

Cloning, expression, characterization, and crystallization of a glutaminyl cyclase from human bone marrow: A single zinc metalloenzyme

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Abstract

Glutaminyl cyclase (QC) catalyzes the N-terminal pyroglutamate formation of numerous hormones and peptides from their glutaminyl precursor. Pyroglutamate is a posttranslational or cotranslational modification important in many physiological and pathological processes. Here, we present the cloning of a QC cDNA from human bone marrow cDNA library. The protein was expressed in *Escherichia coli* system with the yields higher than ~10 mg/L bacterial culture, using a thioredoxin-tagged expression vector with several modifications. Based on high histidine content (~5%) of the protein, a convenient purification step by Ni-affinity chromatography was designed, leading to near homogeneity of the purified human QC. The identity of the recombinant human QC was confirmed by mass spectrometry and circular dichroism spectroscopy. The enzyme was active on both synthetic and physiological substrates, and the activity could be inhibited by several imidazole, triazole, and tetrazole derivatives. An atomic absorption analysis demonstrated that human QC contains one zinc ion per protein molecule. We also obtained the human QC crystals, which belong to cubic, tetragonal, and rhombohedral forms. Our works are useful to acquire new insights into human and animal QCs, particularly for future structural analysis and inhibitor designs.

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Glutaminyl cyclases (QCs)¹ (EC 2.3.2.5.) are acyl-transferases responsible for the conversion of the protein N-terminal glutaminyl residue into pyroglutamic acid

(pGlu) with the concomitant liberation of ammonia. This cyclization reaction is important during the maturation of numerous neuropeptides and cytokines, such as thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), and monocyte chemotactic protein-2 (MCP-2), in the secretory pathway. The role of pGlu on these bioactive peptides is believed to be in (1) developing the proper conformation of the peptides to bind to their targets and/or (2) protecting the peptides from exopeptidase degradation [1,2].

To date, several QCs are found to be present in mammalian neuroendocrine tissues, particularly in hypothalamus

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¹ Abbreviations used: QC, glutaminyl cyclase; pGlu, pyroglutamic acid; TRH, thyrotropin-releasing hormone; GnRH, gonadotropin-releasing hormone; MCP-2, monocyte chemotactic protein-2; SNPs, single nucleotide polymorphisms; AD, alzheimer's disease; DS, down's syndrome; IPTG, isopropyl-β-D-thiogalactopyranoside; CD, circular dichroism.

and pituitary [3–6]. These animal QCs appear to have no sequence homology and distinct protein stability from plant QCs, although they are similar monomeric proteins with close molecular masses. The papaya QC was shown to consist almost solely of β -sheet structure, highly resistant to proteolytic, chemical, acid, and thermal denaturation [7,8]. In contrast, human QC was predicted to adopt an α/β topology, exhibiting a remarkable instability above pH 8.5 and below pH 6.0 [9,10]. Based on the present knowledge, human QC may share a conserved scaffold of the bacterial aminopeptidases [11,12], but, interestingly, no bacterial QCs have been reported thus far.

In human, the aberrant formation of pGlu may be related to some pathological processes, such as osteoporosis and amyloidotic diseases. Ezura et al. [13] reported that 13 single nucleotide polymorphisms (SNPs) in the region of QC gene are strikingly correlated with the osteoporosis susceptibility in adult women. This is probably due to the reduced activity of human QC, resulting in an abnormal homeostasis of GnRH and estrogen. In addition, the plaque-forming peptides, e.g., A β N3(pGlu)-40/42, seem to be directly correlated with the severity and the progression of the amyloidotic diseases, such as Alzheimer's disease (AD) and Down's syndrome (DS) [14,15]. The pGlu on these plaque-forming peptides is converted from a glutamyl residue, and contributes to the hydrophobicity and proteinase resistance of these peptides. Such a glutamyl-to-pGlu conversion was demonstrated to be also catalyzed by human QC in vitro [16].

