. RNA markers (1.6–7.4 kb) were used for determination of molecular size. Subsequent to electrophoresis, the RNA was transferred to nylon membranes by vacuum blotting (Pharmacia) with 20 × SSC (20 × SSC = 3 M sodium chloride, 0.3 M trisodium citrate, pH 7.0) and fixed by baking for 2 h at 80°C.

Nylon membranes were prehybridized for 6 h in a solution containing 50% formamide, 5 × SSC, 5 × Denhardt's (1 × Denhardt's = 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA), 0.1% SDS, and salmon sperm DNA (10 μg ml^{-1}). Hybridization was performed for 18 h at 42°C. The membranes were finally washed at 42°C with 0.1 × SSC containing 0.1% SDS for 15 min. Signals from the autoradiograms were analyzed quantitatively by the 2222–020 Ultro Scan XL Laser densitometer (Pharmacia, Freiburg). The range of linear response was determined by calibration curves.

Results

Sequence analysis

The amino acid sequences of GTP cyclohydrolase I from rat and *E. coli* show considerable homology as shown in Fig. 2. PCR primers (Table I) were designed to match the rat cDNA sequence in areas of maximum homology with the *E. coli* gene. Mixed oligonucleotides were used in the 3'-terminal section of the respective primers.

Pairwise combinations of primers were used for PCR experiments using rat RNA as template, as shown in Table II. cDNA was prepared from RNA by reverse transcription and was subsequently amplified. Products were obtained from rat template with most of the primer combinations tested. The lengths of the respec-

