Human GTP cyclohydrolase I: only one out of three cDNA isoforms gives rise to the active enzyme

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GTP cyclohydrolase I catalyses the first and rate-limiting step of tetrahydrobiopterin biosynthesis. Its expression is regulated by interferon-γ or kit ligand in a tissue-specific manner. Three different cDNA forms have been reported for human GTP cyclohydrolase I [Togari, Ichinose, Matsumoto, Fujita and Nagatsu (1992) Biochem. Biophys. Res. Commun. **187**, 359–365]. We have isolated, from a human liver cDNA library, two clones which contained inserts identical with two of the cDNAs reported by Togari et al. (1992). The three open reading frames corresponding to all reported cDNA sequences were expressed in *Escherichia coli*. Only the recombinant protein corresponding to the longest reading frame catalysed the conversion of GTP into dihydroneopterin triphosphate. The proteins corresponding to the shorter reading frames failed to catalyse not only the generation of dihydroneopterin triphosphate but also the release of formate from GTP, an intermediate step of the reaction. Recombinant human GTP cyclohydrolase I showed sigmoidal substrate kinetics and maximum activity at 60 °C. These findings are well in line with the published properties of the enzyme isolated from rat liver. The data indicate that cytokine-mediated induction of GTP cyclohydrolase I is not due to the expression of enzyme isoforms.

INTRODUCTION

BH_4 serves as an electron donor for hydroxylation of the aromatic amino acids phenylalanine, tryptophan and tyrosine (for review see [1]). More recently it was shown that BH_4 is also involved in the generation of NO from arginine [2,3]. A large body of evidence shows that BH_4 can also be synthesized in cells which do not utilize it as a cofactor [4] for any of these metabolic pathways. These findings have been conducive to the hypothesis that BH_4 is involved in cytokine-directed cell proliferation (for review see [5]). BH_4has been shown to modulate the clonal expansion of T cells [6–8] and the proliferation of erythroid cells [9,10]. It enhances the affinity of the interleukin-2 receptor complex to its ligand and affects various aspects of signal transduction [11].

In accordance with the multiple functions of BH_4, its biosynthesis is individually and specifically regulated in different cell types and tissues. It is synthesized in all tissues competent for phenylalanine degradation or neurotransmitter biosynthesis, such as liver, brain or adrenal medulla [12]. In macrophages [13] or in primed T cells [14], the accumulation of neopterin and BH_4 is selectively triggered by IFN-γ. IFN-γ also appears to control BH_4 formation in NO-producing endothelial cells or macrophages [15,16]. On the other hand, kit ligand has been shown to act as the primary inducer of BH_4 synthesis in bone-marrow-derived murine mast cells [17]. This cytokine is also known as mast-cell growth factor, stem-cell factor or steel factor. It binds to the product of the proto-oncogene c-kit, a receptor with tyrosine kinase activity [18]. In bone-marrow-derived murine mast cells, interleukin-3 triggers an increase in tryptophan 5-mono-oxygenase activity. The co-operation of both kit ligand and interleukin-3 results in a maximum level of 5-hydroxytryptamine formation [17].

The biosynthesis of BH_4 *de novo* begins with GTP, and the first committed step is catalysed by GTP cyclohydrolase I (EC 3.5.4.16) (for reviews see [19,20]). The complex enzyme reaction begins with the release of C-8 of the purine system as formate. It is assumed that subsequently the ribose side chain undergoes an Amadori rearrangement, which is followed by closure of the pyrazine ring to yield dihydroneopterin triphosphate (Figure 1). The subsequent action of 6-pyruvoyl-tetrahydropterin synthase and sepiapterin reductase yields the final product, BH_4.

The regulation of BH_4 synthesis primarily occurs at the level of GTP cyclohydrolase I. High activities of this enzyme are found in liver, brain and adrenal medulla [21]. The induction of BH_4 synthesis by IFN-γ or by kit ligand can be explained satisfactorily by increases in the apparent activity of GTP cyclohydrolase I. The IFN-γ-mediated up-regulation of activity in monocytes/macrophages or in primed T cells [14] correlated with increases in the steady-state mRNA levels specific for GTP cyclohydrolase I [22]. A post-translational control of GTP cyclohydrolase I activity was shown in rat liver, where BH_4 acts as a feedback regulator via a regulatory protein [23].