So far, there is no available 3D structure of QCs in the literature. Although functional human QC has been expressed in yeast and insect cell systems [9,17], expression of the protein still encounters problems with insolubility, low recovery, and heterogeneity of the protein. In this report, we present the cloning of a QC from human bone marrow cDNA library. The protein was successfully expressed in *Escherichia coli* (*E. coli*) system with high yield via the Nus protein- or the thioredoxin-fusion expression vectors with several modifications. The recombinant human QCs were active on the synthetic and physiological substrates, and are comparable to those expressed in yeast and insect cell systems. We demonstrate that, for the first time, human QC contains one zinc ion per molecule of protein, and the QC activity could be inhibited by several triazole and tetrazole derivatives. Finally, we also report the preliminary results from X-ray crystallographic analysis.

Materials and methods

Cloning of human QC cDNA and construction of expression vectors

Using the primers, 5'-GGCTGGGAGAGATGGCA GGCGGAA-3' and 5'-GGATAGATGTTTCCACAC

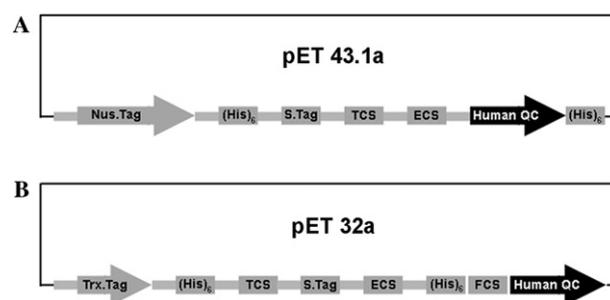


Fig. 1. Strategies to acquire the soluble human QC. (A) Schematic representation of the Nus protein-fusion expression construct of human QC, based on the pET 43.1a vector (Novagen, Madison, WI). (B) The thioredoxin-fusion expression construct, based on the pET 32a vector (Novagen). TCS, thrombin cleavage site; ECS, enterokinase cleavage site; FCS, Factor Xa cleavage site.

AGCATT-3', which are referred to the 5'- and 3'-non-coding regions of human pituitary QC [18], respectively, we have amplified the human QC cDNA from a commercial bone marrow cDNA library (Clontech, Palo Alto, CA) by PCR. The DNA fragment on the PCR product corresponding to the mature coding region of human QC (Ala33–Leu361) was re-amplified and inserted into a pET-43.1a expression vector (Novagen, Madison, WI) via *EcoRI* and *XhoI* cloning sites. The resulting construct, as described in Fig. 1A, contains two (His)₆-tags at the N- and C-terminal sites of human QC. In addition, we also constructed a pET-32a-based expression vector (Fig. 1B). Using the initial PCR product as template, a second PCR was carried out to amplify the mature region of human QC, and to introduce a (His)₆-tag and a Factor Xa cleavage site into its 5'-end. The resulting product was ligated into the *HindIII*–*XhoI* sites of pET 32a vector (Novagen).

Protein expression and purification

The constructed expression vectors were transformed into *E. coli* BL21 (DE3) cells (Novagen). The bacteria were grown in LB media containing 200 μ g/mL ampicillin at 37 °C until a cell density of 0.6–0.8 OD₆₀₀ was reached. The cultures were induced with 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG) for 2 (pET 32a) or 4 (pET 43.1a) days at 20 °C. The cells were harvested by centrifugation at 6000 rpm, followed by freezing at –80 °C. In the case of pET 43.1a vector, frozen bacterial pellet was resuspended in buffer A (300 mM NaCl and 10 mM imidazole in 50 mM sodium phosphate, pH 8.0) and lysed by French Press. The lysate was clarified by centrifugation at 22,000 rpm for 1 h and then loaded onto a Ni-NTA (Amersham Pharmacia, Uppsala, Sweden) column pre-equilibrated with buffer A. After washing the column with buffer A, the Nus-fusion QCs were eluted by a linear gradient of 0–100% buffer B (the same as that in A, except for 300 mM imidazole) on A. The eluates were pooled and

