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Deoxyhypusine synthase is phosphorylated by protein kinase C *in vivo* as well as *in vitro*

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Abbreviations: CHO, Chinese hamster ovary; DAEG, diacyl ethylene glycol; DAG, diacylglycerol; DHS, deoxyhypusine synthase; PKC, protein kinase C; P.Ser, phosphatidylserine; PMA, phorbol 12-myristate 13-acetate

Abstract

Deoxyhypusine synthase catalyzes the first step in the posttranslational synthesis of an unusual amino acid, hypusine, in the eukaryotic translation initiation factor 5A (eIF-5A) precursor protein. We earlier observed that yeast recombinant deoxyhypusine synthase was phosphorylated by protein kinase C (PKC) *in vitro* (Kang and Chung, 1999) and the phosphorylation rate was synergistically increased to a 3.5-fold following treatment with phosphatidylserine (P.Ser)/diacylglycerol (DAG)/Ca^{2+}, suggesting a possible involvement of PKC. We have extended study on the phosphorylation of deoxyhypusine synthase *in vivo* in different cell lines in order to define its role on the regulation of eIF5A in the cell. Deoxyhypusine synthase was found to be phosphorylated by endogenous kinases in CHO, NIH3T3, and chicken embryonic cells. The highest degree of phosphorylation was found in CHO cells. Moreover, phosphorylation of deoxyhypusine synthase in intact CHO cells was revealed and the expression of phosphorylated deoxyhypusine synthase was significantly diminished by diacyl ethylene glycol (DAEG), a PKC inhibitor, and enhanced by phorbol 12-myristate 13-acetate (PMA) or Ca^{2+}/DAG. Endogenous PKC in CHO cell and cell lysate was able to phosphorylate deoxyhypusine synthase and this modification is enhanced by PMA or Ca^{2+} plus DAG. Close association of PKC with deoxyhypusine synthase in the CHO cells was evident in the immune coprecipitation and was PMA-, and Ca^{2+}/phospholipid-dependent. These results suggest that phosphorylation of deoxyhypusine synthase was PKC-dependent cellular event and open a path for possible regulation in the interaction with eIF5A precursor for hypusine synthesis.

Keywords: amino acids; calcium/diglycerides; eukaryotic initiation factors; phosphorylation; protein kinase C; protein kinases

Introduction

The unusual amino acid hypusine [N^{ε}-(4-amino-2-hydroxybutyl)lysine] is found in a singular cellular protein, known as the precursor of eukaryotic translation initiation factor 5A (eIF-5A), and is produced post-translationally in two successive enzyme-catalyzed reactions (Park *et al.*, 1993a; 1993b). In the first step of hypusine synthesis, deoxyhypusine synthase catalyzes transfer of the butylamine moiety of the polyamine spermidine to the ε-amino group of a specific lysine residue of eIF-5A precursor protein to form the intermediate deoxyhypusine [N^{ε}-(4-aminobutyl)lysine] residue (Park *et al.*, 1982; Wolff *et al.*, 1995). Hydroxylation of the side chain of this intermediate by deoxyhypusine hydroxylase completes hypusine biosynthesis and eIF-5A maturation (Abbruzzese *et al.*, 1986).

Hypusine formation is essential for eukaryotic cell proliferation, although its exact mode of action is unclear. Inactivation of the two eIF-5A genes in yeast (Schnier *et al.*, 1991; Wöhl *et al.*, 1993), or the deoxyhypusine synthase gene (Sasaki *et al.*, 1996; Park *et al.*, 1998), causes loss of viability by arresting cell proliferation. As the first enzyme in the hypusine syn-

thetic pathway, deoxyhypusine synthase occupies a critical position, and could provide a potential target for intervention in cell proliferation.

Deoxyhypusine synthase has been purified from different eukaryotic species [rat testis (Wolff *et al.*, 1995), HeLa cells (Klier *et al.*, 1995), *Neurospora crassa* (Tao and Chen, 1995b), and yeast (Sasaki *et al.*, 1996)]. The gene was identified in yeast (Kang *et al.*, 1995; Sasaki *et al.*, 1996), and human and *Neurospora* cDNAs have been cloned (Joe *et al.*, 1995; Tao and Chen, 1995a). The amino acid sequence of the enzyme is highly conserved. The enzyme from all species studied exists as tetramer of identical subunits of 40-43 kDa (depending on the species) and the cofactor requirements and catalytic properties of the enzyme from different species are similar (Joe *et al.*, 1995; Kang *et al.*, 1995; Wolff *et al.*, 1995).

