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Identification of human calphoglin-induced phosphoglucomutase phosphorylation in *Escherichia coli*

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Abstract

Orthologous proteomes, universal protein networks conserved from bacteria to mammals, dictate the core functions of cells. To isolate mammalian protein sequences that interact with bacterial signaling proteins, a BLASTP genome search was performed using catalytic domains of bacterial phosphoryl-transfer enzymes as probes. A [^{32}P]phosphoryl-transfer assay of these mammalian cDNA-expressing *Escherichia coli* cells was used to screen proteins retrieved from the database. Here we report that the expression of a human protein, named calphoglin, resulted in a significant increase in the phosphorylation of a 55-kDa protein in *E. coli*. The phosphorylation of the 55-kDa protein was acid-stable and its isoelectric point was determined to be 5.4. The 55-kDa protein was sequentially purified from an *E. coli* extract using three chromatography and two-dimensional polyacrylamide gel electrophoresis. Finally, the 55-kDa protein was purified 830-fold to homogeneity and the N-terminal amino acid sequence was analyzed. The sequence obtained, AIHNRAGQPAQQ, was identical to the N-terminal amino acids of *E. coli* phosphoglucomutase (PGM). This method may be applicable to the detection and analysis of other orthologous proteomes.

Keywords: Calphoglin; Phosphoglucomutase; Protein phosphorylation; Genome; Orthologue proteome

1. Introduction

Recent studies using yeast expression systems have revealed that a large number of human proteins interact with yeast intracellular signaling proteins thereby interfering with cell division [1]. While *Escherichia coli* (*E. coli*) is more commonly used for expressing human proteins, expressed human proteins generally do not interact with *E. coli* intracellular signaling pathways because mammals and prokaryotes are phylogenetically distant and most of their proteins have lost commonality in structure, folding and function. Recent genomic studies of a broad range of organisms, however, has revolutionized the understanding of prokaryotic cells by revealing that numerous genes are conserved over a broad biological range; from single-celled bacteria to human cells. In addition to nucleotide and protein synthesis-related genes, orthologous genes encoding a number of metabolic enzymes and ion channels were found to be present in the three domains of life: archaea, bacteria and eukaryote [2–4]. Phosphoglucomutase (PGM), a critical enzyme in both glucolysis and gluconeogenesis, is one of the most evolutionarily conserved enzymes and has a common catalytic mechanism in diverse cells. The nature of these universal gene products and their conserved intracellular networks suggest a core function to all cells.

At the molecular level, phosphate is the most versatile and key chemical moiety that dominates biochemical pathways [5]. The genetic building blocks, DNA and RNA, are macromolecular phosphodiesters. Many of the coenzymes and intermediary metabolites are phosphoric or pyrophosphoric acid esters. In addition, phosphate is a part of the basic unit of energy for the cell (ATP) and the molecular

Abbreviations: *E. coli*, *Escherichia coli*; PGM, phosphoglucomutase; PAGE, polyacrylamide gel electrophoresis; CBB, Coomassie brilliant blue; PVDF, polyvinylidene fluoride

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switch for modulating protein phosphorylation, which controls protein function. All these things make it clear that phosphoryl-transfer was selected by early evolution for biochemical transformations and macromolecule control. As the richness and conservation of the protein phosphorylation network in eukaryotes began to manifest over the last two decades, the search for *E. coli* equivalents diminished. In contrast, yeast *Saccharomyces cerevisiae* had many advantages in terms of relevance to higher systems and thus has been used as a unicellular model organism for the study of basic eukaryotic processes in cell biology. However, the complete sequence of the human genome in addition to that of a broad range of other organisms have created a need for screening methods in order to detect protein networks that dictate the core function of cells. In the era of functional proteomics, the universal protein signaling pathways, which are conserved across a broad range of phylogenetic species, have become important clues to functionally annotate the entire assembly of multiple intracellular protein complexes [6]. *E. coli* is particularly advantageous over yeast and other eukaryotic system in that its commonality with human cells is restricted to essential cellular signaling mechanisms.

