The Isolation of Native Glutaminyl Cyclase from *Saccharomyces cerevisiae*

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HONORS THESIS

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By

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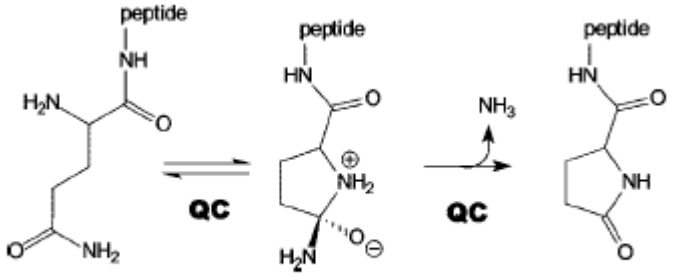
ABSTRACT

Glutaminyl Cyclase (QC)(2.5.2.3) catalyzes the cyclization of the N-terminal glutamine residues of peptides and proteins. Recent interest in QC has been generated by studies that have linked QC activity in human neuroendocrine tissue to diseases such as Alzheimer's and osteoporosis. While QC has been isolated and characterized in multiple mammalian species, no attempt has been made to isolate the QC protein present in *Saccharomyces cerevisiae*. Therefore, this study sought to isolate and begin the characterization of yeast QC protein. A purification scheme was constructed and activity assays were identified to measure QC activity in purified protein samples. Basic characterization of yQC included SDS-PAGE analysis and an imidazole inhibition assay. The results of the study indicate that QC was isolated from *S. cerevisiae*. QC activity was found to have a total activity of 306 nmol/hr using the spectrophotometric assay. QC activity was verified using a more specific fluorometric assay. Furthermore, the partially purified protein was shown to have a molecular mass of 41 kd, which correlates with the theoretical weight of the yQC. While the results of this study verify QC activity, further purification of yQC would allow for the sequence analysis of the isolated protein to conclusively verify its identity. Still, this study has determined a basic purification scheme to isolate QC from *S. cerevisiae*.

INTRODUCTION

Glutaminyl Cyclases (QCs) (EC 2.5.2.3) are acyltransferases that catalyze the cyclization of peptide or protein N-terminal glutaminyl residues into pyroglutamic acid (5-oxoproline) (Scheme 1). The formation of pyroglutamic acid (p-Glu) is a reaction that serves to stabilize peptides and proteins by preventing N-terminal degradation, but under the physiological conditions of the cell, the spontaneous reaction occurs at a negligible rate (1).



SCHEME 1. N-terminal cyclization of glutaminyl peptides by QC.

Aside from the increased molecular stability of p-Glu, the cyclization of N-terminal glutamine (Gln) has also been suggested to serve two important biological functions. First, the presence of p-Glu on several mammalian signaling peptides, hormones, and neurotransmitters (e.g. TRH, GRH) (2,3) appears to enhance the affinity of these ligands for their specific protein receptors (4). Second, p-Glu residues are thought to serve a protective function by inhibiting the N-terminal proteolysis of polypeptides via aminopeptidases (4,5).

Recently, QC has been implicated as a factor in multiple human genetic diseases. For instance, osteoporosis, a disease that results in decreased bone mass due to improper mineral regulation, has been shown to have a high correlation with point mutations of the human QC gene (6). QC has also been suggested that QC functions in the processing of amyloid peptides, many of which contain N-terminal

p-Glu residues. These p-Glu containing amyloid peptides are thought to play a pivotal role in the development of Alzheimer's disease by increasing the hydrophobicity and proteolytic stability of the amyloid plaques (7).

QC was originally isolated and characterized from papaya extract by Messer and Ottesen (8-10) and has been subsequently isolated, cloned, and characterized from mammalian species (11, 12). Kinetic analysis of mammalian QC has determined that the enzyme is responsible for a significant increase in the *in vivo* rate of p-Glu cyclization (12). Later characterization of mammalian pituitary QC by Pohl *et al.* determined that the first 28 amino acids contained a signal sequence that was thought to direct the enzyme to the endoplasmic reticulum (2).

In spite of similarities of kinetic rates and stability, the structural characterization of known forms of QC has determined that plant QC, isolated from papaya, shares virtually no primary sequence homology or secondary folding motifs with mammalian QC forms. Thus, the plant and animal forms of the enzyme are thought to have evolved separately (13,14), and it is the animal structure that yQC is thought to resemble.

The hypothetical yeast gene, Yfr018c, was identified in *Saccharomyces Cerevisiae* during the Yeast Genome Project, and was determined to have a high sequence homology with mammalian QC (15,16). This hypothesized yeast QC (yQC) has not yet been isolated or characterized, so the physical properties and the physiological function of yQC remain a mystery. Computational analysis of yQC DNA sequence has predicted the presence of a 28 amino acid signal sequence, suggesting a similar processing pathway to mammalian QC. However, yQC has been

suggested to be a membrane protein based on transmembrane helix prediction. If yQC turns out to be a membrane protein, its physiological function might differ from the previously characterized human homologues, which process secretion peptides in vesicles being transported for exocytosis.