The sequence of GTP cyclohydrolase I from various species

Abbreviations and trivial names used: BH_4, (6*R*)-5,6,7,8-tetrahydrobiopterin; biopterin, 6-(L-*erythro*-1′,2′-dihydroxypropylpterin); neopterin, 6-(D-*erythro*-1′,2′,3′-trihydroxypropylpterin); 6-pyruvoyl-tetrahydropterin, (6*R*)-(1′,2′-dioxopropyl)-5,6,7,8-tetrahydropterin; IFN-γ, interferon-γ; Fmoc, 9-fluorenylmethoxycarbonyl; Pmc, 2,2,5,7,8-pentamethyl-chroman-6-sulphonyl; tBu, t-butyl; TFA, trifluoroacetic acid; Tween 20, hydroxyethylenesorbitan monolaurate; MBP-2, maltose-binding protein 2.

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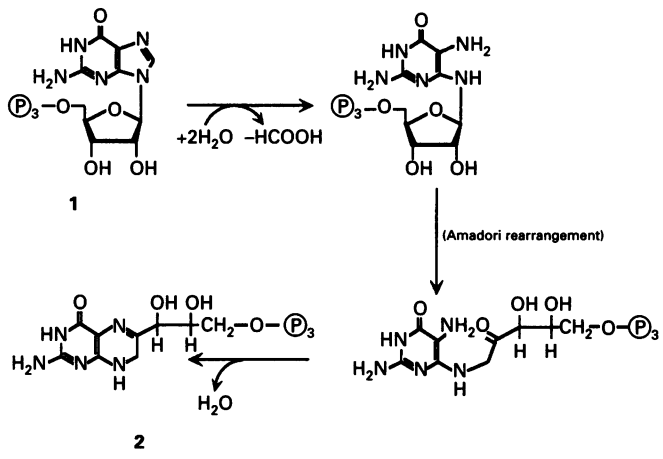
The cDNA sequences described in this paper have been submitted to the GenBank datebase with accession numbers L27626 and L27627.



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2

Figure 1 Hypothetical reaction mechanism of GTP cyclohydrolase I

1, GTP; **2**, dihydroneopterin triphosphate.

has been determined [24–30]. Northern-blot analysis of RNA from rat tissues showed two specific mRNA forms of approx. 1.4 and 3.6 kb [29,31]. The ratio between these two species varies between 0.6 and 2.4 in different tissues [29]. On Northern blots of mRNA from human tissue and cell lines, only one 3.6 kb mRNA species could be detected [29]. On the other hand, Togari et al. [25] reported three different cDNA clones for human GTP cyclohydrolase I which were identical at their central and 5′ region, but diverged at the 3′ ends. However, it is unknown whether all three cDNA isoforms code for catalytically active proteins.

In order to address the question whether the control of GTP cyclohydrolase I can be explained by cell-type-specific synthesis of isoenzymes, we report the expression of the three putative open reading frames in *Escherichia coli*. Our data show that only one of the recombinant proteins has enzymic activity.

MATERIALS AND METHODS

cDNA library screening

Radioactive probes were obtained by labelling the cDNA probes with [α-^{32}P]dATP by random priming [32]. A plasmid-based cDNA library prepared from human liver mRNA by oligo(dT) priming was kindly provided by E. Weiss [33] and was screened with a radiolabelled 555 bp cDNA probe of human GTP cyclohydrolase I [29] hybridizing to positions 177–732 of GTP cyclohydrolase I type 1 mRNA [25]. Hybridization was performed at a concentration of 5×10^6 c.p.m. of labelled fragment/ml of hybridization solution, and the blots were washed as described by Church and Gilbert [34].

Sequence analysis

DNA sequencing was performed by the dideoxy chain-termination method [35], using the T7-polymerase sequencing kit supplied by United States Biochemicals (Braunschweig, Germany). Plasmid DNA was sequenced in both directions by using different oligonucleotides.