The aim of the present study is to reveal heterologous protein interactions by expressing human cDNA in bacterial systems and monitoring their activities of phosphoryl-transfer. The expression of an evolutionarily conserved protein, calphoglin, was found to significantly increase the level of phosphorylation in *E. coli* PGM. Here we represent the calphoglin-induced 55-kDa-protein phosphorylation in *E. coli*, its two-dimensional electrophoresis profile, its purification from *E. coli* extracts, and its identification as an *E. coli* PGM. Upon identification of PGM in this study, we recently performed yeast two-hybrid experiments and identified another conserved enzyme, inorganic pyrophosphatase (IPP), as a common interacting protein between calphoglin and PGM [7]. Other detailed functional studies further revealed that the calphoglin enhanced PGM activity through the activated IPP and more directly on its own in both cells of *E. coli* and mammals. Calphoglin is a novel type PGM activator linked to cellular calcium signaling. This line of evidence shows the striking conservation of the calphoglin-PGM pathway, supporting the notion of an orthologous proteome.

2. Experimental

2.1. Materials

[γ-^{32}P]ATP (3,000 Ci/mmol) was obtained from Amersham Biosciences and the Institute of Isotopes Co., Ltd. (Budapest, Hungary). The *E. coli* strains, BL21-AI and BL21-SI were from Invitrogen. The *E. coli* strain, DH5α was from Toyobo (Osaka, Japan). Q-sepharose, phenyl-sepharose, and butyl-sepharose columns were from Amersham Biosciences. The PVDF (polyvinylidene fluoride) transfer membranes were from Millipore. All other reagents were from Sigma or Wako Pure Chemicals (Osaka, Japan), unless specifically noted.

2.2. Two-dimensional PAGE (polyacrylamide gel electrophoresis)

Partially purified 55-kDa protein was prepared for two-dimensional PAGE by lyophilization and resuspension in sample buffer containing 4% Nonidet P-40, 0.6% SDS, 0.1 M DTT, 5.5% ampholytes (pH 4–9) and 9.5 M urea. Approximately 70–200 μg of sample protein was analyzed in the first dimension on a 13.5-cm tube gel. Tube gels contained 2.8% polyacrylamide, 2% Nonidet P-40, 2% ampholytes (pH 4–9), and 9.5 M urea. The gel was preelectrophoresed at 200, 300, and 400 V for 10 min each, the sample was then loaded and electrophoresed toward the anode for 12 h at 800 V. Upon completion of isoelectric focusing, the tube gels were fixed to the top of 12% polyacrylamide slab gels with 1% agarose and electrophoresed with a constant current of 40 mA/gel.

2.3. Protein expression, E. coli lysates and phosphorylation experiment

Full-length human calphoglin cDNA was subcloned into an *E. coli* expression vector, pDEST14 (Invitrogen). The *E. coli* strains, BL21-AI and BL21-SI (Invitrogen) were transformed with this plasmid and plated on LB and LBON plates containing ampicillin, respectively. These cells were grown in 5 ml LB and LBON medium at 37 °C with vigorous shaking until they reached the mid-log phase. The cultures were induced by 0.2% arabinose and 0.3 M NaCl, respectively to express proteins. The cells were harvested 2 h after the induction by centrifugation. A quantity of 0.3 ml of resuspended cell was sonicated on ice using a microprobe for 1 min. Twenty-five microliters of the phosphorylation solution contained 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 20 μCi of [γ-^{32}P]ATP and 30 μg of lysate protein. Reactions were carried out for 60 min at 25 °C.

2.4. Extraction of E. coli protein fraction

From 200 ml LB cultures, the *E. coli* cells were harvested by centrifugation and the pellet was resuspended in 10 ml of ice-cold 20 mM Tris–HCl pH 8.2, 10 μg/ml leupeptin, 10 μg/ml benzamide, 10 μg/ml aprotinin and 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride. The cell suspension was sonicated on ice using a microprobe for 40 min. The cell lysate was centrifuged at 10,000 $\times$ *g* for 15 min at 4 °C. The collected supernatant was filtered through a 0.45 μm filter to remove cell debris, and subsequently mixed with 300 μl of [^{32}P]-phosphorylated extracts of calphoglin-expressing *E. coli* in order to label the 55-kDa protein.

2.5. Q-sepharose anion exchange chromatography

The pooled 10,000 $\times$ *g* supernatant was injected onto a 1-ml HiTrap Q HP column equilibrated with buffer A con-

taining 20 mM Tris–HCl pH 8.2. After the sample was loaded, the column was washed for 10 min with buffer A at 1 ml/min, and a stepwise ascending gradient of 0.06–0.54 M NaCl was applied. Fractions of 1 ml were collected into ten tubes. The fractions containing the 55-kDa protein were pooled and fractionated by precipitation with 40% ammonium sulfate. After centrifugation at 10,000 × *g* for 10 min, the supernatant was removed and collected for further purification.