The aim of this study was to isolate and characterize yQC. A purification protocol was constructed for yQC, which would enable further biochemical characterization. The identification and purification of yQC might reveal novel information about the QC proteins.

EXPERIMENTAL PROCEDURES

Materials–Saccharomyces cerevisiae YLKL813 (MATα his3-Δ1 leu2-Δ0 lys2-Δu ura3-Δ0 trp::hisG-Ura3-hisG) was provided by Dr. L. Kevin Lewis at Texas State University. Other reagents were purchased as follows. Crude papain powder (EC 232-627-2), and TES were purchased from Sigma (St. Louis, MO), as well as the components of the spectrophotometric assay [α-ketoglutarate, arginine hydroxamate, Gln-NH_2, $NADH/H^+$, and bovine liver glutamate dehydrogenase (38 units/mg, 50% glycerol)]. The *Pfu* pyroglutaminyl aminopeptidase (15.8 units/mg) and the βNA fluorometric substrate (Lot # 50 1353) were from by Takara and Bachem Bioscience, respectively. The Y-Per Yeast Protein Extraction Reagent, the 5 mL polypropylene chromatography columns, the Gel Code Blue Stain Reagent, and the BCA reagents were purchased from Pierce (Rockford, IL). The DEAE-cellulose resin, preswollen microgranular DE52, was a Whatman product; the CM-cellulose, Cellex-CM, was a product of BioRad. The low-range SDS PAGE Silver Stain protein marker, the Mini-Protean 3 gel apparatus, glycine, and the Silver Stain Plus

Kit were also obtained from BioRad. The Benchmark Protein Ladder was purchased from Invitrogen. Tris Base was from Promega, PMSF came from EMD, urea and sodium phosphate were from EM Science, and sodium acetate was from Mallinckrodt. All other chemicals were of analytical grade, and only de-ionized, distilled water was used in the protocols.

Isolation of QC from Yeast

Cell Culturing and Protein Extraction-The *S. cerevisiae* was maintained on YPD plates. A cell culture was prepared by inoculating 30 mL of YPDA liquid media. The cells were incubated for approximately 7 hours at 30˚C with agitation at 250 rpm. Cells were harvested at log phase growth by centrifugation. Cells were pelleted at 3000*g* for 5 minutes at 4˚C. The cell pellet (~225 mg) was then resuspended in Y-Per Protein Extraction Reagent. The Y-per Reagent recommended protocol was followed to isolate the soluble cellular protein (Pierce). During cellular lysis, PMSF (1mM) was used as a protease inhibitor.

Cation and Anion Exchange Chromatography-QC has been isolated previously using ion exchange chromatography (17). Both an anion exchange chromatography (DEAE-cellulose) and a cation exchange chromatography (CM-Cellulose) were initially used to purify yQC. Analytical columns (5 mL) were packed with 3.5 mL of resin, and allowed to swell overnight. The columns were equilibrated using 4 column volumes of 50 mM sodium phosphate buffer, pH 7.0. The yeast protein extract (0.5 mL) was loaded onto each of the columns at 25°C. Fractions (1.5 mL) were collected continuously throughout the chromatography. The column was washed with 2 column volumes of 50 mM sodium phosphate, pH 7.0

(buffer A). Elution of QC was performed with buffer A containing 1.0 M NaCl. The protein concentration of the fractions was determined using the BCA assay protocol designed by Pierce, and the fractions were stored at 4˚C.

Isolation of QC from Papaya

Sample Preparation-Crude papain powder was used to isolate papaya QC (pQC). Papain (5 g) was dissolved in 10 mL of sodium acetate buffer, pH 5.0. The solution was stirred for 30 minutes and then centrifuged at 3000g for 5 minutes. The supernatant was loaded onto a CM-cellulose column.

CM-Cellulose Chromatography-The purification of pQC was based on the method outlined by Messer and Ottesen (10). A 4 mL CM-Cellulose column was regenerated using 5 M urea and then equilibrated using 50 mM sodium acetate buffer, pH 5 (buffer B). The crude papain supernatant (10 mL) was loaded onto the column, and 1.5 mL fractions were collected. The column was eluted in one step using buffer B containing 1.0 M NaCl. Fractions that contained QC activity, as determined by the spectrophotometric activity assay, were pooled together and stored at 4ºC.

Identification of an Activity Assay for yQC

Spectrophotometers-The spectrophotometric were carried out using a SpectraMax 190 microplate reader (Molecular Devices) with SoftMax Pro 4.7.1 software at 25˚C. The fluorometric measurements were carried out using a Perkin-Elmer LS50B Luminescence Spectrophotometer.