In addition, eIF-5A in yeast is phosphorylated on serine (Kang *et al.*, 1993). Therefore eIF-5A undergoes two posttranslational modifications, hypusination and phosphorylation, where the activity of the factor is dependent on the first but is not influenced by the latter *in vitro*. Furthermore, hypusine formation in eIF-5A is not reversed when rates or specificity of protein synthesis is altered (Gorden *et al.*, 1987). However, if a possible reversible modification for the regulation of eIF-5A activity could exit, it will likely be on the synthetic steps. Deoxyhypusine synthase, the first enzyme involved in hypusination, could indeed be a suitable target for the regulation of the factor.

Yeast deoxyhypusine synthase contains putative phosphorylation sequence motifs, (S/T-X-K/R) for protein kinase C (PKC) and (S/T-X-X-D/E) for casein kinase II (CKII). Recently, yeast recombinant deoxyhypusine synthase was found to be phosphorylated by PKC *in vitro* (Kang and Chung, 1999). In this study, we confirmed phosphorylation modification of deoxyhypusine synthase *in vitro* and in intact CHO cells, deoxyhypusine synthase is phosphorylated by endogenous kinases and identified PKC as the primary candidate to catalyze this event.

Materials and Methods

Materials

[γ-^{32}P]ATP (6,000 Ci/mmol) was purchased from NEN Life Science Products. Dulbecco's modified Eagle medium (DMEM), antibiotic-antimycotic mixture, and trypsin-EDTA were from Gibco/BRL Life Technologies (Gaithersburg, MD). PKC was obtained from Upstate Biotechnology, protein size marker from Novex, nitrocellulose membrane from Millipore. Diacylglycerol (DAG) and diacyl ethylene glycol (DAEG) were obtained from Avanti Polar Lipids. Anti-phosphothreonine antibody and secondary antibodies were from Santa Cruz Biotechnology. Fetal bovine serum (FBS) was from Hyclone, and ECL Western blotting detection kit was purchased from Amersham Pharmacia Biotech. Phorbol 12-myristate 13-acetate (PMA), phosphatidylserine (P. Ser), sodium orthovanadate, protein A agarose, Nutrient mixture F-12 HAM, and all other chemicals were from Sigma. Deoxyhypusine synthase protein from rat testis and its specific antibody were prepared as described (Wolff *et al.*, 1995).

Cell culture

Chinese hamster ovary(CHO)-K1 cells and NIH3T3 cells were grown in Nutrient mixture F-12 and DMEM, respectively, supplemented with 100 unit/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, and 10% (v/v) FBS in a humidified 5% CO_2 incubator at 37°C. Serum deprivation was carried out by lowering the concentration of FBS from 10% to 0.5% for the period of 24 h prior to specific treatment.

Analysis of deoxyhypusine synthase phosphorylation

The phosphorylation reaction of yeast recombinant deoxyhypusine synthase by PKC (α+β+γ) was carried out as described (Kang and Chung, 1999). For *in vivo* phosphorylation of deoxyhypusine synthase, growth-arrested CHO cells plated onto 100-mm dishes were treated with or without DAEG, PMA, and Ca^{2+} plus DAG for the indicated times at 37°C. After treatments, cells were rinsed with ice-cold phosphate-buffered saline (PBS) and harvested with a rubber policeman, suspended in 0.5 ml of suspension buffer (10 mM Tris/HCl, pH 7.5, and 2 mM $MgCl_2$) and centrifuged at 2,700 *g* at 4°C for 5 min. Then they were freeze-thawed, suspended in 20 μl of suspension buffer containing 1% Triton X-100, 5 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF), vortexed, and then stood for 10 min on ice. Cell lysates were cleared by centrifugation at 15,000 *g* at 4°C for 10 min. The resulting cell lysates containing equal amounts of protein were used for immunoprecipitation and Western blotting.