2.6. Phenyl-sepharose/butyl-sepharose hydrophobic interaction chromatography

The concentrated 55-kDa protein fractions were diluted with 0.4 volumes of 0.2 M Na_2HPO_4–NaH_2PO_4 (pH 7.0) and then loaded onto a 1-ml HiTrap Phenyl HP column equilibrated with buffer B containing 1.56 M ammonium sulphate and 60 mM Na_2HPO_4–NaH_2PO_4, pH 7.0. After sample loading, the column was washed for 10 min with buffer B at 1 ml/min. The column was then eluted using a stepwise descending gradient 1.43–0 M ammonium sulphate over 13 ml. One milliliter fractions were collected into thirteen tubes. The phenyl-sepharose fractions-containing the 55-kDa protein were pooled, adjusted to 1.56 M ammonium sulphate, and then loaded onto a 1-ml HiTrap Butyl FF column equilibrated with buffer B.

2.7. N-terminal amino acid sequencing

The partially purified 55-kDa protein (200 μg) was electrophoresed by two-dimensional PAGE and electrotransferred onto a Immobilon-P^{SQ} PVDF membrane. The proteins on the membrane were submitted to microsequencing with an Applied Biosystems Procise 494 HT Protein Sequencer (APRO Life Science Institute, Inc., Japan).

3. Result

3.1. Candidate cDNA

In a BLASTP genome search, catalytic domains of numerous bacterial histidine kinases and other phosphoryl-transfer enzymes were used as probes to obtain mammalian protein sequences that interact with *E. coli* signaling proteins. Fifty mammalian gene products homologous to these domains were revealed, having expect values of ∼0.001. Although this degree of homology is generally considered to be insufficient to predict protein function, these unannotated gene products were candidates for bioactive molecules in bacterial phosphoryl-transfer signaling pathways.

3.2. Calphoglin-induced phosphorylation of the 55-kDa protein in E. coli

These cDNAs were transfected to the *E. coli* strains, BL21-AI and BL21-SI. The resultant colonies from both strains were induced using 0.2% arabinose and 0.3 M NaCl, respectively in order to express the proteins. The cell lysates from these culutures were phosphorylated with [^{32}P]ATP. The expression of a human cDNA (named calphoglin) in BL21-AI using arabinose as an inducer exhibited a significant increase in the [^{32}P]phosphorylation of the 55-kDa protein (left arrow in Fig. 1). Similarly, the [^{32}P]phosphorylation of the 55-kDa protein increased significantly when calpholgin was expressed in BL21-SI induced by NaCl (right arrow, Fig. 1). In contrast, upon expression of other clones in the same system, no effect on the protein phosphorylation was observed. These results indicate that the calphoglin protein, not the inducer, is responsible for the increase of phosphorylation. When calphoglin cDNA in pBluescript was transfected into *E. coli* BL21 where basal expression is leaky, calphoglin significantly suppressed the growth of the host cells.

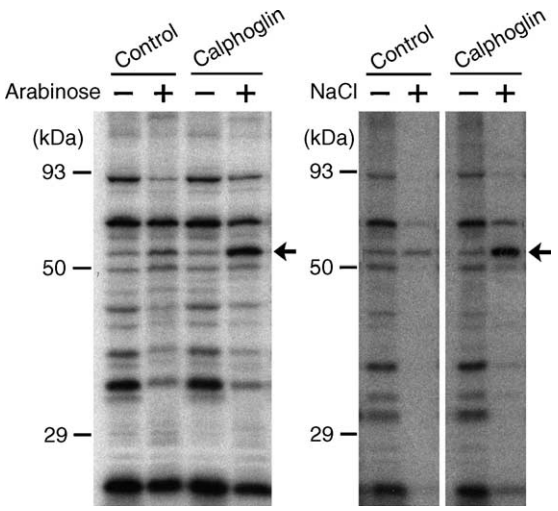

Fig. 1. The specific increase in the phosphorylation of the 55-kDa protein in calphoglin-expressing *E. coli*. Control vector- and calphoglin cDNA-tranfected *E. coli* cultures induced using 0.2% arabinose and 0.3 M NaCl in order to express the proteins. The cell lysates were phosphorylated with 20 μCi of [^{32}P]ATP. Ten micrograms of phosphorylated proteins were separated by SDS–PAGE on 12% gels, transferred onto PVDF, and autoradiography analyzed. Arrows indicate the position of the 55-kDa protein.