then digested with thrombin (0.1 U/mL) (Novagen) in a dialysis bag dialyzed against buffer C (300 mM NaCl in 50 mM sodium phosphate, pH 8.0) at 25 °C overnight. The cleaved Nus proteins were eliminated from the digests by employing an S protein–agarose (Novagen) column, and then the QCs in the flow-throughs were pooled and further purified by a Ni–NTA column. For the construct of pET 32a, the buffers A and B above was replaced with buffer D (150 mM NaCl and 20 mM imidazole in 50 mM Tris–HCl, pH 8.0) and buffer E (the same as that in D, except for 250 mM imidazole), respectively. The thioredoxin–fusion QCs eluted from the first Ni–NTA column, after a similar procedure stated above, were pooled and then digested with Factor Xa (0.2 U/mL) (Novagen) in a dialysis bag against buffer F (150 mM NaCl in 50 mM Tris–HCl, pH 8.0) at 25 °C for 2 days. The mature human QCs were obtained by an additional Ni–

NTA column using a linear gradient of 0–30% buffer E on F. The proteins were concentrated and further desalted by a Sephadex G-50 column (Amersham Pharmacia). Purity of the recombinant human QCs was checked by SDS–polyacrylamide gel electrophoresis (Fig. 2A).

Mass spectrometry analysis

Mass spectrometry analysis was carried out on a Finnigan LCQTM ion trap mass spectrometry (ThermoFinnigan, San Jose, CA) with electrospray ionization interface. The ESI source was operated in positive ion mode. Sample solution was infused using HPLC pump with loop injection. ESI operation conditions involved a spray voltage of 4.5 kV, a heated capillary temperature of 160 °C, and flow rate of 50 μ l/min. The mass spectrometer was operated in full-scan profile mode, scan