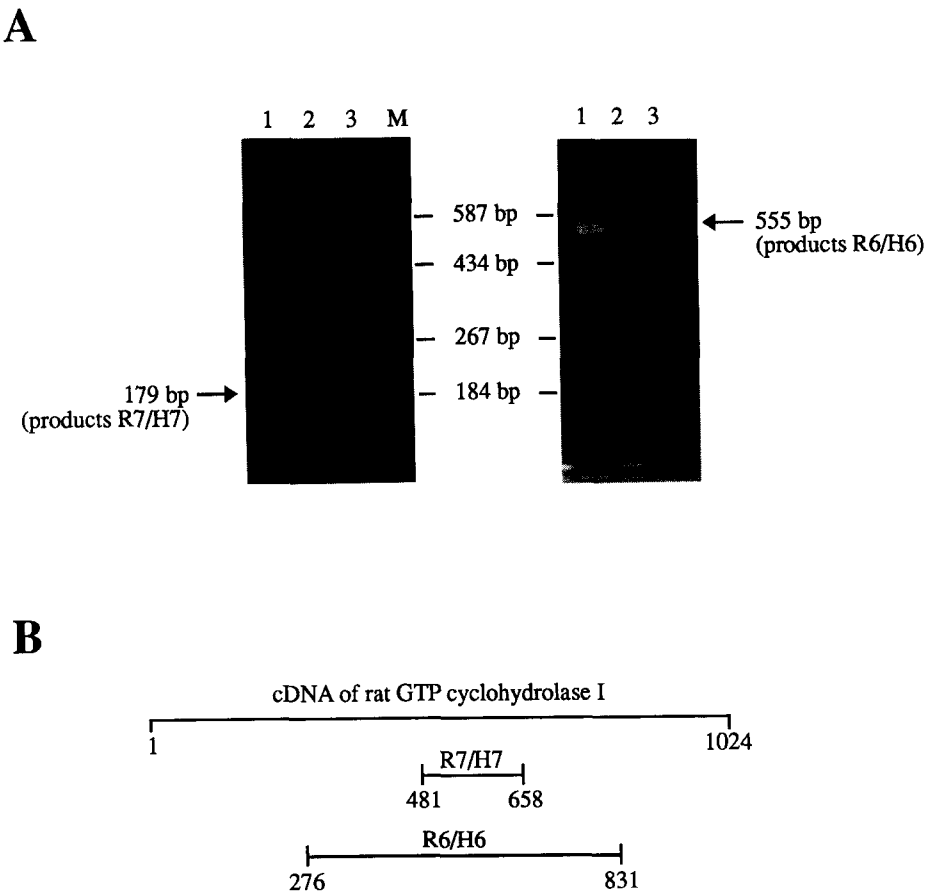

Fig. 3. Products of reverse transcription-PCR. (A) Electrophoresis on a 12% polyacrylamide gel. Template RNAs were from rat liver cell line HTC (lane 1), rat liver (lane 2), and human liver cell line HuH7 (lane 3). Primers: cyclo 3 and cyclo 4 (left), cyclo 2 and cyclo 6 (right). M, molecular weight markers; (B) Map of PCR products. Numbers refer to the cDNA sequence in Ref. 17.

TABLE II

Amplification products obtained in PCR experiments

Primers		Product		Length bp
forward	reverse	rat	human	
Cyclo 1	cyclo 4	R1	n.p. [a]	515
Cyclo 1	cyclo 5	n.p.	n.p.	544
Cyclo 1	cyclo 6	R3	n.p.	688
Cyclo 2	cyclo 4	R4	H4	382
Cyclo 2	cyclo 5	R5	n.p.	411
Cyclo 2	cyclo 6	R6	H6	555
Cyclo 3	cyclo 4	R7	H7	179
Cyclo 3	cyclo 5	R8	n.p.	208
Cyclo 3	cyclo 6	R9	H9	352

[a] n.p., no product observed.

tive PCR products were in agreement with the published cDNA sequence. The PCR product obtained with primer cyclo 2 and cyclo 6 was sequenced after purification by gel electrophoresis and was shown to consist of 555 bp which were identical with the published sequence [17].

The same primer combinations were used in PCR experiments using human cDNA as template. Efficient amplification was observed with primers cyclo 2 and cyclo 6 yielding the DNA species H6 (Fig. 3). This PCR product was purified by gel electrophoresis and HPLC and was sequenced on both strands using DNA primers shown in Table I. The DNA segment encompassed 555 bp in agreement with the corresponding rat fragment R6.

The partial sequence of the human GTP cyclohydrolase I cDNA differed from the rat sequence by 46 nucleotides as shown in Fig. 4. It should be noted that most of the exchanges are located in the wobble positions of the respective codons so that only five different amino acids are coded. The deduced amino acid sequence shows 97% identity with the rat sequence. Among the five amino acid residue exchanges, two were conservative.

An alignment of deduced amino acid sequences from man, rat and *E. coli* is shown in Fig. 2. Moreover, a data bank search showed homology between the proteins under discussion and the product of the mtr A gene of *Bacillus subtilis* [27]. Whereas an enzymatic function of the mtr A gene product from *B. subtilis* has not been established directly, the sequence homology suggests that the gene codes for a GTP cyclohydrolase I. The sequence homology between GTP cyclohydrolase I from bacteria and mammals is remarkably high over a very large evolutionary distance. The 50 absolute consensus amino acids and the conservative exchanges are shown in the top line of the alignment.

The 555 bp fragment H6 from human cDNA was reamplified using primers cyclo 2* and cyclo 6* with extended 5'-ends containing *Sal*I restriction sites. The