Spectrophotometric Assay for pQC and yQC-The spectrophotometric assay was originally developed by Bateman (18). The assay buffer (0.1 M TES, pH 7.0, 0.5

M NaCl) contained 0.65 mM NADH, 13.0 mM α-ketoglutarate, 0.65 mM Gln-NH_2, 0.4 units/mL of glutamate dehydrogenase, and an appropriate amount of enzyme in a final volume of 60 μL. The assay cocktail was prepared fresh. Arginine hydroxamate (0.65 mM) and bestatin were used as aminopeptidase inhibitors. A negative control was prepared by substituting the assay buffer for enzyme.

The coupled reaction was carried out on a microplate, and initiated by adding yQC or pQC to the assay cocktail. The reaction was allowed to pre-incubate for 2 minutes at 30˚C, and then the absorption was measured at 340 nm. After which, the activity assay microplate was incubated for 1 hour at 30˚C, and the absorbance at 340 nm was measured again.

Fluorometric Assay-The spectrofluorometric assay was based on the method described by Schilling et al. (19). The presence of the specific cyclization of Gln to pyroglutamate was assayed for using the fluorometric substrate Gln-βNA. The assay buffer (20 mM Tris-HCl, pH 8.0, 200 mM KCl) contained 0.05 mM Gln-βNA, 0.25 units of pyroglutaminyl aminopeptidase, and an appropriate amount of QC in a final volume of 60 μL. Gln-βNA had excitation/emission wavelengths of 320/410 nm. The reaction was initiated by adding QC at 30°C, after which the activity of QC was monitored by measuring the fluorescence of βNA for 1-minute increments.

Imidazole Inhibition-The spectrophotometric protocol outlined by Bateman was used (18). Imidazole, 1 mM was added to one reaction mixture (assay cocktail and enzyme), and replaced with assay buffer in another. The control substituted assay buffer for QC. The reaction was carried out in cuvettes for 1.5, 2, and 2.5 hours.

Gel Electrophoresis

SDS PAGE Analysis-The protein fractions from the DEAE-cellulose chromatography were analyzed using SDS polyacrylamide gel electrophoresis. A 1.5 mm discontinuous mini gel was prepared that contained a 4% stacking gel and a 10% resolving gel. A tris-glycine running buffer (0.250 M Tris, pH 8.3, 1.92 M glycine, 1% (v/v) SDS) was prepared, and the Mini-Protean 3 cell (BioRad) gel apparatus was used. QC samples (varying concentrations) were loaded onto the gel, along with a low-range SDS-PAGE Silver Stain Protein Marker (BioRad) and the Benchmark Protein Ladder (Invitrogen). Loading samples were prepared by adding maximal volumes of protein to 1X sample buffer [0.5 M Tris-HCl, pH 6.8, 10% Glycerol, and 2 % (w/v) SDS and 0.01% (v/v) bromophenol blue] and 2.5% (v/v) β-mercaptoethanol (Amresco), followed by heating at 95°C for 5 minutes. The gel was run at 150 volts for 1 hour.

Gel Code Blue and Silver Staining--The SDS-PAGE gels were initially stained using the Gel Code Blue Stain Reagent protocol (Pierce); however, the Gel Code Blue did not provide adequate protein detection. The gels were destained in a 40% (v/v) methanol, 10% (v/v) acetic acid solution. The gels were restained using the Silver Stain Plus protocol (BioRad). A Kodak Image Station 440 was used to image and photograph the stained gels.

RESULTS

This study partially purified the yeast QC protein that is homologous to previously characterized mammalian forms of QC (2, 11, 12). Two chromatography

steps, G-25 gel filtration and DEAE-cellulose, were used to develop a purification protocol for native yeast QC. Spectrophotometric and fluorometric activity assays were used to examine the presence of QC activity in the partially purified protein samples. Basic enzyme characterization consisted of inhibition studies using a known human QC inhibitor.

Purification of yQC- The partial purification of yQC in this study was achieved using two chromatography steps. The yeast crude protein extract (1.12 mg/mL), which was isolated from *S. cerevisiae* cells using the Y-PER Reagent recommended protocol, was loaded onto a G-25 column, and the protein was eluted with 50 mM sodium phosphate buffer, pH 7.2 (Fig. 1). Fractions (1mL) were collected continuously throughout the chromatography, and protein concentration was estimated by measuring the absorbance at 280. As seen in Fig. 1, the majority of the cellular protein eluted with the void volume of the column. QC activity was measured using the spectrophotometric assay developed by Bateman (18). An activity peak was measured after the majority of the protein had eluted, but appeared to result from the presence of contaminating reagents such as ammonia, and therefore was not used for further purification. The total activity of the G-25 fractions were calculated using the relationship determined by Bateman, which observed that a 0.01 AU decrease in absorbance at 340 nm was equivalent to the formation of 1 nmol of product. The total activity of QC obtained after G-25 purification was 122 nmol/hr/mL. The purified protein was further purified using anion exchange chromatography.