Kinase assays using cell lysates were performed by adding total cell extract with 1 μg of recombinant deoxyhypusine synthase protein. Cell extracts were generated from 100-mm tissue culture dishes using repeated freeze-thawed cycle. Reactions were incubated with kinase buffer (50 mM Hepes, pH 7.6, 1.0 mM DTT, 250 μM Na_3VO_4, 1 mM $MgCl_2$, and 1.5 mM $MnCl_2$) in the presence of [γ-^{32}P]ATP at 37°C for 30 min. The phosphorylated reaction mixtures were processed for immunoprecipitation.

Immunoprecipitation

Cell lysate was equally mixed with same volume of IP buffer (50 mM Hepes, pH 7.8, 150 mM NaCl, 1% Triton X-100, and 1 mM PMSF). Immunoprecipitation was carried out for 2 h at 4°C with 5 μl of polyclonal rabbit anti-deoxyhypusine synthase antibody. The immune complex was precipitated by incubation with protein A agarose (Sigma, MO) for 30 min at 4°C with constant rocking. Immunoprecipitate was washed four times with 1 ml of IP buffer and the final pellet was fractionated on a 12% SDS-PAGE followed by Western blot analysis with monoclonal anti-phosphothreonine antibody or polyclonal anti-deoxyhypusine synthase antibody.

Western blot analysis

For detection of phosphorylated or unphosphorylated deoxyhypusine synthase, monoclonal mouse anti-phosphothreonine antibody or polyclonal rabbit anti-deoxyhypusine synthase antibody were used. Immunoblots were then incubated with goat anti-rabbit or anti-mouse antibodies coupled to horseradish peroxidase followed by chemiluminescence using ECL reagent (Amersham Pharmacia Biotech) with manufacturer's instructions.

Results

Phosphorylation of deoxyhypusine synthase by recombinant PKC [PKC(α+β+γ) mixture, and PKCδ] was reported and PMA, an activator of PKC, specifically stimulated the phosphorylation of this enzyme (Kang and Chung, 1999). PKC is a family of ten structurally related enzymes that have been implicated in a variety of cellular responses. PKC-mediated phosporylation is defined as a Ca^{2+}/phospholipid-dependent, DAG or phorbol ester activated serine/threonine protein kinase with exceptional atypical PKCs. A large number of cellular proteins are phosphorylated by PKC. Of these PKCα, β, and γ isoforms, classical PKCs (cPKCs), is inactive in its basic state, but is completely activated by P.Ser and DAG in the presence of Ca^{2+}. To examine the effects of various PKC activators on the phosphorylation of deoxyhypusine synthase by cPKC, yeast recombinant deoxyhypusine synthase was allowed to react in the presence of different sets of P.Ser (200 μg/ml), DAG (40 μg/ml), and Ca^{2+} (0.5 mM) (Figure 1A). Preincubation of PKC (α+β+γ) with Ca^{2+} or DAG in kinase assay resulted in 2-fold and 1.4-fold increase in the phosphorylation of deoxyhypusine synthase, respectively. Whereas, only slight increase in the amounts of radioactivity was observed in deoxyhypusine synthase treated with P.Ser alone. Treatment of Ca^{2+} plus P.Ser, Ca^{2+} plus DAG, or P.Ser plus DAG resulted in 3.3-fold, 3.1-fold, and 2.6-fold increase in the degree of phosphorylation, respectively. Maximal kinase activity was observed after the addition of Ca^{2+}, P.Ser, and DAG (3.5 folds). This PKC-mediated phosphorylation was blocked by the PKC inhibitor DAEG up to 320 μg/ml treatment in a dose-dependent manner (Figure 1B).