Calphoglin was initially a protein of unknown functions, but had a unique structure that differed from the “kinase” protein family. Calphoglin mRNA encodes a protein of 691 amino acids (AY563137) that has an evolutionarily conserved domain, one that is also found in Smc ATPase, Sbc ATPase, myosin tail, and metallopeptidase [7].

3.3. Two-dimensional gel electrophoresis of the 55-kDa protein and acid-stable phosphorylation

In order to characterize the 55-kDa protein, its isoelectric point was determined by two-dimensional PAGE of [^{32}P]-phosphorylated extracts of intact *E. coli* and calphoglin-expressing *E. coli* (Fig. 2). The 55-kDa protein migrated in two predominant spots with pI values of 5.3–5.4

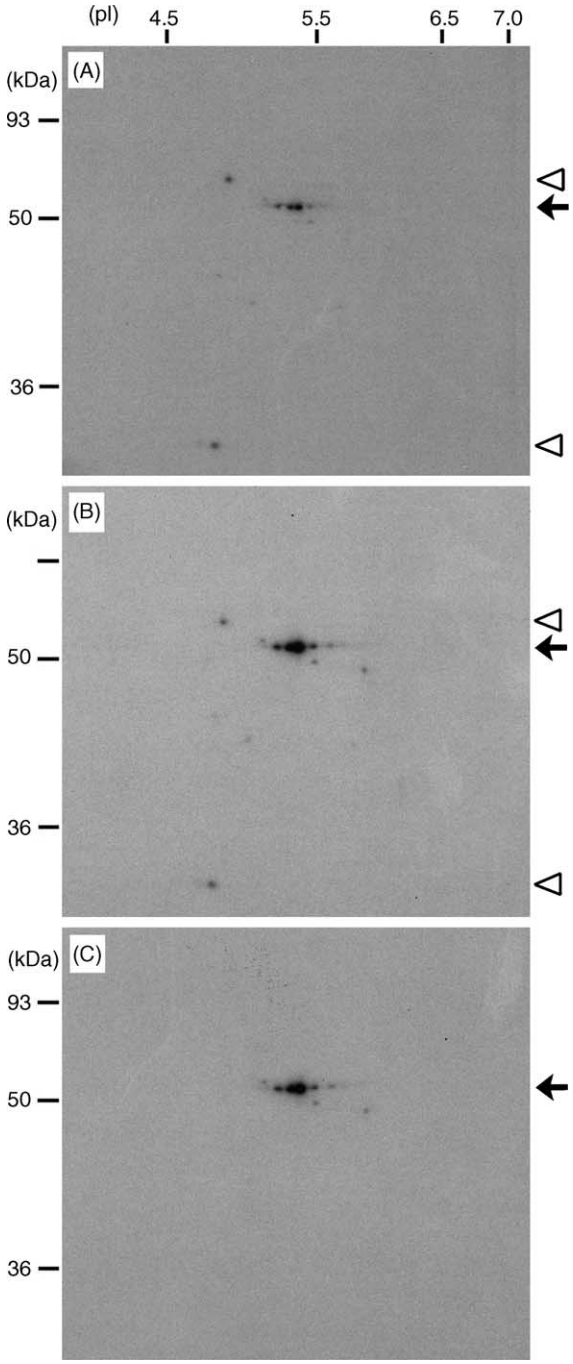

Fig. 2. Two-dimensional PAGE autoradiography of extracts of control *E. coli* (A) and calphoglin-expressing *E. coli* (B and C). Seventy micrograms protein was subjected to two-dimensional PAGE, with isoelectric focusing at pH 4–9, followed by SDS–PAGE on a 12% gel. The black arrows indicate the position of the 55-kDa protein. The white triangles indicate the acid-labile phosphorylation of *E. coli* proteins. (A) Gel pattern of the control vector-transfected *E. coli* [^{32}P]-phosphorylated protein (B) Gel pattern of the calphoglin cDNA-tranfected *E. coli* [^{32}P]-phosphorylated protein (C) Acid treatment of the calphoglin cDNA-tranfected *E. coli* [^{32}P]-phosphorylated protein.

and a molecular mass of 55-kDa (black arrow, Fig. 2A). The phosphorylation of the 55-kDa protein increased significantly upon expression of calphoglin (black arrow, Fig. 2B), whereas the phosphorylation of other protein spots appeared to be unaffected by the expression of calphoglin (white triangles, Fig. 2A and B).