The G-25 purified protein sample that contained QC (0.728 mg/mL) was loaded onto the DEAE-cellulose column, washed with 4 mL of 50 mM sodium phosphate, pH 7.2, and eluted using 50 mM sodium phosphate, pH 7.2, 1 M NaCl. Fractions (1.0 mL) were collected continuously and assayed to determine protein concentration and QC activity (Fig. 2). The majority of protein was obtained in the initial flow through fractions, after which negligible protein was observed until the column was eluted with 1 M NaCl. The elution fraction with the highest protein concentration had a total activity of 306 nmol/hour. When Bateman developed the spectrophotmetric assay for hQC, he observed an activity of approximately 18 nmols/hr for 45 μg of partially purified hQC, which equates to a total activity of 360 nm/hr/mL. Thus, the observed activity of yQC is similar to previously determined activities of bovine and human QC (18). The pH at which the column was eluted was varied to optimize QC elution. It should be noted that no increase in activity was obtained when the column was equilibrated and eluted using 50 mM Tris-HCl at pH values of 7.6 and 8.0 (data not shown). During the rest of the study, the G-25 column/DEAE-cellulose two-step purification was used to obtain partially purified protein samples, which could be used for further protein analysis.

As seen in Table I, the chosen chromatographic steps resulted in the partial purification of QC activity. The initial protein extract could not be assayed for activity due to the presence of ammonia. The G-25 fraction with the highest protein concentration had a total activity of 122 nmol/hr/mL. After the DEAE chromatography, the purified protein sample had a total activity of 306 nmol/hr/mL. The purification protocol resulted in a 30-fold increase of QC activity.

Isolation of pQC- Papaya QC was isolated form crude papaya latex to serve as a positive control for the spectrophotometric and fluorometric assays. Papaya QC was isolated using a method derived from Messer and Ottesen (8). A 3 mL CM-Cellulose column was used to isolate the pQC. Fractions (1 mL) were assayed for activity and protein concentration (data not shown). Comparison of the protein concentration and activity assay show that pQC was isolated by elution with 1 M NaCl. This confirmed that the spectrophotometric assay parameters were measuring QC activity. The fractions containing pQC activity were pooled and used as a positive control during the subsequent fluorometric assay.

Fluorometric Assay- The yQC fractions obtained from DEAE-cellulose chromatography and the pQC obtained from the CM-cellulose column had been analyzed using the spectrophotometric activity assay. The fractions that contained QC activity were further analyzed using a more specific fluorometric assay developed by Schilling (19) to verify the presence of yQC. The aminopeptidase inhibitors bestatin (50 µg/mL) and arginine hydroxamate (0.01 mM) were included in the reaction mixture to prevent nonspecific cleavage of the Gln-βNA residue. The fluorometric assay measured the formation of product as the increase in fluorescence of β-NA during one-minute increments for 20 minutes. Isolated pQC was used in the assay to verify that the assay was functional. As shown in Fig. 3A, the pQC activity showed linear increase during the time of analysis; whereas, the yQC showed a slower linear increase over 3 hours (Fig. 3B). The pQC activity is similar to previous data (19), which indicated the assay was monitoring QC activity. The negative

controls, which excluded the enzyme and the pyroglutaminyl aminopeptidase, did not show any increase at any time (data not shown).

Inhibition with Imidazole- Imidazole, a known competitive inhibitor of human pituitary QC, was added to the spectrophotometric assay to a final concentration of 1 mM, and examined for an ability to inhibit the enzymatic reaction occurring. If yQC was the enzyme responsible for the measured activity, imidazole should inhibit the reaction. Since the K_I of hQC was been found to be 0.103 mM for imidazole (20), it was determined that 1 mM imidazole should be sufficient to see a decrease in QC activity. The reaction was monitored over a period of two hours using the spectrophotometric assay. As seen in Figure 4, the reaction that contained imidazole showed a 12% decrease in total activity at 1 hour when compared to the sample that did not contain imidazole, and an 18% decrease at 1.45 hours. The control, which consisted of assay buffer substituted for the enzyme, showed no decrease in absorbance.

SDS-PAGE Analysis- The fractions obtained from the DEAE chromatography were analyzed using SDS-PAGE to confirm the presence yQC and to isolate yQC for subsequent identification with mass spectrometry. Aliquots of 200 µL were taken from each fraction and concentrated using a speed vacuum. The concentrated fraction, 50 µL, were then added to 6X sample buffer, heated at 95ºC, and the total volume of 60 µL was loaded onto an SDS tris-glycine discontinuous polyacrylamide gel. As seen in Fig. 5, a 41 kd band can be seen in the elution fractions, but not in the prior washes, which is thought to be QC.

DISCUSSION

QC enzymes (EC 2.3.2.5) have previously been isolated and characterized from papaya and bovine species (2,3). QC's are known to catalyze the cyclization of N-terminal glutamine residues of peptides and proteins in mammals and plants (11,12). Recent studies have implicated hQC in disease processes that involve amyloidotic peptides, such as Alzheimer's and British dementia (21), which has thrust QC into the biomedical spotlight. Previous genomic studies have identified the presence of a structural gene, Yfr018c, on the yeast chromosome VI (16) that appears to code for a QC homolog, yet no attempt has been made to isolate this protein. This study sought to isolate and characterize native QC from *Saccharomyces cerevisiae*, which could then be used to elucidate the physiological function of QC in eukaryotic cells.