Expression of recombinant proteins

For expression of recombinant GTP cyclohydrolase I in *E. coli* either as fusion proteins or as unmodified enzyme, the following plasmids and strains were used: pQE-6, pQE-9 and pQE-40 (Qiagen, Chatsworth, CA, U.S.A.), pMAL-C2 (New England Biolabs, Schwalbach, Germany) and XL-1 blue (Stratagene, La Jolla, CA, U.S.A.). Bacteria were cultivated at 37 °C with agitation in LB medium [36] supplemented with ampicillin (50 μg/ml) and tetracycline (15 μg/ml). When the A_{600} of the culture reached a value of 0.6, isopropyl β-D-1-thio-galactopyranoside was added to a final concentration of 1 mM. Bacteria were harvested 5 h after induction and were lysed by ultrasonication (Sonifier B-12; Branson, Danbury, CT, U.S.A.). The cell debris was removed by centrifugation at 10000 *g*, and phenylmethanesulphonyl fluoride was added to the supernatant to a final concentration of 0.25 mM. The crude extract was either used immediately for further purification of the recombinant proteins, or mixed with an equal volume of glycerol and stored at −20 °C. Fusion proteins of MBP-2 and GTP cyclohydrolase I were purified according to the manufacturer's instructions (New England Biolabs). This involved capture on an amylose resin for binding of fusion proteins, followed by elution with 10 mM maltose. Purification of histidine and dihydrofolate reductase fusion proteins was carried out under denaturing (8 M urea) or non-denaturing conditions on Ni-chelating agarose (Ni-NTA-agarose) according to the manufacturer's instructions (Qiagen).

Peptide synthesis and coupling

The peptides A and B were synthesized by the solid-phase method utilizing the Fmoc/tBu strategy [37,38] on a manual peptide synthesizer Biolynx 4174 (LKB/Pharmacia, Freiburg, Germany) in the continuous-mode technique with Fmoc amino acid pentafluorophenyl esters on polyamide resins (Pepsyn-KA resins, Milligen-Biosyntech, Hamburg, Germany). Arginine side chains were protected by Pmc. Acylation and deprotection reactions were controlled by u.v. monitoring (Ultrospec-II; Pharmacia, Freiburg, Germany).

The final cleavage of protecting groups and resins was performed with TFA/water (19:1, v/v). The crude peptides were purified by preparative h.p.l.c. on a reversed-phase Hire-Sil C_{18} column (10 μm; 250 mm × 20 mm; Chemdata, Gross Zimmern, Germany) with gradient elution (solvent A, 0.1 % TFA in water; solvent B, 0.08 % TFA in acetonitrile). The purified peptides were characterized by analytical h.p.l.c. on a reversed-phase Nucleosil C_{18} column (5 μm; 200 mm × 4 mm; Macherey & Nagel, Düren, Germany) and by fast-atom-bombardment mass spectrometry.

The peptides were coupled to carrier proteins by using glutaraldehyde [39]. The synthetic peptide (1 mg) was dissolved in 1 ml of PBS. BSA (1 mg) or ovalbumin (1.2 mg) was added to the solution. An equal volume of glutaraldehyde (0.2 % in PBS) was added with stirring. The mixture was incubated for 1 h at room temperature. Glycine was added from a 1 M stock solution in PBS to a final concentration of 200 mM. After 1 h at room temperature, the mixture was dialysed against PBS overnight. The conjugate was stored in portions at −20 °C.

Immunization

New Zealand White rabbits (female, 8 weeks) were immunized subcutaneously with 250 μg of the recombinant protein His-p21 or with 1 mg of peptide/protein conjugate in complete Freund's adjuvant. Four additional injections with the antigen suspended

in incomplete Freund's adjuvant were given at intervals of 2 weeks. Antisera were tested by enzyme-linked immunoassay and Western blotting.

Enzyme-linked immunoassay

Peptide–ovalbumin conjugate was dissolved in 20 mM sodium carbonate buffer, pH 9.6, at a final concentration of 0.1 mg/ml. Microtitre plates (96-well) were coated overnight, blocked with 2% dried skimmed milk in PBS and washed with PBS. Rabbit antisera were diluted in PBS containing 1% BSA in order to adsorb anti-BSA antibodies. Staining was carried out with peroxidase-conjugated goat anti-rabbit Ig antibody (Cappel-Organon Technica, Eppelheim, Germany; no. 32120231, diluted 1:250 in PBS) and with *o*-phenylenediamine by using standard protocols [40].

