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Biochemical characterization of Pkn2, a protein Ser/Thr kinase from *Myxococcus xanthus*, a Gram-negative developmental bacterium

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Abstract Pkn2, a protein Ser/Thr kinase, from the developmental bacterium *Myxococcus xanthus* was expressed under a T7 promoter in *Escherichia coli* and purified. Purified Pkn2 retained the autophosphorylation activity with the K_m value of 177 μM for ATP and 73 nmol/min/mg for V_{max}. The optimum pH and temperature were determined to be 7.5 and 35°C, respectively. The autophosphorylation activity was inhibited by staurosporine with the IC_{50} value of 400 nM while H-7 and genistein had little effect on this kinase. Pkn2 appears to be unique for its higher manganese dependence. This is the first biochemical characterization of the prokaryotic protein Ser/Thr kinase.

Key words: Pkn2; Biochemical characterization; Gram-negative bacteria; *Myxococcus xanthus*

1. Introduction

Many cellular responses such as adaptation and differentiation are carried out by signal transduction systems that often use protein phosphorylation as a molecular switch. However, the prokaryotes and eukaryotes have been found to employ structurally different protein kinases, protein His kinases and protein Ser/Thr and Tyr kinases, respectively.

Interestingly, a eukaryotic-like protein Ser/Thr kinase gene, *pkn1*, was identified in a Gram-negative bacterium, *Myxococcus xanthus*, which showed sequence similarities with eukaryotic protein kinases [1]. A large family of eukaryotic-like protein kinase genes were consequently discovered in this bacterium, suggesting that eukaryotic-like protein Ser/Thr kinases play important roles in signal transduction systems in *M. xanthus* together with protein His kinases [2]. Eukaryotic-like protein kinases have been also identified in other bacteria such as *Yersinia* [3], *Streptomyces* [4] and *Anabaena* [5]. These kinases in *Myxococcus*, *Streptomyces* and *Anabaena* except for *Yersinia* are also found to be more homologous to each other than to eukaryotic protein kinases and relatively distant from the eukaryotic protein kinases [6,7]. It is intriguing to note that *Myxococcus*, *Anabaena* and *Streptomyces* species undergo characteristic cellular development induced by nutrient starvation.

Although a number of eukaryotic-like protein kinases have been identified in prokaryote, it has not been determined how this prokaryotic protein Ser/Thr kinases are biochemically related to eukaryotic protein kinases. Therefore, we have undertaken some biochemical experiments to characterize Pkn2 to compare with eukaryotic protein kinases. Pkn2 was identified as the first eukaryotic-like transmembrane Ser/Thr kinase ever found in prokaryotes [8]. Pkn2 consists of 830 amino acid residues with a single transmembrane domain. It has been suggested that Pkn2 is required for the normal development in response to extracellular stimuli. In this report, we purified Pkn2 expressed in *E. coli* and characterized its biochemical properties. The present paper is the first to show that a prokaryotic protein Ser/Thr kinase shares similar biochemical properties with eukaryotic protein Ser/Thr kinases except for its higher manganese dependence.

2. Materials and methods

2.1. Strains and medium

E. coli JM83 [9] and BL21(DE3) [10] were used for the genetic manipulation and the overproduction of protein, respectively. *E. coli* strains were grown in LB or glucose-M9 medium [11]. As necessary, kanamycin (30 μg/ml) or ampicillin (100 μg/ml) was supplemented. Pkn2 was overproduced using a T7 expression system as described previously [10].