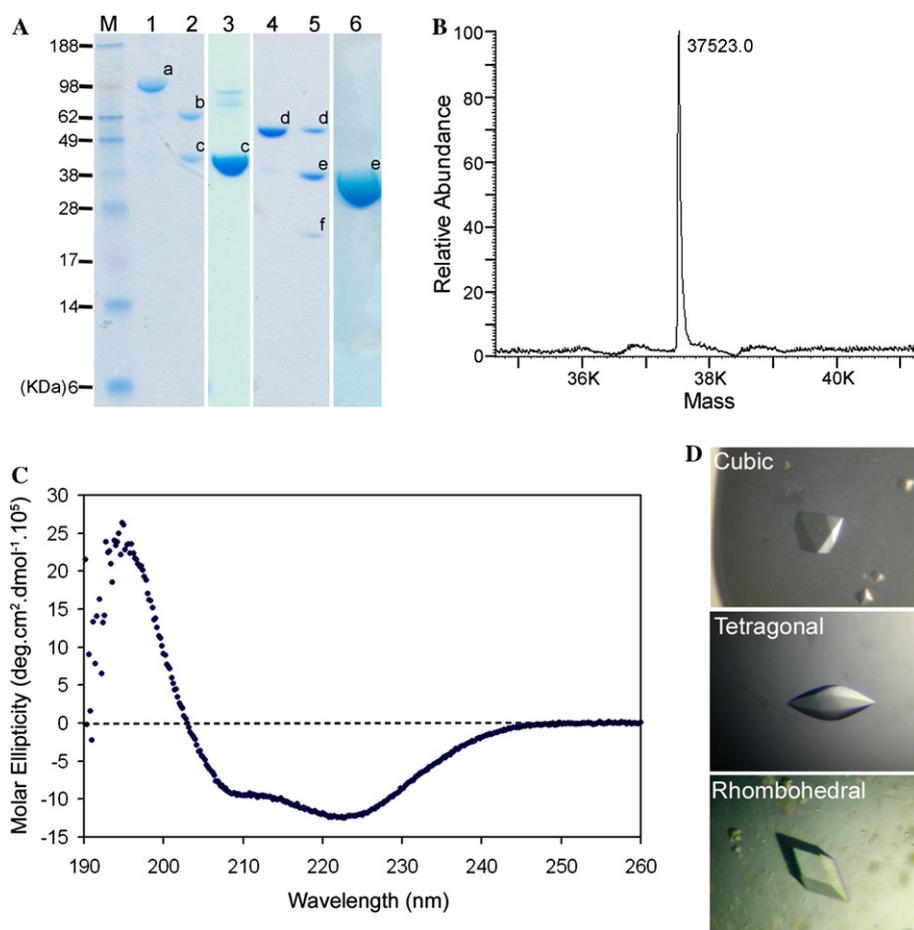


Fig. 2. Purification, characterization, and crystallization of recombinant human QC. (A) SDS–PAGE analysis of the recombinant human QCs. Lane M, protein markers. Lanes 1 and 4, human QC fused to Nus and Trx fusion proteins, respectively, after first Ni–NTA column purification (see Materials and methods). Lanes 2 and 5, the Nus-tagged and Trx-tagged human QCs digesting with thrombin (0.1 U/mL) and Factor Xa (0.2 U/mL), respectively. Lanes 3 and 6, final purified products of human QC from the pET 43.1a and pET 32a constructs, respectively. Protein bands: a, Nus-tagged human QC; b, Nus-tag part in a; c, human QC part in a; d, Trx-tagged human QC; e, mature human QC; f, Trx-tag part in d. (B) Mass spectrometry analysis of the mature human QC. Experiment was carried out on a Finnigan LCQTM ion trap mass spectrometry (ThermoFinnigan, San Jose, CA) with electrospray ionization interface. The molecular weight determined was 37523.0, close to the predicted molecular mass, 37515.5. (C) Circular dichroism spectroscopy analysis of the mature human QC. (D) Photographs of the human QC crystals. The cubic form crystals were grown using the pET 43.1a products, while the tetragonal and rhombohedral forms were obtained using mature human QC expressed from the pET 32a construct.

range from m/z 150 to 2000. Data requisition and analysis was done with Xcalibur (version 1.3, ThermoFinnigan) and BioWorks™ 3.0 (ThermoFinnigan).

Circular dichroism spectroscopy analysis

Circular dichroism (CD) spectra of mature human QC (0.3 mg/mL) were measured at 25 °C on a Jasco J-715 spectropolarimeter using a 0.1 cm light-path cuvette. The mean of 10 scans between 190 and 260 nm was calculated and calibrated by subtraction of the buffer spectra (50 mM Tris–HCl, pH 8.0).

Atomic absorption analysis

The zinc content of human QC was estimated by employing an atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) as described previously [19]. The protein concentration was spectroscopically determined in 6.0 M guanidine hydrochloride using an extinction coefficient of $55,190 \text{ M}^{-1} \text{ cm}^{-1}$ at 280 nm estimated from the amino acid sequence of human QC.

Peptide synthesis

The peptide [Gln¹]-TRH, with a C-terminal amide, was synthesized by the standard Fmoc peptide chemistry. After cleavage from the resin, the peptides were lyophilized and then purified by reversed-phase HPLC. Purified peptides were verified by N-terminal sequencing and mass spectrometry.

QC activity analysis and QC inhibitor assay

QC activity was analyzed based on reversed-phase HPLC and spectrophotometric measurement. In the case of HPLC, the 30 μl reaction mixture that contains 0.6 μM mature human QC and 14.8 mM [Gln¹]-TRH in 50 mM Tris–HCl, pH 8.0, was incubated at 25 °C for a variety of period ranging from 0 to 20 min. Subsequently, a 20 μl aliquot of the mixture was subjected to HPLC analysis on a Waters liquid chromatograph using a C₁₈ column (Waters, Milford, MA). The bound materials were eluted by a three-step linear gradient of methanol containing 0.1% TFA. The eluates were detected at 214 nm and identified by mass spectrometry and N-terminal sequencing.

For the spectrophotometric method, the 500 μl assay solutions consisted of 15 U glutamate dehydrogenase, 12 mM α -ketoglutarate, 0.6 mM NADH, and varying concentrations of synthetic substrate in 50 mM Tris–HCl, pH 8.0. Reactions were started by the addition of QC (0.04–2.5 μg), and activity was monitored by recording the decrease of NADH absorbance at 340 nm.