Preparation of antigen–agarose and purification of antisera

CNBr-activated Sepharose (Pharmacia; 4 g) was swollen for 4 h in 1 mM HCl to a volume of about 15 ml and washed with water. Samples (3 mg) of peptide or BSA were dissolved in 6 ml of 50 mM carbonate buffer, pH 8.3. The insoluble protein His-p21 was suspended in 6 ml of the same buffer by ultrasonication. Agarose (5 ml) was added to each respective mixture. After incubation for 2 h at room temperature, 2 ml of a solution containing of 1.5 M Tris/HCl and 200 mM glycine, pH 8.3, was added, and the mixture was incubated for 2 h at room temperature. The gel was washed with 0.1 M sodium acetate, pH 4.0, and equilibrated with 50 mM Tris/HCl, pH 8.0, containing 137 mM NaCl and 27 mM KCl (Tris-buffered saline). Antisera (10 ml) were dialysed against Tris-buffered saline overnight and applied to a column of Blue Sepharose (Pharmacia; 1 cm × 1.5 cm) equilibrated against the same buffer. Fractions containing protein were collected and passed through a BSA–agarose column (1 cm × 1.5 cm). The effluent was applied to the specific antigen–agarose column (1 cm × 1 cm), which was washed with 10 ml of Tris-buffered saline. The bound antibodies were eluted with 5 ml of 100 mM glycine, pH 2.5. The column was then washed with Tris-buffered saline, and a second elution was performed with 5 ml of 100 mM triethylamine, pH 11.5 (freshly prepared). Fractions (300 μl) were collected and were neutralized by addition of 500 μl of 1 M Tris/HCl, pH 7.5. Fractions containing protein were analysed by enzyme-linked immunoassays and by Western blotting. Fractions found to contain antibody were combined, dialysed against PBS and stored in batches with 50% glycerol at −20 °C.

Western blotting

Proteins were separated by SDS/PAGE [41] in 6–20% slab gels. Polyacrylamide gels and poly(vinylidene difluoride) membranes (Immobilon P; Millipore, Eschborn, Germany) were equilibrated with transfer buffer (48 mM Tris, 39 mM glycine, 0.0375% SDS, 20% methanol) for 30 min. Transfer was performed by semi-dry blotting (Hoefer Scientific Instruments, Heidelberg, Germany; 10 min at 0.5 mA/cm^2 + 60 min at 1 mA/cm^2). The membrane was blocked with 5% dried skimmed milk in Tris-buffered saline containing 0.05% Tween 20 for 2 h. Antisera were diluted 1:50 in Tris-buffered saline containing 0.05% Tween 20 and 1% BSA. The membrane was washed with Tris-buffered saline containing 0.05% Tween 20 and was stained with peroxidase-conjugated goat anti-rabbit Ig antibody (Cappel-Organon Technica; 1:250 in PBS) and diaminobenzidine, as described in [42].

Determination of GTP cyclohydrolase I activity

Protein solutions were dialysed against 50 mM Tris/HCl, pH 8.0, and were incubated at 37 °C with 0.5 mM GTP as described previously [14]. GTP cyclohydrolase I activity was assayed (i) by the formation of dihydroneopterin triphosphate which was oxidized by acidic iodine and monitored by h.p.l.c. [14] or (ii) by the release of formic acid from [8-^{14}C]GTP (Amersham, Braunschweig, Germany) as described by Fukushima et al. [43].

Protein concentrations were determined by the method of Bradford [44], using dye reagent from Bio-Rad (München, Germany) and BSA as a standard.

RESULTS

Antisera to GTP cyclohydrolase I

A 555 bp cDNA segment of human GTP cyclohydrolase I [29] spanning the nucleotide positions 177–732 was cloned into the *Sal*I site of the plasmid pQE-9. The resulting construct coded for a fusion protein consisting of a 185-amino-acid fragment of GTP cyclohydrolase I (see Figure 2, protein a) preceded by 6 histidine residues. This recombinant protein (His-p21) was expressed in *E. coli* and could be purified under denaturing conditions (8 M urea) on Ni-NTA-agarose. The precipitate formed during dialysis was used to raise an antiserum in rabbits.