An essential difference generally emerges when comparing protein phosphorylation in eukaryotes and prokaryotes [8]. In eukaryotes, phosphorylation of hydroxyamino acids (serine, threonine, tyrosine) in substrate proteins is accomplished by protein kinases. This type of phosphorylation is chemically stable. In contrast, another type of phosphorylation, by histidine kinase, established the generality of phosphoryl-transfer signal transduction in bacteria. The histidine kinase system is represented by sensor kinase/response regulator element that responds to a wide spectrum of environmental stimuli and requires acid-labile phosphorylation at histidine and aspartate residues [9]. As shown in Fig. 2C, the chemical stability of the phosphorylated proteins was examined by treating PVDF transfer membranes with 1 N HCl for 2 hrs. In contrast to the acid-labile nature of the most *E. coli* phosphoproteins (white triangles, Fig. 2B and C), the phosphorylation of the 55-kDa protein was acid-stable, which is characteristic of eukaryotic proteins (black arrows, Fig. 2B and C).

3.4. Purification of the 55-kDa protein

E. coli DH5α was grown in 200 ml of LB culture broth. The harvested cell was resuspended in a 10 ml solution volume and sonicated using a microprobe. The cell lysate was centrifuged at 10,000 × *g* for 15 min, the supernatant was removed and collected. At the initial and some other steps of purification, the 55-kDa protein was phosphorylated with 240 μCi [^{32}P]ATP in a 300 μl reaction volume, and added to the large scale sample as a radiolabelled tracer protein. Prior to chromatographic purification, the extraction efficiency of the 55-kDa protein was examined. The majority of the radioactive labeled 55-kDa protein present in the lysate was recovered in the supernatant after 10,000 × *g* centrifugation.

The supernatant was loaded onto a Q-sepharose column, and the protein was eluted with an increasing stepwise gradient of NaCl, as shown in Fig. 3A and B. The phosphorylated 55-kDa protein was observed to elute from the Q-sepharose column at a 0.24–0.3 M concentration of NaCl as a single peak (fractions 4 and 5 in Fig. 3C). Fractions 4 and 5 were pooled and utilized for further purification. The 55-kDa protein was purified 2.6-fold after Q-sepharose column chromatography purification, and had a 49% yield. The results are summarized along with those for the other purification steps in Table 1. Fractionation using 40% ammonium sulfate provided only a slightly more purified protein (Table 1), however it proved to be a necessary step as it prepared the sample buffer for the hydrophobic interaction chromatography step that would follow. The 40% ammonium sulfate supernatant was removed and immediately applied to a phenyl-sepharose column. As shown in Fig. 4B, the 55-kDa protein was strongly retained on the phenyl-sepharose column, and finally eluted at an ammonium sulfate concentration between 0.13 and 0 M (fractions 11 and 12), resulting in at least 5.5-fold purifi-