Sequence comparison shows the Yfr018c gene has a 31% identity with characterized forms of mammalian QC. A previous genetic analysis that compared the yeast QC gene to other mammalian forms of QC determined that yeast QC contained four completely conserved histidines (22). Two of the conserved histidine residues have been shown to participate in zinc complexation in mammalian forms (20). The presence of these conserved histidine residues suggests that yQC also complexes a zinc ion *in vivo*. More recent X-ray crystallography studies of QC has identified all the amino acid residues critical for hQC's activity and substrate binding (23), and the conservation of all but one of the active-site residues between yQC and hQC suggest the enzymes are carrying out the same reaction.

The partial purification of yQC entailed the development of a purification protocol, which consisted of multiple steps of chromatography. First, CM-cellulose and DEAE-cellulose columns were run and the chromatograms of both were compared to determine which form of ion exchange chromatography would purify yQC at physiological pH. The QC bound DEAE-cellulose at a pH of 7.2, which indicates yQC has an acidic pK_a. This correlates with the theoretical pI of yQC, which is 5.01. Various chromatography parameters were varied to achieve the greatest yQC purification, including different pH values (6.9, 7.0, 7.2, 7.4, 7.6, 8.0). Modification of the pH did not seem to increase the measured QC activity, but did increase the affinity of the protein for the DEAE resin. Desalting the protein sample using a G-25 gel filtration resin increased the total protein eluted from the column, since it removed ions in solution that interfered/competed with the QC/DEAE interaction.

The spectrophotometric assay developed by Bateman (18) was used during the purification to identify which fractions contained QC activity. The assay was carried out under the conditions specified by the author, since modification of the pH and temperature are detrimental to the activity of the coupling enzyme, glutamate dehydrogenase. One limitation of this assay is that the reaction needs to be carried out in an ammonia-free solution, and the early protein samples contained ammonia. Since the yeast cell extract obtained was not ammonia free and contained other salts that interfered with the DEAE purification, the cell extract had to be desalted using a G-25 column. The activity of the partially purified yQC obtained from DEAE purification correlated strongly with the QC activity that Bateman found for hQC

(18). Bateman determined that hQC had an activity of 360 nmol/hour; whereas, the partially purified yQC in this study had an activity of 306 nmol/hour (Table I). It should be noted that further purification of yQC would result in a more accurate measurement of activity.

The fluorometric assay developed by Schilling for pQC was used to verify the observed activity was due to QC (19). This assay was designed to be a more specific assay for QC that utilizes a pyroglutaminyl peptidase to specifically cleave pyroglutamatic acid residues and does not necessitate an ammonia-free environment. The fluorometric assay provided a more accurate confirmation of QC activity than the spectrophotometric assay, since it is less prone to interference. However, the observed activity of yQC was much lower than the observed activity of pQC (Fig. 3A), and the data previously obtained by Schilling for pQC (19). The increase in fluorescence of the yQC sample took place over an extended period of analysis. Possible explanations of yQC's diminished rate include enzyme inhibition and nonspecific substrate cleavage. Aminopeptidase inhibitors, bestatin and arginine hydroxamate, where used to prevent the non-specific cleavage of Gln from the Gln-ßNA substrate. Interesting, the lowered activity of yQC might have resulted from partial inhibition by bestatin. After running multiple series of the spectrophotometric assay, bestatin was determined to inhibit the activity of yQC (data not shown); whereas, bestatin did not seem to affect pQC in the fluorometric assay. The reason for this discrepancy could lie in structural differences between the two molecules. Bestatin is able to bind to zinc via two coordination sites (24). Therefore, the decreased yQC activity might have resulted from the presence of bestatin, since

bestatin could potentially interfere with the zinc atom complexed with yQC. A fluorometric assay should be carried out that excludes bestatin from the reaction mixture to see if the QC activity increases. Also, despite the precautions taken to prevent non-specific cleavage, there is a possibility that an aminopeptidase was responsible for the cleavage of the Gln-ßNA substrate.

The inhibition study of yQC (Fig 4) with 1 mM imidazole indicated that the observed QC activity was inhibited by the addition of imidazole. Imidazole has previously been determined to competitively inhibit hQC (20). Furthermore, imidazole is not known to inhibit aminopeptidase activity. Therefore, the ability of imidazole to inhibit yQC suggests the observed activity was due to QC, and not an aminopeptidase. The imidazole is thought to inhibit the formation of pyroglutamic acid by resembling the reactive intermediate.

SDS-PAGE analysis of DEAE purified protein samples further suggests that the yQC protein was isolated. As seen in Figure 5, the protein band at 41 kd correlates with the theoretical molecular weight of QC. It should be noted that the yQC protein is predicted to have an N-terminal signal sequence that resembles an ER-directing sequence. If the sequence was cleaved from the protein, the weight would be reduced. However, predicted glycosylation could also serve to increase the apparent molecular weight of the QC protein. Therefore, while SDS-PAGE analysis does not conclusively verify the identity of the isolated protein, it does provide further suggest that QC had been purified. Once the isolated protein band is digested with trypsin and analyzed with MALDI-TOF, the protein identification will be conclusive.