Rabbit antisera were also raised against two peptides named A (LSSLGENPQRQ) and B (KGGTREEFLTLIRS) corresponding to the amino acids 79–83 and 240–250 of human GTP cyclohydrolase I, respectively (see Figure 2, protein b). The three N-terminal amino acids of peptide B are not present in the GTP cyclohydrolase I sequence, but were added to facilitate coupling to BSA. As shown in Figure 3, the recombinant polypeptide His-p21 was detected by its specific antiserum and by the antiserum to peptide A, but not by an antiserum to peptide B. This result is in line with the expectation, since the sequence of peptide B is not part of the recombinant polypeptide.

Expression and immunological mapping of recombinant proteins

A human liver cDNA library consisting of 3×10^5 colonies was screened by using a 555 bp cDNA probe for GTP cyclohydrolase I described previously [29]. Two clones containing cDNA inserts of 1072 and 1752 bp were isolated and sequenced. The inserts were found to be identical at their 5′ region, but diverged at their 3′ ends. The predicted amino acid sequences corresponding to the respective reading frames were named GTP cyclohydrolase I type 1 and type 2. Their sequences were identical with those described by Togari et al. [25]. The predicted sequence for GTP cyclohydrolase I type 1 encompassed 250 amino acid residues with a calculated molecular mass of 27903 Da (see Figure 2, protein b). The predicted sequence for GTP cyclohydrolase I type 2 encompassed only 213 amino acids, of which the first 209 were identical to type 1 (see Figure 2, protein c). The 1752 bp clone coding for GTP cyclohydrolase I type 2 extended the published cDNA sequence [25] by 727 bp and 67 bp of the non-coding 3′ and 5′ sequences, respectively. Although the library was constructed by using oligo(dT) for priming, a poly(A) tail was not found in either sequence.

For the construction of type 3 GTP cyclohydrolase I cDNA [25] (see Figure 2, protein d), the clone that coded for type 2 was cleaved at its single *Hinf*I site (position +602), and a 47 bp synthetic adaptor including a stop codon was added to the 3′ end.

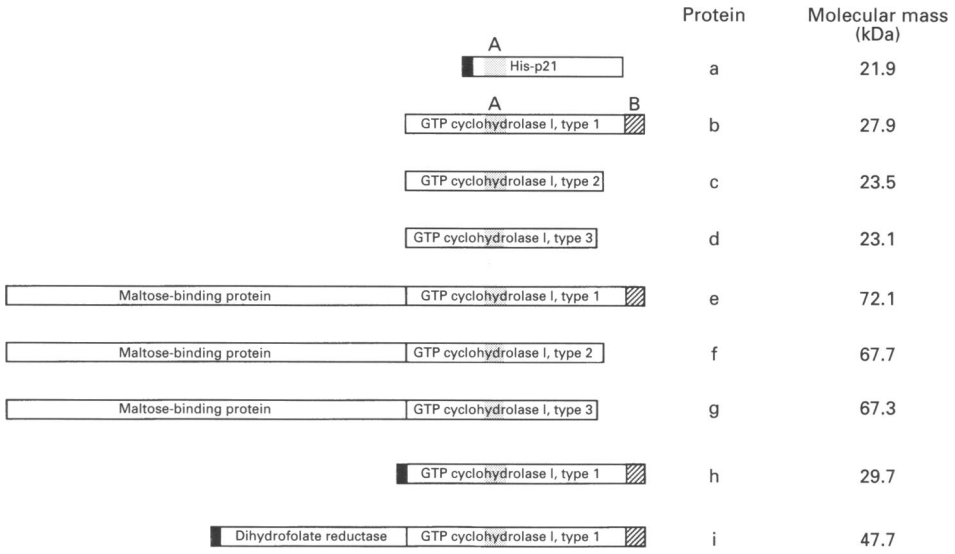

Figure 2 Expressed recombinant proteins corresponding to the open reading frames of GTP cyclohydrolase I types 1–3

Positions of the 6 × histidine peptide (black bar), of peptide A (stippled) and of peptide B (hatched) are indicated.