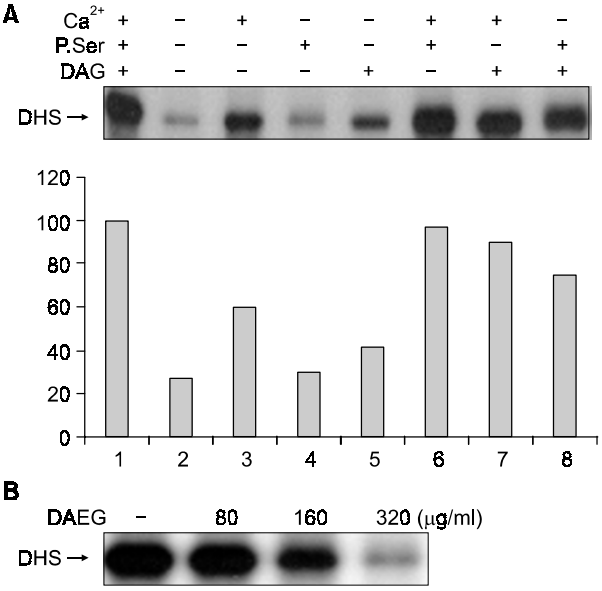

Figure 1. Influence of activators or inhibitor on the phosphorylation of yeast recombinant deoxyhypusine synthase by PKC. (A) Deoxyhypusine synthase was incubated for 30 min at 37°C with [γ-^{32}P]ATP, PKC(α+β+γ) mixture, and various activators such as Ca^{2+} (0.5 mM), P.Ser (200 μg/ml), and DAG (40 μg/ml). Phosphorylated deoxyhypusine synthase with a molecular mass of 43 kDa was visualized by SDS-PAGE followed by autoradiography (upper panel). Lower panel shows histogram depiction of the relative levels of phosphorylation of deoxyhypusine synthase in the presence of Ca^{2+}, P.Ser, and DAG served as 100 percent control. (B) Deoxyhypusine synthase was phosphorylated by PKC in the absence (lane 1) or presence of DAEG (PKC inhibitor) of the following concentrations: 80 μg/ml (lane 2), 160 μg/ml (lane 3), and 320 μg/ml (lane 4). Phosphorylated deoxyhypusine synthase was detected by autoradiography.

To verify the endogenous phosphorylatin of deoxyhypusine synthase, NIH3T3, CHO, and chicken embryonic cell lysates were incubated in kinase reaction buffer in the presence of exogenous ATP for 30 min at 37°C, and then immunoprecipitated with anti-deoxyhypusine synthase antibody. The immune complexes were processed for Western blot analysis with anti-phosphothreonine antibody. By this procedure an immunoreactive band of the expected molecular mass, in the size range of 41-42 kDa, was clearly detectable in all cells examined. Figure 2 demonstrates that endogenous deoxyhypusine synthase appears in Western blot with anti-phosphothreonine antibody, and the highest level of phosphorylated deoxyhypusine syn-

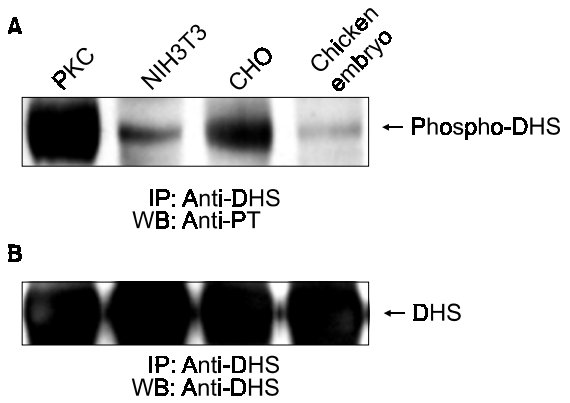

Figure 2. *In vitro* phosphorylation of deoxyhypusine synthase with endogenous kinases. Equivalent amounts of total cell or tissue lysates (1 mg each) of NIH3T3, CHO, and chicken embryo were precleared with 20 μl protein A agarose, and incubated in kinase reaction buffer containing 1 μg of purified rat deoxyhypusine synthase and 250 μM cold ATP at 37°C for 30 min, and then further incubated with anti-deoxyhypusine synthase (DHS) antibody for 2 h at 4°C. The immune complexes were bound to protein A agarose, washed, and separated by SDS-PAGE followed by Western blot analysis with anti-phosphothreonine (PT) antibody(A) or anti-DHS antibody (B). PKC (α+β+γ) was used as a control kinase (lane 1). Positions of unphosphorylated or phosphorylated DHS are indicated to the right.