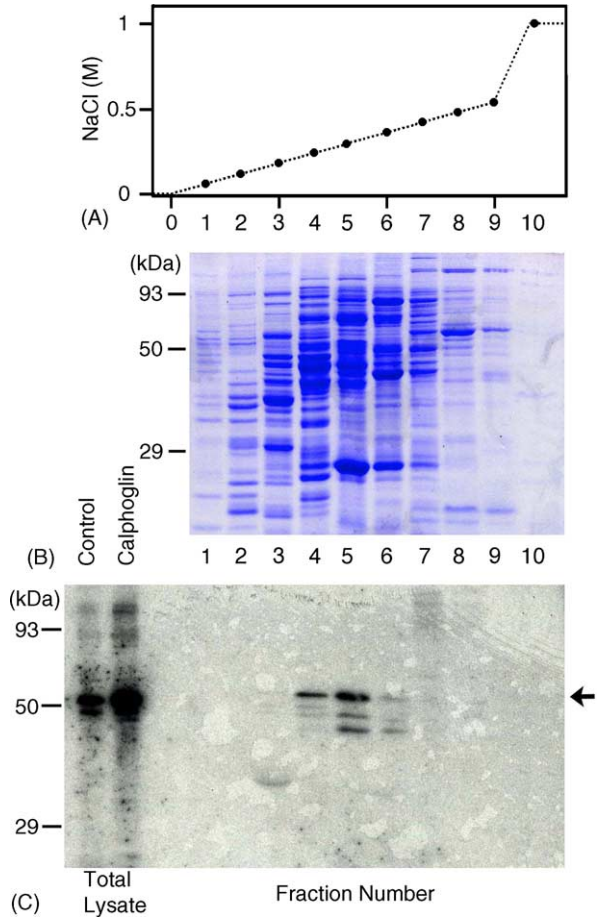

Fig. 3. Anion exchange chromatography of the 55-kDa protein on Q-sepharose. (A) The 55-kDa protein was extracted in the 10,000 × *g* centrifuged supernatant of the *E. coli* lysates, and applied to a Q-sepharose column equilibrated with 20 mM Tris–HCl pH 8.2, and the protein was eluted by an increasing stepwise gradient of NaCl (0–0.54 M). (B) Aliquots of the fractions were separated by SDS–PAGE on 12% gels and visualized using CBB staining. (C) Fractions separated by SDS–PAGE were transferred onto PVDF, and autoradiographed. The radiolabeled 55-kDa protein eluted in fractions 4 and 5, and the fractions were combined for further purification. The first and second lanes contain the phosphorylated total lysate of vector- and calphoglin cDNA-transfected *E. coli*, respectively. The arrow indicates the position of the 55-kDa protein.

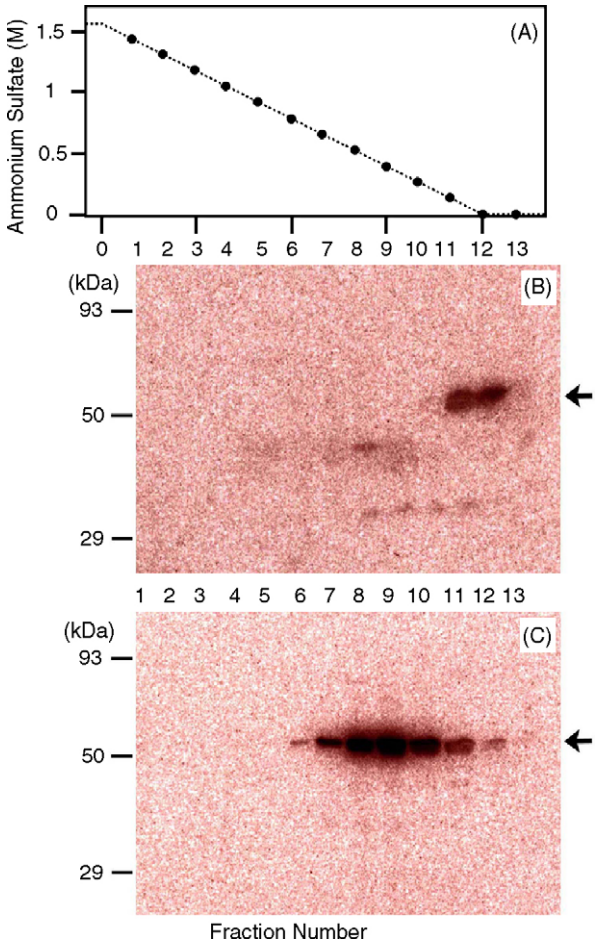

Fig. 4. Purification of the 55-kDa protein by phenyl-sepharose and butyl-sepharose chromatography. (A) Ammonium sulfate was added to the 55-kDa-protein-containing fraction that eluted from the Q-sepharose column to obtain a final concentration of 40% ammonium sulfate. The 40% ammonium sulfate supernatant was removed and applied to a phenyl-sepharose column, and the protein was eluted using a decreasing stepwise gradient of ammonium sulfate (1.43–0 M). After adding ammonium sulfate to obtain a final concentration of 40%, the fraction from the phenyl-sepharose column containing the 55-kDa protein was applied to a butyl-sepharose column, and the protein was eluted using a decreasing stepwise gradient of ammonium sulfate (1.43–0 M). (B) Aliquots of the fractions from the phenyl-sepharose column were separated by SDS–PAGE and transferred onto PVDF. The PVDF onto which the protein fractions were transferred was autoradiographed. The radiolabeled 55-kDa protein eluted in fractions 11 and 12 as indicated by the arrow, the fractions were pooled for further purification. (C) Aliquots of the fractions from the butyl-sepharose column were separated by SDS–PAGE and transferred onto PVDF. The radiolabeled 55-kDa protein eluted in fractions 7–11 as indicated by the arrow. Fraction 9 was selected for further purification.