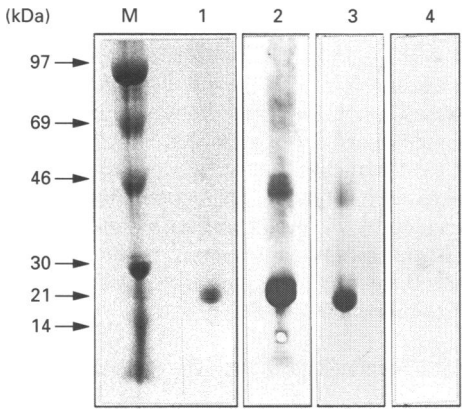

Figure 3 Western-blot analysis of protein His-p21 (see Figure 2, protein a)

A 1 μg portion of the protein was subjected to SDS/PAGE, stained with Coomassie Brillant Blue G-250 (lane 1) and blotted on to poly(vinylidene difluoride) membranes (lanes 2–4). Detection was performed with antisera to His-p21 (lane 2), peptide A (lane 3) and peptide B (lane 4). Lane M, molecular mass markers.

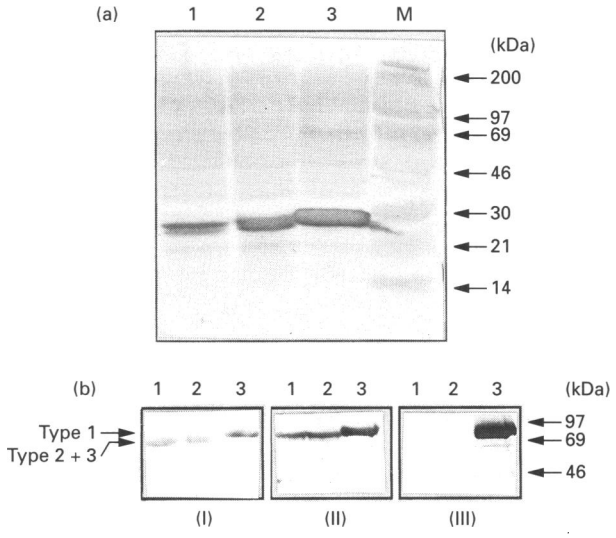

Figure 4 Detection of recombinant proteins by Western-blot analysis

(**a**) Extracts of *E. coli* (50 μg) expressing the unmodified proteins which corresponded to the reading frames of GTP cyclohydrolase I type 3 (lane 1), type 2 (lane 2) and type 1 (lane 3). The molecular masses of these proteins are indicated in Figure 2 (proteins b–d). Detection was performed with an antiserum to His-p21. (**b**) Purified fusion proteins (1 μg) of GTP cyclohydrolase I type 3 (lane 1), type 2 (lane 2) and type 1 (lane 3) were detected with antisera to His-p21 (I), peptide A (II) and peptide B (III). The molecular masses of these fusion proteins are also included in Figure 2 (proteins e–g). Lane M, molecular mass markers.

All three forms of GTP cyclohydrolase I cDNA were expressed in *E. coli* either without modification (see Figure 2, proteins b–d), or as C-terminal fusion proteins to MBP-2 of *E. coli* (see Figure 2, proteins e–g). Type 1 GTP cyclohydrolase I was also expressed with six N-terminal histidine residues and as a C-terminal fusion protein to murine dihydrofolate reductase (Figure 2, proteins h and i). Like His-p21, the histidine-linked type 1 protein did not bind to the resin under non-denaturing conditions. After treatment with 8 M urea, the enzyme activity was lost and could not be recovered after dialysis. The MBP-2 fusion protein was purified about 380-fold on amylose–agarose under non-denaturing conditions.