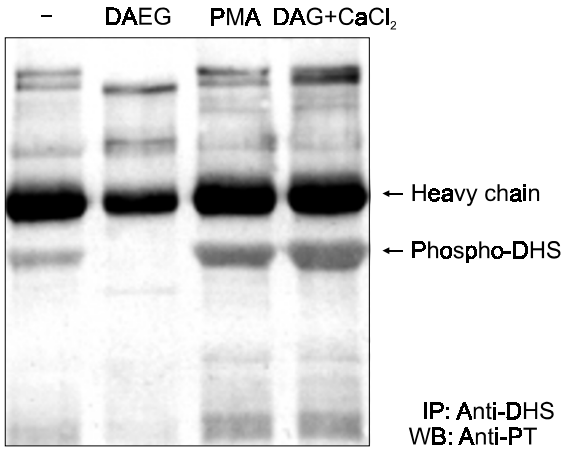

Figure 3. *In vivo* phosphorylation of deoxyhypusine synthase in CHO cells. CHO cells were starved of serum and stimulated with DAEG(300 μg/ml), PMA (10 μM) or $CaCl_2$ (0.5 mM) plus DAG (120 μg/ml) for 10 min at 37°C. The cells were harvested, precleared with protein A agarose, and deoxyhypusine synthase was immunoprecipitated with anti-deoxyhypusine synthase (DHS) antibody. The immunocomplexes were subjected to SDS-PAGE and then immunoblotted with anti-phosphothreonine (PT) antibody. Lane 1 shows untreated control. The strong band in the blot is the IgG heavy chain used in immunoprecipitation. Phosphorylated deoxyhypusine synthase is designated phospho-DHS.

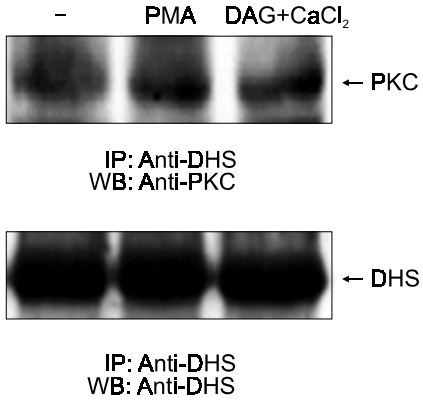

Figure 4. Interaction between PKC and deoxyhypusine synthase *in vitro*. Serum-starved CHO cells were incubated with 10 μM of PMA or 120 μg/ml of DAG plus 0.5 mM of $CaCl_2$ for 10 min at 37°C. The cell lysates (1 mg each) were phosphorylated in the presence of 1 μg of purified rat deoxyhypusine synthase and 250 μM ATP, and then incubated with anti-deoxyhypusine synthase (DHS) antibody for 2 h at 4°C. The immune complexes were bound to protein A agarose, and then subjected to SDS-PAGE followed by Western blot analysis with anti-PKC (α+β+γ) antibody or anti-DHS antibody. Untreated control was shown in lane 1.

thase was observed in CHO cells, followed by NIH3T3 cells and chicken embryo. Since CHO cells had the greatest specific activity of the cellular kinases, we used the cells for further study.

To check the *in vivo* occurrence of deoxyhypusine synthase phosphorylation, CHO cells treated with various stimuli were harvested, immunoprecipitated with polyclonal anti-deoxyhypusine synthase antibody, and then Western blot analysis was performed with anti-phosphothreonine antibody. As shown in Figure 3, phosphorylated deoxyhypusine synthase was definitely visible (lane 1) and remarkably diminished after the addition of DAEG, a typical PKC inhibitor (lane 2). Treatment of PMA or Ca^{2+}/DAG known as PKC activator in intact CHO cells resulted in considerable increase in the expression of phosphorylated deoxyhypusine synthase (Figure 3, lane 3 and 4).

Based on *in vitro* results with recombinant PKC (α+β+γ) (Figure 1), we investigated whether PKC present in cell lysate is involved in the phosphorylation of deoxyhypusine synthase. Figure 4 demonstrates a high interaction between deoxyhypusine synthase and PKC (α+β+γ) leading to the phosphorylation of deoxyhypusine synthase in untreated cells (lane 1 in upper panel). This interaction between two proteins, PKC and deoxyhypusine synthase, was slightly enhanced in the presence of PMA or Ca^{2+} plus DAG (lane 2 and 3), and which in turn increased the phosphorylation of deoxyhypusine synthase (data not shown). These data are in good correlation with those previously obtained with the recombinant PKC (α+β+γ).