cation. The 55-kDa protein was then further purified using a butyl-sepharose column. Fig. 4C shows that the 55-kDa protein eluted from the butyl-sepharose column as a broad peak at a concentration of 0.65–0.13 M ammonium sulfate (fractions 7–11) when eluted using a decreasing concentration gradient of ammonium sulfate. In order to further purify the protein, the fraction with the highest radioactivity (fraction 9) was selected. This butyl-sepharose column purification step resulted in 2.6-fold additional purification. Fraction 9 was concentrated and the buffer was exchanged and separated using two-dimensional PAGE from pH 4 to 9. As shown in Fig. 5A, Coomassie brilliant blue (CBB) staining revealed more than 10 major protein spots on the

Table 1
Purification of 55-kDa phosphoprotein from *Escherichia coli*

		Protein (mg)	Purification (fold)	Yield (%)
1.	*E. coli* extract	83	1	100
2.	Q-sepharose	41	2.6	49
3.	Ammonium sulfate	33	2.9	44
4.	Phenyl-sepharose	2.8	16	21
5.	Butyl-sepharose	0.19	41	3.5
6.	Two-dimensional PAGE	0.009	830	3.5

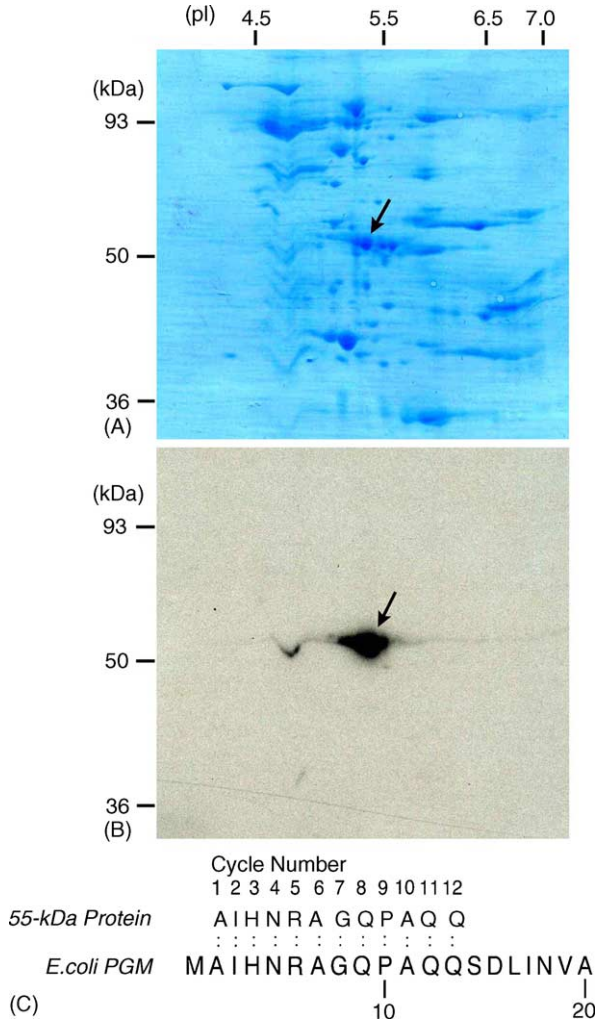

Fig. 5. Two-dimensional PAGE and N-terminal amino acid sequencing of the 55-kDa protein. (A) CBB-stained two-dimensional electrophoresis pattern of the partially purified 55-kDa protein-containing fraction 9 from butyl-sepharose column. (B) Autoradiography of the PVDF membrane revealed a few bands (arrows) that were exclusively radioactive at the molecular mass of 55-kDa had pI values of 5.3–5.4. (C) Identification of the 55-kDa protein as *E. coli* PGM. The spot of the 55-kDa protein (arrows) was cut out of the PVDF membrane and sequenced using a protein sequencer. The identified N-terminal sequence of the 55-kDa protein was aligned with the N-terminal sequence of *E. coli* PGM [10].

fraction-9-electroblotted membrane. In an autoradiographic image, the 55-kDa protein appeared as two continuous spots with pI values of 5.3–5.4 and a molecular mass of 55 kDa (arrow, Fig. 5B), corresponding the spot on the initial two-dimensional gel (Fig. 2). Finally, the 55-kDa protein was purified ~830-fold to homogeneity, with a total recovery of 3.5%.