Further investigation of yQC should begin with an extended purification of yQC. Since previous studies have determined other forms of QC to be glycoproteins, it is probable that yQC also contains sugar residues; therefore, lectin affinity chromatography might result in a significantly purer product. A purer sample could then be used to conduct a kinetic analysis of yQC. Also, sequence analysis of the isolated protein would verify the isolate protein is QC.

After yQC has been adequately purified and characterized, the next step will be to examine its physiological function. In mammals, QC modifies biologically active peptides. Currently, no signaling peptides have been identified in *S. cerevisiae* that contain N-terminal glutamine residues. However, yQC might catalyze the same reaction on proteins and not peptides. This modification, as shown in previous research, can affect the longevity and integrity of proteins by enhancing their resistance to degradation by aminopeptidases. Also, GFP tagging of the yQC gene would determine protein distribution throughout the yeast cell, which could provide valuable information for further enzyme preparation and purification.

In summary, the data presented in this paper further confirms the presence of QC in yeast. A purification protocol for yQC was designed and two distinct activity assays were used to verify the presence of QC activity. SDS-PAGE analysis was used to confirm protein purification and determine the isolated protein's molecular weight. Another significant finding is that 1 mM Imidazole competitively inhibits yQC function, which correlates with the previously determined hQC inhibition by imidazole. While this study has initiated the process of purification and characterization, yQC still needs to be further purified and characterized.

ACKNOWLEDGEMENTS

I would like to thank L. Kevin Lewis for the insight he gave to working with yeast. A deep thanks to Greg and Nick, my lab partners, who filled a sterile environment with life. Finally, I would like to thank Dr. Booth for her willingness to answer my many questions.

Table I. Purification of QC from *Saccharomyces cerevisiae*.

Fraction	Total Protein mg	Total Activity µmol/hr/mL	Specific Activity µmol/hr/mg	Recovery %	Purification fold
G-25	0.728	0.122	0.17	---	---
DEAE	0.060	0.306	5.10	250	30

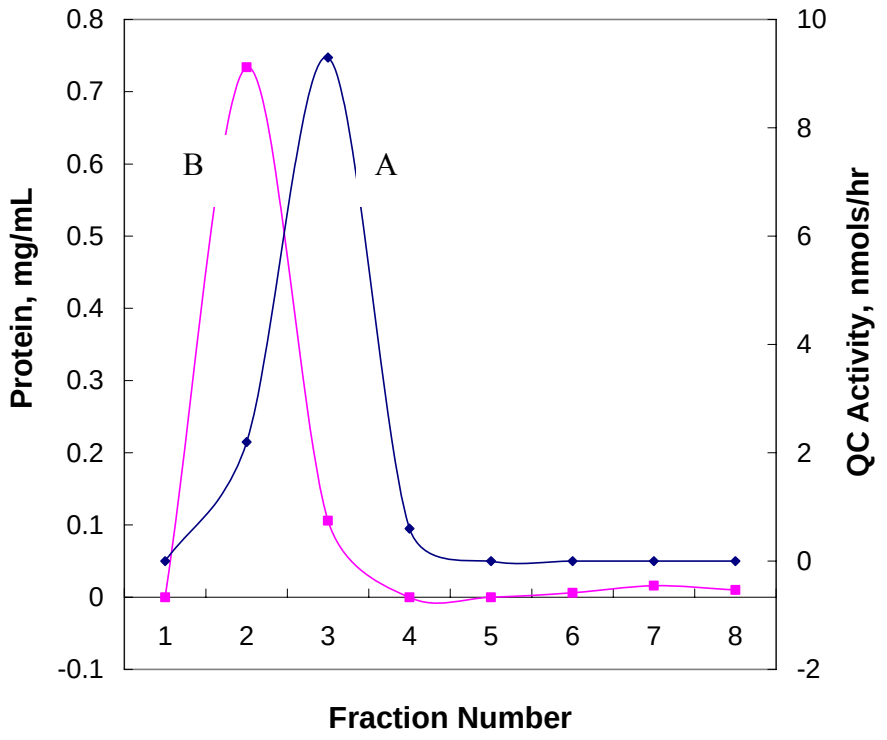


Figure 1. Gel filtration chromatography using G-25 resin. Yeast protein was obtained as described in Materials and Methods. Aliquots of 0.05 mL were taken from fractions and assayed using the spectrophotometric assay (A). Protein concentration was measured using the BCA assay (B).