For inhibitor test, the reaction composition was the same as described above, except for the addition of the

Table 1
Statistics of X-ray data collections

Crystal form	Cubic	Tetragonal	Rhombohedral
Space group	<i>F</i> 432	<i>P</i> 422	<i>R</i> 32
Resolution (Å)	30.0–3.10	25.0–2.25	50.0–1.78
Cell dimensions (Å)	$a = b = c = 273.47$	$a = b = 70.04$ $c = 198.39$	$a = b = 119.26$ $c = 333.73$
Unique reflections	16,234	23,825	84,584
Redundancy	4.5	4.7	4.2
Completeness (%)	98.8 (97.3)	97.7 (99.8)	96.4 (94.1)
$I/\sigma(I)$	11.3 (2.6)	33.7 (4.1)	29.2 (2.6)
R_{merge} (%)	12.0 (51.7)	5.6 (57.1)	4.9 (59.8)
Mol/a.u. ^a	2	1	2
V_{m} ^b	2.839	3.243	3.019

^a Number of molecules per asymmetric unit.

^b Matthews coefficient.

inhibitor compound. Prior to the spectrophotometric analysis on a 96-well microplate, the enzyme was incubated with the inhibitor for 5–10 min. The concentration of substrate (H-Gln-Gln-OH), human QC, and inhibitor in the assay solution were 0.55 mM, 28.8 nM, and 140 μM , respectively.

Crystallization and data collections

Recombinant human QC was concentrated to 8–10 mg/mL using an ultra-filtration membrane YM-10 (Millipore, Billerica, MA). The screening for human QC crystals was achieved using the crystallization kits from Hampton Research (Hampton, Laguna Niguel, CA) by the method of hanging drop vapor diffusion. Cubic crystals for the pET 43.1a products were obtained using equal volume of the protein solution and the reservoir that contains 30% PEG 4000 and 0.2 M MgCl₂ in 0.1 M Tris–HCl, pH 8.5. For the tetragonal crystals of mature human QC, the reservoir consisted of 20% PEG MME 550 and 0.1 M NaCl in 0.1 M Bicine, pH 9.0. For the rhombohedral crystals of mature human QC, the reservoir was 1.8 M (NH₄)₂SO₄ and 4% dioxane in 0.1 M Mes, pH 6.5.

X-ray diffraction experiment was performed at the Institute of Biological Chemistry, Academia Sinica (Taipei, Taiwan) using MSC MicroMax 002 equipped with an R-AXIS IV⁺⁺ image-plate detector. Prior to mounting on X-ray machine, the crystals were flashly soaked in mother liquor containing 20–25% glycerol (v/v) as cryoprotectants. Diffraction data were processed and scaled using the HKL package [20] (listed in Table 1).

Results and discussion

Expression of human QC in *E. coli*

We have amplified the human QC cDNA from a human bone marrow cDNA library, which has an identical amino acid sequence to the pituitary QC published

[18]. To overcome the problems of low recovery and heterogeneity of the recombinant human QC, we have constructed more than 10 expression vectors in *E. coli* system based on a variety of fusion tags. Surprisingly, as judged by SDS-PAGE, the induced human QC in *E. coli* cells are present almost in the inclusion body, except for that of Nus-tagged expression vector. Using double (His)₆ tags on the pET 43.1a construct (Fig. 1A), we have isolated Nus-tagged human QCs from the crude extract of bacterial lysates (Fig. 2A, lane 1). After cleavage with thrombin (Fig. 2A, lane 2), and purification by an S protein-agarose and an additional Ni-NTA column (see Materials and methods), the functional human QCs were obtained with a level reaching 1.5–2 mg/L culture and a homogeneity of ~90% (Fig. 2A, lane 3). However, the resulting human QC contains a 22-residue linker and an uncleaved (His)₆ tag at its N- and C-termini, respectively.