2.2. Purification of histidine-tagged Pkn2

Plasmid pET11/6Hpkn2 was constructed by the insertion of an adapter encoding six histidine residues at the unique *Nde*I site of the plasmid, pET11/pkn2. The positive colonies were selected and confirmed by sequencing. Strain *E. coli* BL21(DE3) harboring pET11/6Hpkn2 was cultured in 4 l of glucose-M9 medium containing 100 μg/ml ampicillin at 37°C until a Klett unit reached 120 (blue filter). The *pkn2* gene was induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 2 h. Cells were centrifuged and washed with 50 mM Tris-HCl (pH 8.0). Sonication buffer (20 ml: 50 mM Tris-HCl (pH 8.0), 1 mM phenylmethane sulphonylfluoride (PMSF), 1 mM ethylenediamine tetraacetic acid (EDTA) and 2 mM 2-mercaptoethanol) was added and the cell suspension was sonicated 5 times at 40 W for 1 min at 4°C. The insoluble inclusion body fraction was obtained by centrifugation at 20000×*g* for 10 min at 4°C and resuspended in 20 ml of solubilizing buffer (50 mM Tris-HCl (pH 8.0), 6 M urea, 0.1 M 2-mercaptoethanol) with gentle shaking for 2 h at 4°C. Solubilized proteins were recovered by centrifugation at 30000×*g* for 20 min at 4°C. Ni-NTA resin (2 ml) was incubated with the solubilized 6HPkn2 for 10 h at 4°C. The resin which bound 6HPkn2 was centrifuged at 3000×*g* for 5 min at 4°C, washed with 100 ml of the solubilizing buffer and then packed into a column (1×8 cm). The refolding of Pkn2 was carried out by reducing the urea concentration using a linear gradient from the solubilizing buffer to the washing buffer (50 mM Tris-HCl (pH 8.0), 0.3 M NaCl, 2 mM 2-mercaptoethanol) and the resin was further washed with 100 ml of the washing buffer. The renatured enzyme was eluted with 6 ml of the elution buffer (50 mM Tris-HCl (pH 8.0), 0.2 M EDTA, 2 mM β-2-mercaptoethanol) and dialyzed against 10 mM Tris-HCl (pH 7.5) and 2 mM 2-mercaptoethanol overnight at 4°C. The enzyme solution was concentrated and the protein concentration was determined by the Bradford method [12]. The final preparation was divided into small aliquots and kept at −70°C.

2.3. Autophosphorylation of Pkn2

Purified Pkn2 (50 ng or 0.722 pmol) was incubated with 100 mM [γ-^{32}P]ATP or [γ-^{32}P]GTP (6000 cpm/mmol) in the protein kinase buffer (100 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$ and 1 mM dithiothrei-

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tol (DTT)) in a total volume of 20 μl for 1 h at 30°C. The reaction was stopped by SDS-PAGE sample buffer (80 mM Tris-HCl (pH 6.8), 2% SDS, 0.2 M 2-mercaptoethanol, 10% glycerol) and analyzed by 15% SDS-PAGE. The gel was dried and autoradiographed.

To obtain the optimum pH, piperazine–glycylglycine–NaOH buffer was used from pH 6.0–10.5. For the optimum temperature and salt concentrations, Pkn2 was incubated at different temperatures (0–65°C) and NaCl or KCl concentrations (0–200 mM). Protein kinase inhibitors, staurosporine [13], 1-(5-isoquinolinyl sulfonyl)-2-methylpiperazine (H-7) [14] and genistein [15] were used at concentrations of 0–10 μM, 0–200 μM and 0–200 μM, respectively. For divalent metal ions, different concentrations of $MgCl_2$, $MnCl_2$, $CoCl_2$, $ZnCl_2$, $CuCl_2$ and $CaCl_2$ were used.

2.4. Autophosphorylation of Pkn2

Purified Pkn2 was incubated with 1 mM [γ-^{32}P]ATP or [γ-^{32}P]GTP in protein kinase buffer (0.1 M Tris-HCl (pH 7.5), 5 mM $MgCl_2$ and 1 mM DTT) in a total volume of 20 μl for 1 h at 30°C. The reaction was stopped by the addition of trichloroacetic acid (TCA) solution to a final concentration of 10%. After 1 h on ice, insoluble materials were precipitated by centrifugation at 15000×*g* for 10 min at 4°C. Pellets were washed with 10% TCA twice, cold enthanol once and then dried. Proteins were solubilized with 10 μl of SDS-PAGE sample buffer (80 mM Tris-HCl (pH 6.8), 2% SDS, 0.2 M 2-mercaptoethanol, 10% glycerol) and analyzed by 15% SDS-PAGE. The gel was dried an autoradiographed.

2.5. Kinetics of Pkn2 autophosphorylation

Purified Pkn2 (29 pmol) was incubated with different concentrations (0–200 mM) of [γ-^{32}P]ATP. The reaction was carried out for 5 min at 30°C and stopped by adding TCA to a final concentration of 10%. The radioactivity of the samples was measured in a scintillation counter.