On Western blots, the antisera to His-p21 and to peptide A detected all three forms of GTP cyclohydrolase I (Figure 4). The sequence of peptide B is only present in GTP cyclohydrolase I type 1, which is larger than type 2 and 3 (see Figure 2). Therefore, GTP cyclohydrolase I type 1 is the only form that is detected by antibody to peptide B. This confirmed the appropriate length and sequence of the recombinant proteins. The antiserum to His-p21 or that to the peptides did not immunoprecipitate the native enzyme or modify its catalytic properties. In enzyme-linked

Table 1 Specific activity of recombinant enzymes determined by formation of dihydroneopterin phosphates or by release of [^{14}C]formate from [8-^{14}C]GTP

The data (in pmol/min per mg) represent the means ± SD of three experiments. Abbreviation: DHFR, dihydrofolate reductase.

	Measured by formation of dihydroneopterin triphosphate				Measured by release of [^{14}C]formate
Enzyme	Crude extracts Unmodified protein	MBP-2 fusion protein	DHFR-fusion protein	Purified proteins MBP-2 fusion proteins	Purified proteins MBP-2 fusion proteins
Type 1	374 ± 15	771 ± 92	110 ± 8	292000 ± 6000	249000 ± 34000
Type 2	1.6 ± 0.9	0.9 ± 0.4	–	< 0.01	< 4000
Type 3	0.9 ± 0.5	1.8 ± 1.1	–	< 0.01	< 4000

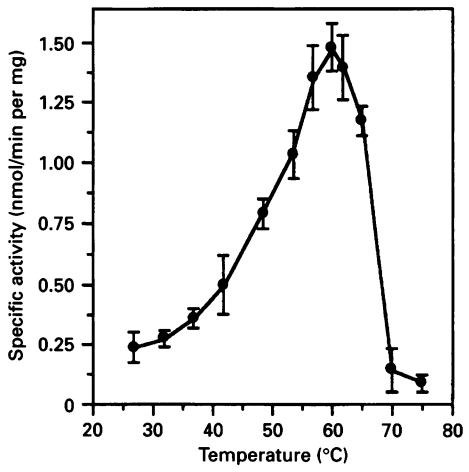

Figure 5 Formation of dihydroneopterin triphosphate by recombinant GTP cyclohydrolase I type 1

Extracts of *E. coli* expressing GTP cyclohydrolase I type 1 were incubated with GTP (500 μM) at different temperatures for 3 h. Neopterin phosphates were determined by h.p.l.c. The values represent means ± SD of three experiments.

immunoassays, only anti-His-p21 reacted slightly with a crude extract of *E. coli* expressing recombinant type 1 human GTP cyclohydrolase I (results not shown).

Enzymic properties of recombinant GTP cyclohydrolase I

The catalytic properties of the purified MBP-2 fusion proteins and the unmodified proteins in extracts of *E. coli* are summarized in Table 1. Only type 1 GTP cyclohydrolase I catalysed dihydroneopterin triphosphate formation from GTP. Moreover, the release of formic acid from GTP could only be detected with GTP cyclohydrolase I type 1 (Table 1).

The recombinant GTP cyclohydrolase I type 1 proved to be a heat-stable enzyme. The maximum turnover rate was found at 60 °C (Figure 5). The enzyme has a broad pH optimum between 7.4 and 8.2. Positive co-operativity for GTP was found with the human protein, in close similarity to the purified rat enzyme [45]. The unmodified protein showed Hill coefficients between 1.6 and 1.9, depending on the KCl concentration (Figure 6). Sigmoidal kinetics was shown for both the unmodified protein and the histidine fusion protein (results not shown). KCl shifted the $K_{0.5}$ value to higher GTP concentrations, whereas the $V_{max.}$ was only slightly influenced (Figure 6). Both fusion proteins showed sigmoidal kinetics in the absence of KCl. Addition of > 100 mM