On the other hand, based on the putative importance of a disulfide bridge in human QC [9], attempts on modifying a thioredoxin-tagged (pET 32a) expression vector of human QC were made. Fortunately, a yield of ~6 mg/L was achieved due to a longer linker inserted between the human QC and the thioredoxin genes on the construct. We put an additional (His)₆ tag on the linker region (Fig. 1B) so that it is convenient to isolate the thioredoxin-tagged human QC from *E. coli* components by Ni-NTA column (Fig. 2A, lane 4). In addition, we also introduced a Factor Xa cleavage site to the N-termini of human QC to obtain the mature form by proteolytic digestion (Fig. 2A, lane 5). Since human QC molecule contains 16 (~5%) histidine residues, the protein was found to weakly bind to the Ni-NTA resin. Therefore, we used a Ni-NTA column for the final purification step. Mature human QCs were eluted from the column using a low concentration imidazole, which showed near homogeneity on SDS-PAGE (Fig. 2A, lane 6), with final expression levels up to ~10 mg/L bacterial culture. Mass spectrometry analysis revealed that the mature human QC has a molecular weight of 37523.0 (Fig. 2B), consistent with the predicted molecular mass (37515.5). The circular dichroism spectrum of mature human QC (Fig. 2C) corresponds well to the spectra of the proteins expressed in yeast and insect cell systems [9,17].

QC activities on physiological and synthetic substrates

Recombinant human QCs expressed from either the pET 43.1a or the pET 32a constructs showed similar activities on synthetic substrates. We used the synthetic [Gln¹]-TRH (Gln-His-Pro), one of the putative physiological substrates of human QC, to analyze the QC activity of our recombinant protein based on the HPLC elution profile of the peptide. As shown in Fig. 3, [Gln¹]-TRH was almost completely converted by the enzyme into its corresponding product, [pGlu¹]-TRH, within 20 min. In contrast, less than 20% turnover was

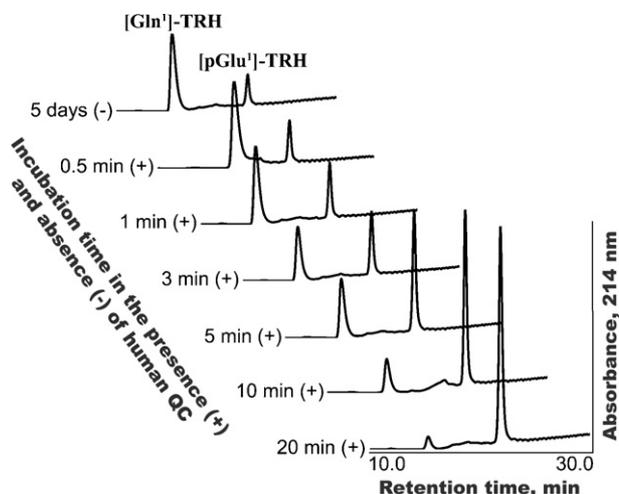


Fig. 3. Analysis of glutaminyl cyclase activity on physiological substrate. One of the putative physiological substrates of human QC, [Gln¹]-TRH (Gln-His-Pro), was incubated with the recombinant proteins of mature human QC for various periods as indicated (see Materials and methods). Conversion from glutaminyl substrate to its respective pGlu product was monitored by reversed-phase HPLC and confirmed by mass spectrometry and N-terminal sequencing. A reaction without the addition of human QC was used as control.

observed in the absence of human QC for 5 days under the assay condition (50 mM Tris-HCl, pH 8.0, at 25 °C). This minor formation of pGlu product might be due to the acidic condition (0.1% TFA) of the running buffer during the HPLC separation by a C₁₈ column.

The recombinant human QC was active on other synthetic substrates, i.e., H-Gln-*t*-butyl ester and H-Gln-Gln-OH (Table 2), based on a spectrophotometric experiment (see Materials and methods). These two substrates had also been used by other groups in detecting the human QC activity expressed in yeast and insect cell systems [10,17]. The K_m and k_{cat} values determined for our recombinant protein are very similar to the human QC expressed in *Drosophila* S2 cells (Table 2).