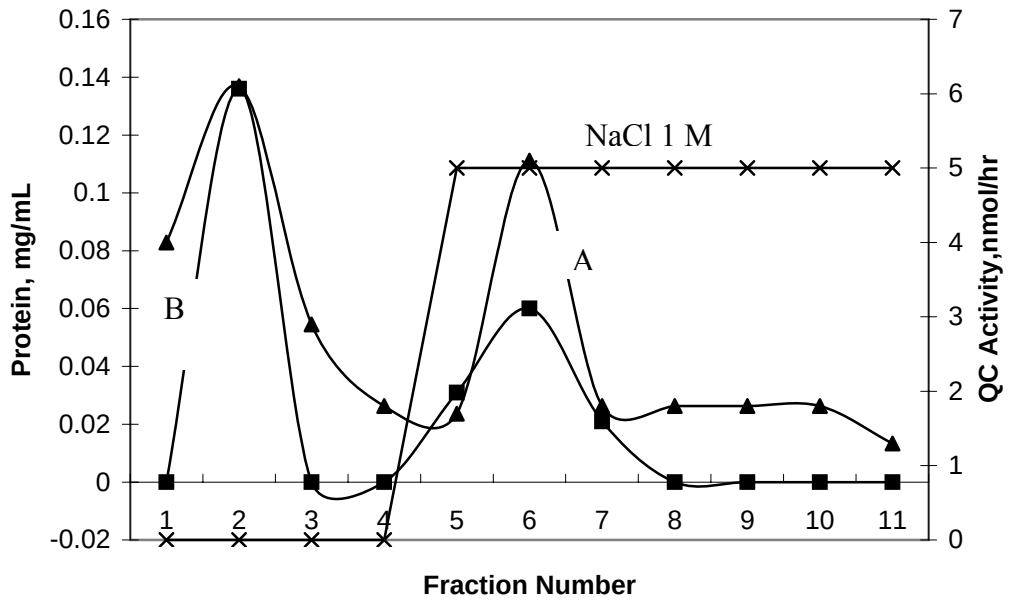


Figure 2. Anion exchange chromatography of yeast protein extract. Column fractions were assayed using the spectrophotometric assay (A). Protein concentration was measured using the BCA assay (B).

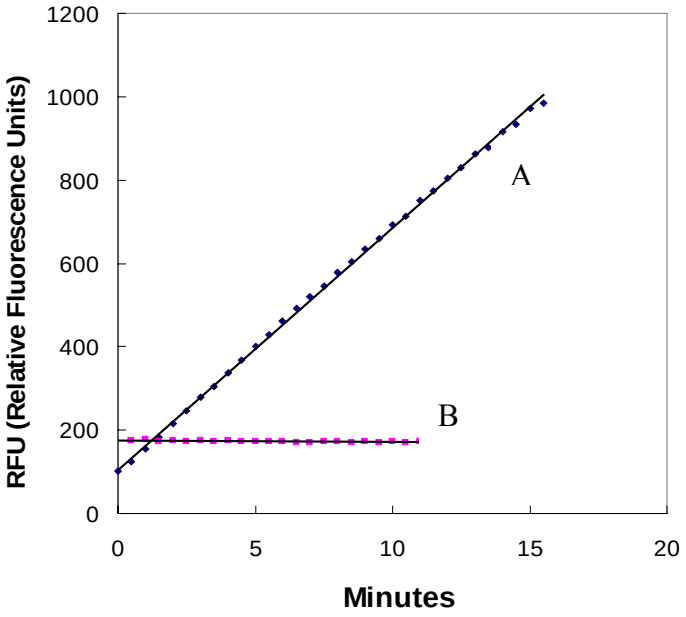


Figure 3A. Fluorometric analysis of pQC and yQC activity. The QC activity was monitored by recording the fluorescence emission at 410 nm. The formation of product by pQC (A) was linear during the time of analysis, and resembled prior results (19). The yQC (B) showed a reduced activity compared to the pQC during the time of analysis. The negative control, which excluded purified QC, showed no increase (data not shown).