To further confirm that PKC is directly involved in PMA-or Ca^{2+}/DAG-induced deoxyhypusine synthase phosphorylation, we looked for an *in vivo* association between PKC (α+β+γ) and deoxyhypusine synthase.

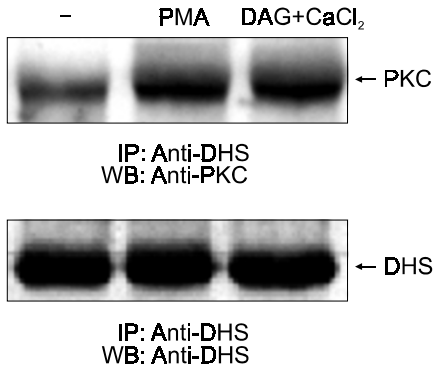

Figure 5. Association of PKC with deoxyhypusine synthase *in vivo*. Growth-arrested CHO cells (2 mg each) were treated with PMA (10 μM) or DAG (120 μg/ml) plus $CaCl_2$ (0.5 mM) for 10 min at 37°C. Same amounts of total cell lysates were precleared with protein A agarose and incubated with anti-deoxyhypusine synthase (DHS) antibody. The immune complexes bound to protein A agarose were separated by SDS-PAGE and subsequent Western blot analysis with anti-PKC (α+β+γ) antibody or anti-DHS antibody. Lane 1 denotes untreated control. The positions of PKC (α+β+γ) and DHS are indicated to the right.

As shown in Figure 5, PKC (α+β+γ) and deoxyhypusine synthase could be co-immunoprecipitated from CHO cells, and this interaction was markedly enhanced in the presence of PMA or Ca^{2+}/DAG, which suggests that the association of deoxyhypusine synthase with PKC in CHO cells is PMA-, and Ca^{2+}/phospholipid-dependent.

Discussion

Deoxyhypusine synthase catalyzes the first reaction in a two-step conversion of inactive eIF-5A to its activated form. The deoxyhypusine synthase-mediated reaction entails the transfer of a butylamine residue from spermidine to a conserved lysine of inactive eIF-5A forming the unusual amino acid, deoxyhypusine, which is then converted to hypusine by deoxyhypusine hydroxylase (Park *et al.*, 1997). Hypusine-modified eIF-5A, the active form of the protein, is the only cellular protein known to contain hypusine. It is present in all eukaryotic cells, and although its precise function has not been elucidated, it appears to facilitate mRNA translation (Park *et al.*, 1997).

Hypusine modification, which is essential for eIF-5A activity, is actually irreversible. Despite of eIF-5A being a constitutive component, for maintaining a homeostasis of cell, there should be some point of regulating it's production due to this protein's function as one of the translation initiation factors involved in the synthesis of other cellular proteins. The most probable target for reversible regulation of eIF-5A is deoxyhypusine synthase, the first enzyme involved in its hypusination. Yeast recombinant deoxyhypusine synthase was phosphorylated *in vitro* with PKC and CKII, but not phosphorylated in the presence of tyrosine kinases such as $p56^{lck}$ and $p60^{c\text{-}src}$ (Kang and Chung, 1999). Results in this study indicated that PKC is a powerful candidate for *in vivo* phosphorylation.

PKC, the major receptor for a number of tumor-promoting agents, utilizes DAG and/or other phospholipids to modulate various cellular functions. The finding that tumor-promoting phorbol esters such as PMA are able to replace the endogenous activator DAG in the stimulation of PKC has provided insight into the role of this enzyme in the regulation of a variety of cellular processes such as gene expression, proliferation, and tumor promotion (Geiges *et al.*, 1997; Kim *et al.*, 2001).

Single treatment with Ca^{2+}, P.Ser or DAG increased up to 2-fold increase in the phosphorylation of deoxyhypusine synthase by PKC. However, double treatment resulted in more than 3-fold increase in the amount of ^{32}P-incorporation into deoxyhypusine synthase. Maximal activity (3.5-fold) was obtained from triple treatment. These data may suggest that DAG, essential for PKC activation, stimulates the P.Ser/Ca^{2+}-dependent protein kinase C activity (Chen *et al.*, 1992), supporting classic PKC pathway is implicated in the control of this system (Nishizuka, 1992).