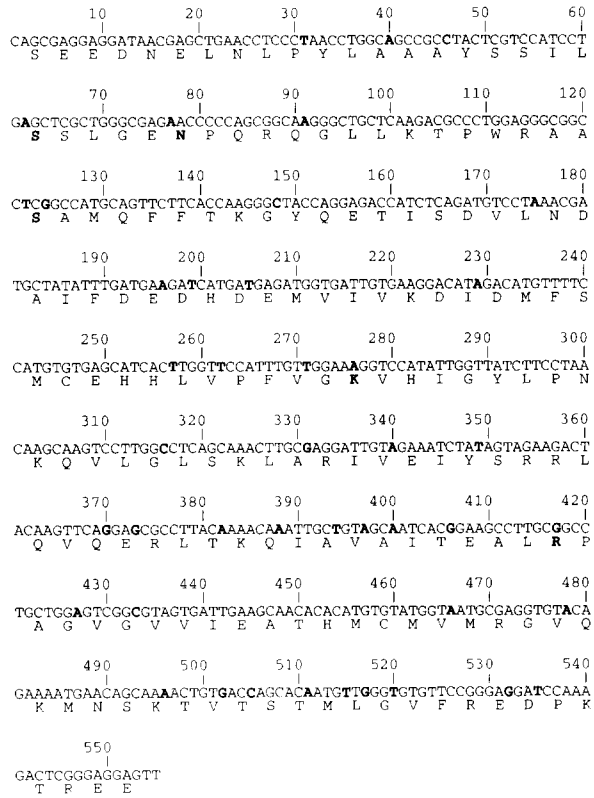

Fig. 4. Partial cDNA sequence of human GTP cyclohydrolase I. Marked nucleotides are different from the rat cDNA sequence [17].

product was purified by electrophoresis and HPLC, digested with *Sal*I endonuclease, and cloned into the *Sal*I site of the plasmid pUC19. Sequencing of the plasmid confirmed the results obtained by direct sequencing of the primary PCR product. The cloned fragment was used as template for radiolabelling of human cDNA probes.

Tissue specifity

Total RNA was obtained from liver, kidney, bone marrow, spleen and brain from rats and from human peripheral blood lymphocytes. Messenger RNA was prepared from several rat and human cell lines. Northern blot analysis was performed using ^{32}P-labelled

TABLE III

Ratio of GTP cyclohydrolase I mRNA species in different rat tissues

Tissue	Relative amount [a]
Liver	0.8 [b]
Kidney	2.4
Bone marrow	0.6
Spleen	1.2
Brain	2.4

[a] 3.6 kb mRNA/1.4 kb mRNA

[b] the values represent the mean of 5 scans of the same autoradiogram which differed < 10%.

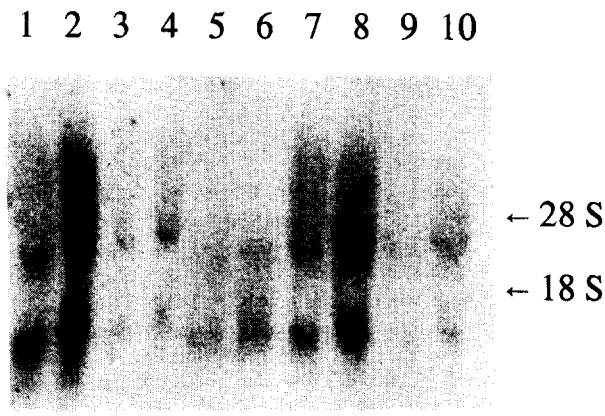

Fig. 5. Northern blot of total RNA from rat organs. Total mRNA (20 or 40 μg) of liver (lanes 1 and 2), kidney (lanes 3 and 4), bone marrow (lanes 5 and 6), spleen (lanes 7 and 8), and brain (lanes 9 and 10) were separated on a 2% agarose gel and hybridized with the radiolabelled rat PCR fragment R7.

probes H6, H7, R6 and R7 (see Table II), which were prepared by PCR and purified as described under Materials and Methods.

The radiolabelled probes R6 and R7 encompassing 555 and 179 nucleotides, respectively, hybridized with two rat mRNA species with approximate sizes of 1.4 and 3.6 kb. The two different species were detected in all rat RNA preparations analyzed (Fig. 5). Studies of hybridization stringency indicated that the probes bound with comparable efficiency to both mRNA species. This indicates that rats produce two different messengers for GTP-cyclohydrolase I. The ratio of the two presumed mRNA species varied between 0.6 and 2.4 in different rat organs (Table III). The large mRNA species was relatively more abundant in brain and kidney.