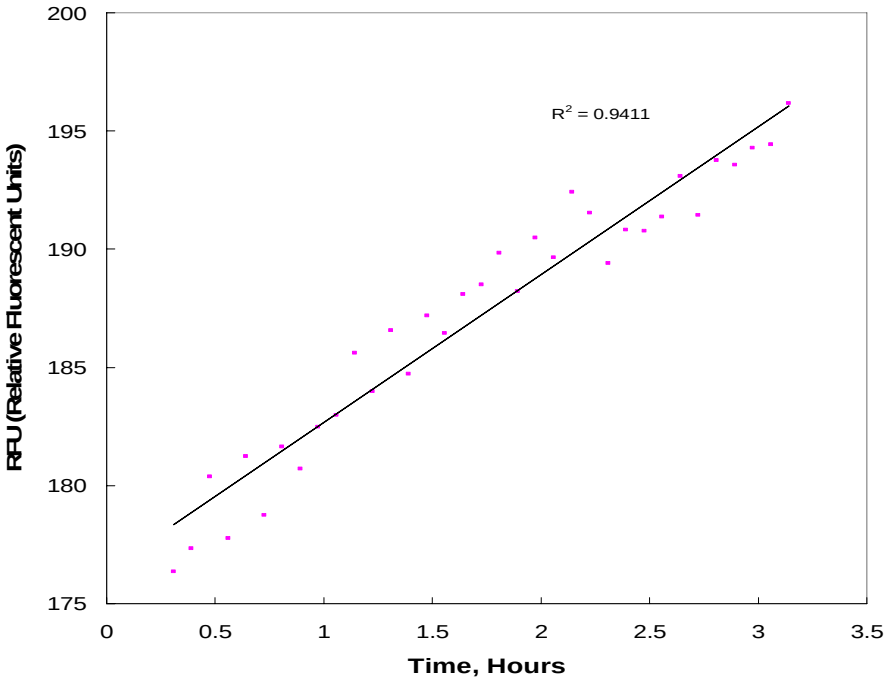


Figure 3B. Fluorometric Analysis of yQC. The progress curve of 2-naphthylamine formation showed a linear increase, which indicated QC activity. The control, which excluded purified QC, showed no increase in activity (data not shown).

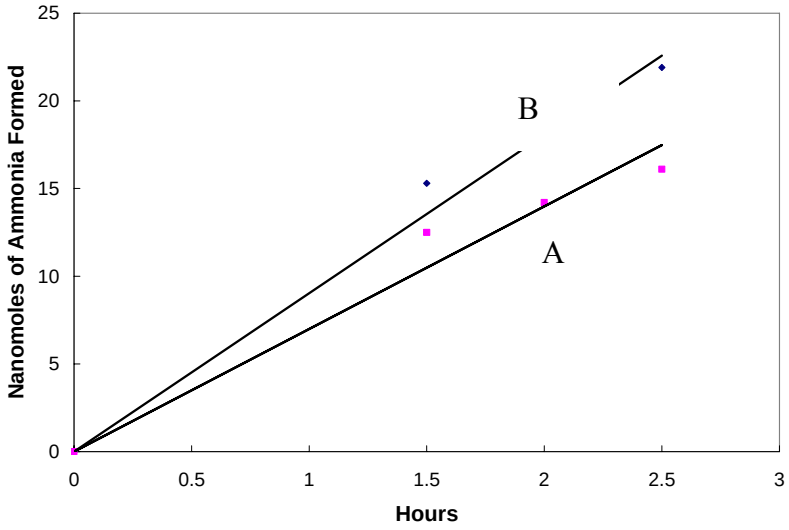


Figure 4. Inhibition of yQC with 1 mM Imidazole. Incubations contained 60 µg/mL of partially purified protein with (A) or without (B) imidazole. The QC activity was measured using the spectrophotometric assay.

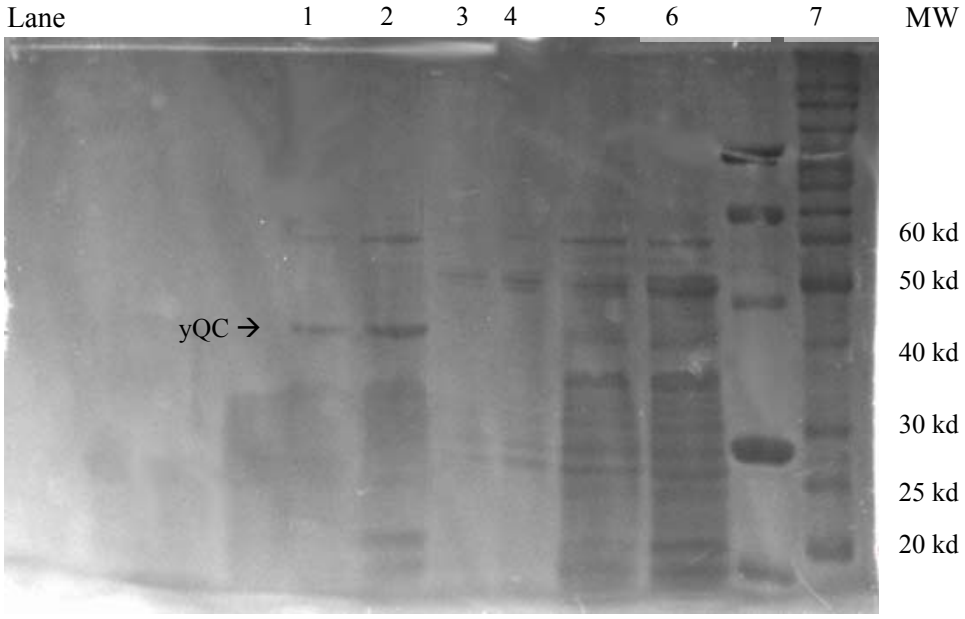


Figure 5. SDS-PAGE Analysis of DEAE Chromatography. The protein molecular marker that was used was the Benchmark Protein Ladder from Invitrogen (Lane 7). Examination of the elution fractions reveal the presence of a protein band at 41 kd (Lanes 1 and 2), which is the theoretical mass of yQC. Lanes 3 – 6 are the fractions collected prior to elution.

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