Table 2
Kinetic parameters for human QC expressed in *E. coli*, yeast, and insect cell systems

	K_m (mM)	k_{cat} (s ⁻¹)	k_{cat}/K_m (mM ⁻¹ s ⁻¹)
<i>Escherichia coli</i> cell			
Gln- <i>t</i> -butyl ester	4.1 ± 0.6 ^a	20.9 ± 2.1	5.1 ± 0.8
Gln-Gln	0.6 ± 0.1	8.6 ± 0.5	13.7 ± 0.5
<i>Pichia pastoris</i> cell ^b			
Gln- <i>t</i> -butyl ester	1.2 ± 0.1	6.7 ± 0.2	5.4 ± 0.2
Gln-Gln	0.1 ± 0.0	20.7 ± 0.2	140.0 ± 2.0
<i>Drosophila</i> S2 cell ^b			
Gln- <i>t</i> -butyl ester	6.7 ± 2.0	16.0	2.4
Gln-Gln	0.7 ± 0.4	10.0	14.3

^a Mean ± SD ($n = 2$).

^b Human QCs expressed in *Pichia pastoris* and *Drosophila* S2 cells were reported by Schilling et al. [9,10] and Booth et al. [17], respectively.

They are also comparable to the results of QC expressed in *Pichia pastoris* cells, except for the parameters analyzed on H-Gln-Gln-OH. This inconsistency is probably due to the different methods for calculating the initial velocities of catalysis. The activity determined by Schilling et al. [10] was based on a standard curve of ammonia. In contrast, the activity reported in this paper was according to the decrease of NADH concentration in the reaction mixture, that was calculated based on the extinction coefficient of NADH. The optimum pH value for the activity of our recombinant QC was approximately 8.0.

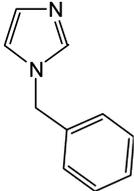
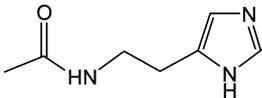
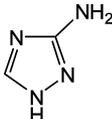
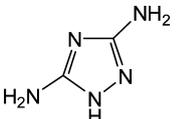
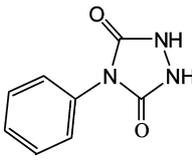
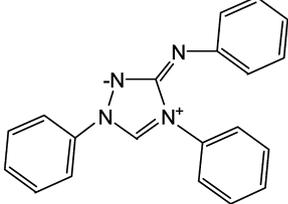
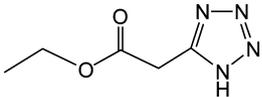
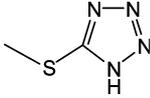
Inhibition of the QC activity by imidazole, triazole, and tetrazole derivatives

Recently, several imidazole derivatives were identified as competitive inhibitors of human QC [11]. We present here 8 out of the 25 tested imidazole, triazole, and tetrazole derivatives to be potent inhibitors of our recombinant human QC (Table 3), through a quickly screening procedure using a 96-well microplate. The imidazole-derived inhibitors listed in Table 3 had also been reported by Schilling et al. [11], whose K_i values fell in the low micrometer range. Interestingly, the hydrophobic multiple-phenyl triazole, nitron, exhibited the similar inhibitory effect of *N*- ω -acetylhistamine ($K_i = 17 \mu\text{M}$), suggesting a hydrophobic environment of the human QC active site. It is noticeable, from the 25 tested compounds, that the inhibition effect of the imidazole derivatives appears to be better than the triazole and tetrazole compounds. The nitrogen atoms in the imidazole, triazole or tetrazole rings are probably the good candidates to coordinate to the active-site zinc ion of human QC.