3. Results

3.1. Purification of Pkn2 expressed in E. coli

The expression of the *pkn2* gene in *M. xanthus* is transient at a very low level [8]. Therefore, Pkn2 was overproduced by a T7 expression system in *E. coli* and six histidine residues were attached to the N-terminal end of Pkn2. *E. coli BL21*(DE3) harboring pET11/6Hpkn2 was cultured in glucose-M9 medium and the *pkn2* gene was induced with 1 mM IPTG. Pkn2 was produced as inclusion bodies which were solubilized in 6 M urea and applied to a Ni-NTA chelating column. Pkn2 was renatured on a column and eluted with EDTA (Fig. 1, lane 1). Pkn2 was found to be soluble, suggesting that the transmembrane domain of Pkn2 may be imbedded in the Pkn2 structure. Pkn2 thus obtained was approximately 90% pure judged by SDS-PAGE followed by staining with Coomassie brilliant blue and used for biochemical characterization.

3.2. Autophosphorylation of Pkn2

As shown in lane 2 of Fig. 1, purified Pkn2 was clearly phosphorylated when [γ-^{32}P]ATP was added. Pkn2 has been shown to autophosphorylate both at Ser and Thr residues [8] and the ^{32}P-labeled Pkn2 band was stable upon TCA treatment. Pkn2 was also found to use [γ-^{32}P]GTP as a phosphate donor less effectively with the ratio of ATP/GTP to be 1:28.

3.3. Optimal conditions for the autophosphorylation activity

The autophosphorylation activity was examined at different temperatures, pHs, and salt concentrations as shown in Fig. 2. The maximum activity was observed at pH 7.5 and more than 50% of the activity was retained at pH range 6.3–9.6. The optimal temperature for maximal activity was determined to be 35°C. The maximal autophosphorylation activity was obtained in lower ionic conditions with either NaCl or KCl. Eukaryotic protein kinases such as cAMP-dependent protein kinase (PKA) [16,17], cGMP-dependent protein kinase (PKG) [18], calmodulin-dependent protein kinase [19] and Src [20] have been shown to prefer weak alkaline conditions, the optimal temperature of around 30°C and a low ionic strength for their phosphorylation activity.

3.4. Effect of protein kinase inhibitors

A variety of inhibitors for eukaryotic protein kinases have been discovered and used in vitro and in vivo assays. The following three inhibitors; staurosporine [13], H-7 [14] and genistein [15], known as a general protein kinase inhibitor, PKA, PKG and PKC inhibitor and a tyrosine kinase inhibitor, respectively, were examined for Pkn2 activity as shown in Table 1. Pkn2 was inhibited by staurosporine with a relatively high IC_{50} value of 400 nM. The IC_{50} values for PKA, PKC and Src with staurosporine are shown to be less than 10 nM, while it was 630 nM for epidermal growth factor receptor [13]. H-7 and genistein have little effect on Pkn2 activity with the IC_{50} values of higher than 500 μM and 200 μM, respectively. It has been reported that PKA and PKC are inhibited by H-7 with a K_i value of less than 15 μM, and EGFR and Src are inhibited by genistein with a IC_{50} value of 22–26 μM [14,15].

3.5. Effect of metal ions

Protein kinases require divalent metal ions for their phosphate transfer reaction and especially magnesium ion is widely used for protein kinase assays. The effect of the divalent metal ions Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+} and Ca^{2+} on Pkn2 kinase activity is shown in Fig. 3. Among them, Mn^{2+}, Co^{2+}, Zn^{2+} as well as Mg^{2+} were found to support the Pkn2 auto-

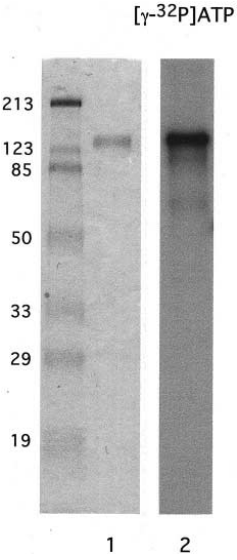

Fig. 1. Purification of Pkn2 and its autophosphorylation. *E. coli* BL21(DE3) harboring pET11/6HPkn2 was grown in glucose-M9 medium and Pkn2 was induced with 1 mM IPTG. Proteins were applied to a Ni-NTA column and Pkn2 was eluted with EDTA. Approximately 0.5 μg of the purified Pkn2 was subjected to SDS-PAGE and stained with Coomassie brilliant blue (lane 1). The purified Pkn2 (50 ng) was incubated in 20 μl of protein kinase buffer containing 100 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, and 100 μM [γ-^{32}P]ATP for 5 min at 30°C. The sample was then subjected to SDS-PAGE and autoradiographed (lane 2). Molecular weight markers were applied at the left lane and their molecular weights are indicated at the left-hand side of the lane (kDa).