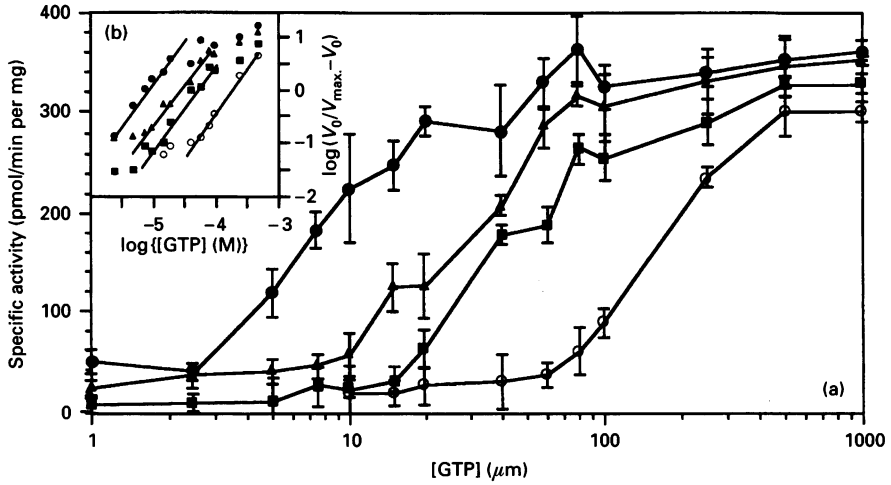

Figure 6 Substrate kinetics of recombinant GTP cyclohydrolase I type 1

(**a**) Influence of KCl on the activity of recombinant GTP cyclohydrolase I type 1. Formation of dihydroneopterin triphosphate was determined at 37 °C in the presence of 0 mM (●), 100 mM (▲), 200 mM (■) and 400 mM (○) KCl. The values represent means ± SD of five experiments. (**b**) Hill plot: influence of various concentrations of KCl on the Hill coefficient. Symbols indicating molarities of KCl are the same as in (**a**). Hill coefficients, evaluated from slopes, were 1.6 (●), 1.6 (▲), 1.8 (■) and 1.9 (○).

Table 2 Specific activities reported for purified GTP cyclohydrolase I

Species	Specific activity (nmol/min per mg)	Assay	Reference
Human	9.4	H.p.l.c	44
Rat	45	H.p.l.c.	41
Mouse	1.5	H.p.l.c.	45
Drosophila melanogaster	6.8	Formate release	46
E. coli	700	Formate release	47
E. coli	46	H.p.l.c.	*
Human recombinant fusion protein	290	H.p.l.c.	

* A. Bacher (unpublished work).

KCl changed the kinetics of the fusion proteins from sigmoidal to hyperbolic type (results not shown).

DISCUSSION

Three different cDNA forms for human GTP cyclohydrolase I, implicating open reading frames with different 3′ portions, have been reported by Togari et al. [25]. We have independently confirmed two of these reading frames by sequencing of two clones obtained from an oligo(dT)-primed human liver cDNA library.

It should be noted that Northern blots of mRNA from human tissues and cell lines hybridized with a probe corresponding to the central part of the cDNA showed only one signal of 3.6 kb. Although our clones encompassed longer untranslated 5′ and 3′ regions than the clones of Togari et al. [25], the full-length sequence of any human GTP cyclohydrolase I mRNA has not yet been determined.

The proteins corresponding to each of the three putative open reading frames were expressed in *E. coli* in their native forms and as fusion proteins. Only the protein that was encoded by the longest reading frame showed enzymic activity. The other protein forms did not only fail to produce dihydroneopterin triphosphate, the end product of the enzymic reaction; they also failed to release formate from GTP, which is believed to occur as an intermediate step before closure of the pyrazine ring [46]. The protein encoded by the longest open reading frame can therefore be considered to represent the functional enzyme.

Our data indicate that the regulation of GTP cyclohydrolase I activity by cytokines occurs via increased expression of the type 1 protein, rather than through switching to other isoforms. A potential function of both catalytically inactive recombinant proteins, not immediately related to dihydroneopterin triphosphate formation, remains to be determined.

The reported specific activities of GTP cyclohydrolase I purified from different species differed by two orders of magnitude (Table 2). These differences are obviously due to the different levels of purification which in one case reached only 200-fold [47]. In other cases, sequencing of the protein [45] or crystallization [48] proved highest purity. The recombinant human protein is in the upper activity range.

The sequences of our clones were identical with the published sequence [25] up to position 626. In the larger clone, the next two nucleotides were GT, the characteristic beginning of introns. Therefore it cannot be ruled out at present that an incompletely spliced mRNA was reverse-transcribed and isolated.

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