Human QC is a single zinc metalloenzyme

Up to now, the metal ion content of human QC is still unclear. Schilling et al. [11] proposed that human QC is a metalloenzyme based on the inhibition by several metal ion chelators and the reactivation of apoenzyme by zinc ions. However, Booth and co-workers [12] reported lower than 0.3 molecule zinc ion per molecule of human QC by mass spectrometry. Here, we demonstrate, for the first time, that human QC contains one zinc ion per protein molecule as analyzed by atomic absorption spectrophotometer. As shown in Fig. 4, the zinc to human QC molar ratio, being ~ 1 , was determined in triplicate at various protein concentrations. The zinc concentration in the protein solutions was estimated according to the absorbance at zinc wavelength (307.6 nm) and a standard curve made by using zinc sulfate in a high correlation coefficient (0.998). The protein concentration was carefully quantified based on the extinction coefficient.

Table 3
Effects of imidazole, triazole, and tetrazole derivatives on glutaminy cyclase activity of the recombinant human QC

Compound	Structure	QC activity (% of control)
None		100.0
1-Benzylimidazole		41.5
<i>N</i> - ω -acetylhistamine		66.3
3-Amino-1,2,4-triazole		84.5
3,5-Diamino-1,2,4-triazole		89.9
4-Phenyl-1,2,4-triazole-3,5-dione		78.2
Nitron		61.4
Ethyl-1 <i>H</i> -tetrazole-5-acetate		90.6
5-(Methylthio)-1 <i>H</i> -tetrazole		89.1

Preliminary X-ray crystallographic analysis

After a number of crystallization screening, three human QC crystals, which belong to cubic, tetragonal, and rhombohedral forms, have been obtained (Fig. 2D). The cubic crystals were grown using the pET 43.1a products, while the others were made from the mature proteins. Among these, the rhombohedral crystals were best to diffract X-ray (Table 1). A crystallographic asymmetric unit of the cubic, tetragonal, and rhombohedral crystals contain two, one, and two human QC molecules, with the solvent content of 55, 61, and 58% [21],

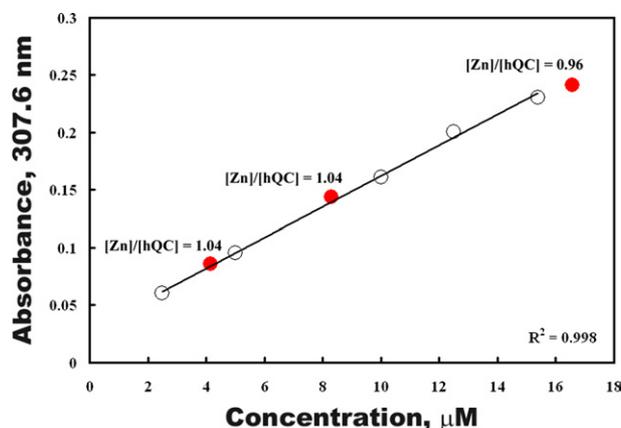


Fig. 4. Analysis of the zinc ion content in human QC. Open circles represent the standards made by using ZnSO_4 in the concentration range of 2.5–15.5 μM . Closed circles are the human QC sample at various concentrations. The zinc to human QC ratio for each the triplicate tests are indicated.

respectively. We found obvious fluorescence peak emitted from the tetragonal and rhombohedral crystals using synchrotron radiation with X-ray energy set on the zinc-absorption range (data not shown). This is consistent with the result of atomic-absorption experiment as described above. Since human QC is a zinc metalloenzyme, structural determination of the protein by the multiple-wavelength anomalous diffraction method is being undertaken.

Conclusion

Currently, owing to limited amount of animal QCs obtained, several arguments concerning the properties, distributions, and structure of animal QCs remain to be answered. In this paper, we present the expression of mature human QC in a relatively high level, a high homogeneity, and a convenient purification procedure. Our works are useful to acquire new insights into human and animal QCs, particularly for the future structural analysis and structure-based drug development for diseases associated with the biological activity of QC enzyme.

Acknowledgments

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of $[\text{Gln}^1]\text{-TRH}$ and helpful discussion on enzyme kinetic analysis, respectively. We are also grateful to National Core Facility of Proteomics (NSC 93-3112-B-001-010-Y) for assistance in the mass spectrometry analysis.

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