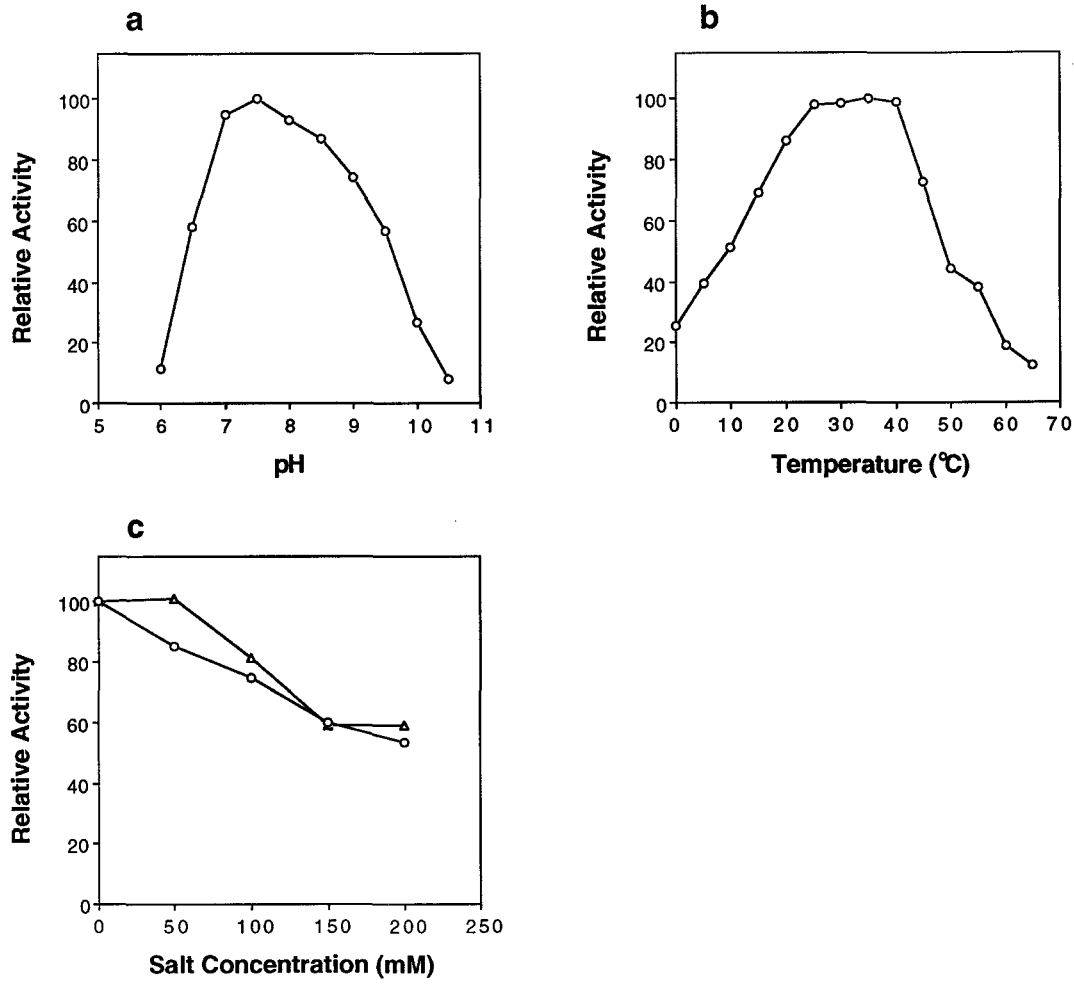

Fig. 2. Effect of temperature, pH and salts on Pkn2 autophosphorylation activity. a: The optimal pH was determined in piperazine–glycylglycine–NaOH buffer from pH 6–10.5. The relative activities to the value at pH 7.5 were plotted. b: The enzyme was incubated in the reaction mixture at different temperatures (0–65°C). The activity at 30°C was taken as 100%. c: The reaction was carried out at different concentrations of NaCl (△) or KCl (○). The activity in the absence of the salts was taken as 100%.

phosphorylation activity. Very low autophosphorylation activity was observed with $ZnCl_2$. Cu^{2+} and Ca^{2+} did not support the phosphorylation. Interestingly, manganese was found to be very effective and the enzyme activity in 1 mM $CoCl_2$ is also comparable to that in 5 mM $MgCl_2$.