3.5. Identification of the 55-kDa protein

In order to identify the 55-kDa protein, N-terminal sequencing was performed on the 55-kDa protein spot in the membrane. The sequence obtained for the 55-kDa protein was AIHNRAGQPAQQ, corresponding to the sequence of the N-terminal (2–13) amino acids in *E. coli* PGM protein (Fig. 5C). *E. coli* PGM is a 546 amino-acid protein, with a pI value of 5.35, and a molecular mass of 58.3 kDa [10]. It is known to act as an active enzyme when it is in its phosphorylated state [11]. This is consistent with the molecular profile of the 55-kDa protein observed in the purification process. Thus, the 55-kDa protein has been confirmed as *E. coli* PGM.

4. Discussion

Calphoglin was an evolutionarily conserved protein with a unique structure and an unknown function. A calphoglin protein homology search was conducted and revealed the presence of a conserved structure for various species of proteins with equally balanced divergence [7]. Calphoglin showed high homology with two human functionally unknown proteins; tumor necrosis factor receptor-associated factor 6-binding protein and T-cell leukemia virus type I binding protein, with expect value/percent identity of 3e-59/28 and 4e-59/26%, respectively. In addition, calphoglin showed an intermediate level of homology to prokaryotic, yeast and plant proteins with expect values of 1e-9 to 5e-17: Smc1 (1e-9/20%); prokaryote, Nuf1p (4e-16/19%); yeast, and Myosin heavy chain-like protein (5e-17/23%); plant. Furthermore, a conserved domain database search revealed that the conserved domains on these calphoglin homologues were found in Smc ATPase, DNA repair ATPase, myosin tail, and metallopeptidase.

Similar to calphoglin, PGM is an evolutionarily conserved protein that catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate. This reaction is central to energy metabolism in all cells and is essential to the synthesis of the polysaccharides in the cell walls of bacterial cells. PGM derived from a diverse range of species has a molecular weight between 55 and 65-kDa and is known to be active in its phosphorylated form [12]. In this enzyme mechanism, a phosphoryl group is transferred from the active phosphoenzyme to glucose-1-phosphate, forming a dephosphoenzyme-glucose-1,6-diphosphate intermediate, which then rephosphorylates the enzyme to yield glucose-6-phosphate. In rabbit muscle PGM, the formation of the intermediate is facilitated by phosphorylated ^{116}Ser [13]. The presence of the PGM phosphorylated enzyme has been observed in all cellular organisms (archaea, bacteria and eukaryote) with a highly conserved structure [10,14]. These facts coupled with the observations found in this evaluation, support the presence of a universal cellular signaling mechanism for PGM across all domains of life.

In addition, other members of the hexosephosphate mutase family (phosphoglucosamine mutase and phosphomannomutases) are only enzymatically active in their phosphorylated forms [15,16]. Knowlegde of the structure and function of this family is rapidly growing due to the completion of various genome-sequencing projects. Enzyme phosphorylation is a key factor involved in regulating the flow of sugar metabolites in these pathways. The initial in vivo phosphorylation

of these enzymes remains unknown [17]. Calphoglin was found to work as an in vivo regulator that phosphorylated PGM. Since the protein structures of calphoglin and PGM are phylogenetically conserved, the protein-interaction pathways are likely to be conserved, and this phosphorylation is expected to occur according to the same mechanism in diverse species (human cells to *E. coli*). Thus, the identification of calphoglin-induced PGM phosphorylation is significant in terms of understanding a common cellular mechanism regulating the flow of glucose metabolites between eukaryotes and prokaryotes.

Recent analysis of yeast protein complexes on a proteome-wide scale revealed that orthologous proteins interact preferentially with complexes enriched with other orthologues [6]. The orthologous gene products are thought to be responsible for essential cellular activities. Similarly, the likelihood of essential gene products to interact is higher for essential- than for nonessential-proteins [6]. These observations support the existence of an 'orthologous proteome' that may represent the core functions of cells. The presence of orthologous protein interactions provides insight into the mechanism of the calphoglin-induced *E. coli* PGM phosphorylation in this study. The calphoglin-induced activation of *E. coli* PGM suggests that the inter-species orthologous protein interaction may be applicable to the analysis of other conserved protein networks, such as essential metabolic enzymes, and nucleotide and protein synthesis-related proteins.

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