Another finding of deoxyhypusine synthase phosphorylation with recombinant PKC (α+β+γ) is autophosphorylation of PKC. P.Ser/DAG/ Ca^{2+}-dependent PKC activation has been shown to result in autophosphorylation (data not shown). Inhibition study using DAEG indicates that PKC is involved in deoxyhypusine synthase signaling mechanism (Figure 1B). The *in vitro* phosphorylation of deoxyhypusine synthase by recombinant PKC prompted us to investigate the phosphorylation of deoxyhypusine synthase with endogenous kinases. As shown in Figure 2A, all cell lysates showed the presence of phosphorylated form of deoxyhypusine synthase. Phosphoamino acid analysis of yeast recombinant deoxyhypusine synthase phosphorylated by PKC revealed that this enzyme was phosphorylated mainly on serine and threonine (Kang and Chung, 1999). We used polyclonal anti-phosphoserine antibody (Chemicon, CA) as well as monoclonal anti-phosphothreonine antibody (Santa Cruz Biotechnology, CA) for the detection of endogenous phosphorylation in deoxyhypusine synthase. However, only indistinguishable bands were obtained using polyclonal anti-phosphoserine antibody.

Deoxyhypusine synthase is indeed phosphorylated in intact CHO cells. Such a phosphorylation is especially enhanced if CHO cells are incubated in the presence of PMA or Ca^{2+}/DAG (Figure 3). It is also detectable, however, in untreated cells. Such an *in*

vivo phosphorylation is markedly reduced by specific inhibitor of PKC, DAEG. On the basis of these results, it could be possible to suggest that PKC might be the *in vivo* kinase in the phosphorylation of deoxyhypusine synthase.

To strengthen our *in vitro* data, the interaction of PKC ($\alpha+\beta+\gamma$) with deoxyhypusine synthase was examined in CHO cells which showed the higher cellular kinase activity in the phosphorylation of deoxyhypusine synthase. The fact that deoxyhypusine synthase and PKC ($\alpha+\beta+\gamma$) can be co-immunoprecipitated from cells and the association became increased after treating the cells with PMA or Ca^{2+}/DAG, definitely support specificity of interaction as well as possible cellular signal link. Based on the results showing different intensities upon treatment with PMA or Ca^{2+}/DAG in the association of PKC ($\alpha+\beta+\gamma$) with deoxyhypusine synthase between the *in vitro* (Figure 4) and *in vivo* (Figure 5), one could speculate that a signaling mechanism turning off the stimulation of deoxyhypusine synthase by PKC ($\alpha+\beta+\gamma$) other than the direct molecular interaction between the two proteins resides in intact cells.

We have demonstrated that deoxyhypusine synthase is phosphorylated by PKC *in vivo*. Furthermore, the hypusinated eIF-5A undergoes phosphorylation on serine residue (Kang *et al.*, 1993). However, intracellular role of phosphorylation of this enzyme and substrate is still unclear. Phosphorylation is one of the most important mechanisms for regulating the activity of protein factors involved in transcription (Hunter and Karin, 1992) and translation (Hershey, 1991). In particular, regulation of the activity of eukaryotic translation initiation factors eIF-2 and eIF-4E by phosphorylation is well established as a mechanism to control the rate of protein synthesis. It is therefore possible that phosphorylation of deoxyhypusine synthase, the essential enzyme for the production of mature eIF-5A, regulates its activity as well. To remark this issue more definitely, we are in the process of identifying the site of phosphorylation, which will allow us to alter specific serine and/or threonine residue(s) by site-directed oligomutagenesis of deoxyhypusine synthase gene. Substituting the mutated gene for the wild-type gene in mammalian cells may result in changes in the growth characteristics or physiology of the cells and thereby shed light on the role of phosphorylation of deoxyhypusine synthase.