In contrast to rat RNA, Northern blots of RNA from human origin with probe H7 showed only one band with a size comparable to the large mRNA species

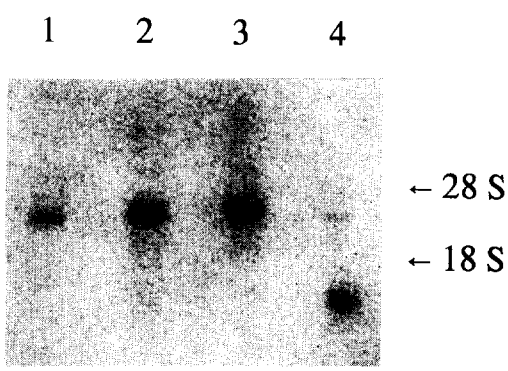

Fig. 6. Northern blot of human and rat RNA. Hybridization was performed with the human PCR fragment H7. Lane 1, 15 μg of total RNA of human peripheral blood lymphocytes stimulated by PHA for 48 h; lane 2, 2 μg of mRNA of human T cell line HUT 102; lane 3, 2 μg of mRNA of human liver cell line HuH7; lane 4, 2 μg of mRNA of rat liver cell line HTC.

found in rat. The human probe detected both mRNA species of the rat (Fig. 6). The rat probe also detected the single human mRNA species (data not shown). The observed cross-hybridization between the rat and human sequences is in line with the high degree of DNA sequence homology.

Discussion

A segment of the human cDNA coding for 185 amino acids of GTP cyclohydrolase I has been cloned and sequenced. The deduced amino acid sequence of this segment shows 97% identity with rat enzyme.

Radiolabelled cDNA probes derived from the human and rat genes detected mRNA species of about 3.6 kb in Northern blots from various human and rat tissues and cells. In addition, all rat RNA specimens contained a smaller mRNA of about 1.4 kb.

It appears likely that the 1.4 kb species from rat corresponds to the cDNA of 1024 bp reported by Hatakeyama et al. [17]. This mRNA would appear to be sufficient for the formation of the GTP cyclohydrolase I subunit from rat as studied by Hatakeyama. The larger mRNA species has not been reported by Hatakeyama et al. [17].

The two different mRNA species observed in rat could arise by differential splicing, but the specific role of the 3.6 kb mRNA species is at present unknown. In light of the various metabolic and regulatory functions of H_4biopterin, the occurrence of different mRNAs could have regulatory significance. It should be noted that the ratio of the two mRNA species shows significant differences in the rat organs studied.

In Northern blots of RNA from human cell lines and tissue, only one mRNA species was found. The size of this band is comparable to the large mRNA species of rat.

The molecular weights reported for mammalian GTP cyclohydrolase I vary over a surprisingly wide range. An early report suggested a subunit mass of about 9.2 kDa for the enzyme from rat brain [28]. More recently, a molecular mass of about 30 kDa was found in studies with the rat liver enzyme [29]. This value was confirmed by the cDNA sequence [17]. A 55.7 kDa subunit was recently suggested for the enzyme from mouse liver [30]. Subunit masses of 50 kDa [31] and 150 kDa [32] were reported for the enzyme from human liver.

Since we have only cloned a segment of the human cDNA, the size of the human protein remains an open question. However, the human mRNA consisting of about 3.6 kb could at best code for a peptide with a mass up to 120 kDa.

It appears that the evolution of GTP cyclohydrolase I has proceeded rather conservatively despite the fact that the enzyme serves in two different pathways, i.e., biosynthesis of tetrahydrofolate in microorganisms and

of H_4biopterin in mammals. The available sequences from two mammalian species and two bacteria show 50 absolutely conserved amino acid residues and a high overall similarity. It is therefore surprising that much larger molecular weights have been reported for the human GTP cyclohydrolase I as compared to the rat and the *E. coli* enzyme. This problem requires further study.

The cloned human cDNA probe provides a tool for the study of GTP cyclohydrolase I regulation at the genetic level in mammalian cell systems.

Acknowledgments

We thank Sabine Lazis for excellent technical assistance and Dr. G.J. Arnold (Laboratorium für Molekulare Biologie, Genzentrum der Ludwigs Maximilians Universität München, Martinsried) for valuable discussions. This work was supported by grants from Deutsche Forschungsgemeinschaft, Bundesministerium für Forschung und Technologie, and Thyssen-Stiftung.