Acknowledgements

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References

Abbruzzese A, Park MH, Folk JE. Deoxyhypusine hydroxylase from rat testis. Partial purification and characterization. J Biol Chem 1986;261:3085-9

Chen SG, Kulju D, Halt S, Murakami K. Phosphatidylcholine-dependent protein kinase C activation. Biochem J 1992; 284:221-6

Geiges D, Meyer T, Marte B, Vanek M, Weissgerber G, Stabel S, Pfeilschifter J, Fabbro D, Huwiler A. Activation of protein kinase C subtypes α, γ, δ, ε, ζ, and η by tumor-promoting and nontumor-promoting agents. Biochem Pharmacol 1997;53:865-75

Gordon ED, Mora R, Meredith SC, Lindquist SL. Hypusine formation in eukaryotic initiation factor 4D is not reversed when rates or specificity of protein synthesis is altered. J Biol Chem 1987;262:16590-5

Hershey JWB. Translational control in mammalian cells. Annu Rev Biochem 1991;60:717-55

Hunter T, Karin M. The regulation of transcription by phosphorylation. Cell 1992;70:375-87

Joe YA, Wolff EC, Park MH. Cloning and expression of human deoxyhypusine synthase cDNA. J Biol Chem 1995; 270:22386-92

Kang HA, Schwelberger HG, Hershey JWB. Translation initiation factor eIF-5A, the hypusine-containing protein, is phosphorylated on serine in *Saccharomyces cerevisiae*. J Biol Chem 1993;268:14750-6

Kang KR, Chung SI. Characterization of yeast deoxyhypusine synthase: PKC-dependent phosphorylation *in vitro* and functional domain identification. Exp Mol Med 1999;31: 210-6

Kang KR, Wolff EC, Park MH, Folk JE, Chung SI. Identification of *YHR068w* in *Saccharomyces cerevisiae* chromosome VIII as a gene for deoxyhypusine synthase. J Biol Chem 1995; 270:18408-12

Kim YW, Hur SY, Kim TE, Lee JM, Namkoong SE, Kim I-K, Kim JW. Protein kinase C modulates telomerase activity in human cervical cancer cells. Exp Mol Med 2001;33:156-63

Klier H, Csonga R, Steinkasser A, Wöhl T, Lottspeich F, Eder J. Purification and characterization of human deoxyhypusine synthase from HeLa cells. FEBS Lett 1995;364:207-10

Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 1992; 258: 607-14

Park MH, Cooper HJ, Folk JE. The biosynthesis of protein-bound hypusine (N^{ε}-(4-amino-2-hydroxybutyl)lysine). J Biol Chem 1982;257:7217-22

Park MH, Joe YA, Kang KR. Deoxyhypusine synthase activity is essential for cell viability in the yeast *Saccharomyces cerevisiae*. J Biol Chem 1998;273:1677-83

Park MH, Lee YB, Joe YA. Hypusine is essential for eukaryotic cell proliferation. Biol Signals 1997;6:115-23

Park MH, Wolff EC, Folk JE. Hypusine: its post-translational formation in eukaryotic initiation factor 5A and its potential

role in cellular regulation. Biofactors 1993a;4:95-104

Park MH, Wolff EC, Folk JE. Is hypusine essential for eukaryotic cell proliferation? Trends Biochem Sci 1993b;18: 475-9

Sasaki K, Abid MR, Miyazaki M. Deoxyhypusine synthase gene is essential for cell viability in the yeast *Saccharomyces cerevisiae*. FEBS Lett 1996;384:151-4

Schnier J. Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae*. Mol Cell Biol 1991;11:3105-14

Tao Y, Chen KY. Molecular cloning and functional expression of *Neurospora* deoxyhypusine synthase cDNA and identification of yeast deoxyhypusine synthase cDNA. J Biol Chem 1995a;270:23984-7

Tao Y, Chen KY. Purification of deoxyhypusine synthase from *Neurospora crassa* to homogeneity by substrate elution affinity chromatography. J Biol Chem 1995b;270:383-6

Wöhl T. The HYP2 gene of *Saccharomyces cerevisiae* is essential for aerobic growth: characterization of different isoforms of the hypusine-containing protein Hyp2p and analysis of gene disruption mutants. Mol Gen Genet 1993; 241:305-11

Wolff EC, Lee YB, Chung SI, Folk JE, Park MH. Deoxyhypusine synthase from rat testis: purification and characterization. J Biol Chem 1995;270:8660-6