References

1 Kaufman, S. and Fisher, D.B. (1962) In Oxygenases (Hayaishi, O., ed.), pp. 285–37, Academic Press, New York.
2 Duch, D.S., Bowers, S.W., Woolf, J.H. and Nichol, C.A. (1984) Life Sci. 35, 1895–1901.
3 Tayeh, M.A. and Marletta, M.A. (1989) J. Biol. Chem. 264, 19654–19658.
4 Kwon, N.S., Nathan, C.F. and Stuehr, D.J. (1989) J. Biol. Chem. 264, 20496–20501.
5 Ziegler, I. (1990) Med. Res. Rev. 10, 95–114.
6 Ziegler, I., Hamm, K. and Berndt, I. (1983) Cancer Res. 43, 5356–5359.
7 Webber, S. and Jaye, D.L.(1986) In Chemistry and Biology of Pteridines (Cooper, B.A. and Whitehead, V.M., eds.), pp. 235–238, Walter de Gruyter, Berlin.
8 Shen, R.S. and Zhang, Y. (1991) Biochem. Arch. 7, 21–31.
9 Tanaka, K., Kaufman, S. and Milstien, S. (1989) Proc. Natl. Acad. Sci. USA 86, 5864–5867.
10 Kerler, F., Hültner, L., Ziegler, I., Katzenmeier, G. and Bacher, A. (1990) J Cell. Physiol. 142, 268–271.
11 Ziegler, I. and Schwuléra, U. (1989) J. Cell. Biochem. 265, 17026–17030.
12 Nichol, C.A., Smith, G.K. and Duch, D.S. (1985) Annu. Rev. Biochem. 54, 729–764.
13 Duch, D.S. and Smith, G.K. (1991) J. Nutr. Biochem. 411–423.
14 Kerler, F., Ziegler, I., Schwarzkopf, B. and Bacher, A. (1989) FEBS Lett. 250, 622–624.
15 Ziegler, I., Schott, K., Lübbert, M., Herrmann, F., Schwuléra, U. and Bacher, A. (1990) J. Biol. Chem. 265, 17026–17030.
16 Schott, K., Brand, K., Hatakeyama, K., Kagamiyama, H., Maier, J., Werner, T. and Ziegler, I. (1992) Exp Cell Res., in press.
17 Hatakeyama, K., Inoue, Y., Harada, T. and Kagamiyama, H. (1991) J. Biol. Chem. 266, 765–769.
18 Katzenmeier, G., Schmid, C. and Bacher, A. (1990) FEMS Microbiol. Lett. 66, 1–3.
19 Katzenmeier, G., Schmid, C., Kellermann, J., Lottspeich, F. and Bacher, A. (1991) Biochem. Hoppe-Seyler 372, 991–997.
20 Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
21 Sawadogo M. and Van Dyke M.W. (1991) Nucleic Acids Res. 19, 674.
22 Kawasaki, E.S. (1990) In PCR Protocols (Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., eds.), pp. 21–27, Academic Press, San Diego.
23 Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
24 Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
25 Bachmann, B., Luke, W. and Hunsmann, G. (1990) Nucleic Acids Res. 18, 1309–1314.
26 Feng, D.F. and Doolittle, R.F. (1987) J. Mol. Evol. 25, 351–360.
27 Babitzke, P., Gollnick, P. and Yanofsky, C. (1992) J. Bacteriol. 174, 2059–2064.
28 Gál, E.M., Dawson, M.R., Dudley, D.T. and Sherman, A.D. (1979) Neurochem. Res. 4, 605–626.
29 Hatakeyama, K., Harada, T., Shuhei,.S., Yasuyoshi, W. Kagamiyama, H. (1989) J. Biol. Chem. 264, 21660–21664.
30 Cha, K.W., Jacobson, K.B. and Yim, J.J. (1991) J. Biol. Chem. 226, 12294–12300.
31 Schoedon, G., Redweik, U. and Curtius, H.C. (1989) Eur. J. Biochem. 178, 627–634.
32 Shen, R.S., Alam, A. and Zhang, Y. (1989) Biochimie 71, 343–349.