The requirement of calcium ion for Pkn2 was examined in the presence of $MgCl_2$ since calcium has been shown to be required for the gliding motility, aggregation and germination of *M. xanthus* [21,22] and for the activation of Pkn1 (unpublished data) and Pkn6 [23]. In addition, some eukaryotic protein kinases such as PKA [17] was inhibited by Ca^{2+}, while protein kinase C (PKC) [24], Ca^{2+}/calmodulin dependent protein kinase [25] and myosin light chain kinase [26] are activated by Ca^{2+} or Ca^{2+}/calmodulin. In contrast, Ca^{2+} had no effect on Pkn2 within the range of 0–10 mM $CaCl_2$ (data not shown).

3.6. *Effect of magnesium and manganese on Pkn2*

Since manganese was found to activate Pkn2 effectively, we further examined the optimal concentrations and the relative activities as shown in Fig. 4A. Pkn2 was not active without metal ions. The maximum activity was obtained at 10 mM $MgCl_2$ and 5 mM $MnCl_2$. In eukaryotic protein kinases known to utilize manganese, the optimal concentration of manganese (0.5–5 mM) is generally lower than that of magnesium (5–20 mM) in the case of Pkn2. The activity in 5 mM $MnCl_2$ was calculated to be 23 times higher than that in 10 mM $MgCl_2$.

Fig. 4B shows a Lineweaver-Burk plot of the Pkn2 autophosphorylation. The K_m value for ATP and the V_{max} value were determined as 177 and 68 μM, and 0.73 and 2.1 μmol/min/mg in 5 mM $MgCl_2$ or $MnCl_2$, respectively. The K_m value for ATP was relatively high compared with eukaryotic protein kinases, since their K_m values for ATP are usually less than 100 μM except that myosin light-chain kinase has a high K_m value of 175 μM. The V_{max} value of Pkn2 was found to be comparable to that of PKA [16]. The Pkn2 activity was dram-

Table 1
Effects of protein kinase inhibitors on Pkn2 autophosphorylation activity

Inhibitors	IC_{50} (μg)
Staurosporine	0.4
H-7	>500
Genistein	>200

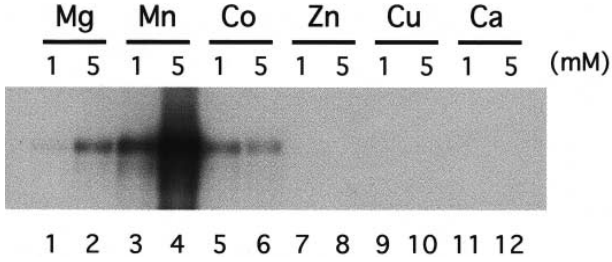

Fig. 3. Effect of divalent metal ions on Pkn2 autophosphorylation activity. Pkn2 was incubated in the presence of $MgCl_2$ (lanes 1 and 2), $MnCl_2$ (lanes 3 and 4), $CoCl_2$ (lanes 5 and 6), $ZnCl_2$ (lanes 7 and 8), $CuCl_2$ (lanes 9 and 10) or $CaCl_2$ (lanes 11 and 12). The concentrations of metal ions are 1 mM for lanes 1, 3, 5, 7, 9 and 11 and 5 mM for lanes 2, 4, 6, 8 and 10.

atically enhanced in the presence of manganese (28 fold compared with magnesium). The enhancement of the kinase activity by manganese seems to be mainly due to the γ-phosphate transfer reaction rather than the affinity of ATP, since the K_m value for ATP decreased 2.6-fold. Many eukaryotic protein kinases including PKA [17] and PKC [24] were reported to utilize manganese. However, a few are known to be activated by manganese. Manganese–phospholipid-activated protein kinase [27], Src [20] and Varicella-Zoster virus ORF 47 [28] are found to be more active with Mn^{2+} than with Mg^{2+} (2-, 3- and 13-fold, respectively). Therefore, Pkn2 appears to be more dependent on manganese.

4. Discussion

In this report, a prokaryotic protein Ser/Thr kinase, Pkn2, from *M. xanthus* was purified and its enzymatic properties were characterized to compare with eukaryotic protein kinases. This is the first report to biochemically characterize a prokaryote protein Ser/Thr kinase. The kinase domain of Pkn2 has been shown to have 27% and 25% identity to rat Ca^{2+}/calmodulin kinase [29] and *Bos taurus* rhodopsin kinase [30], respectively. It is suggested that prokaryotic kinases may be evolutionary related to eukaryotic kinases. However, no biochemical studies of eukaryotic-like protein kinases have been reported to elucidate how these prokaryotic enzymes are related to eukaryotic protein Ser/Thr kinases at the level of catalytic functions.

We examined Pkn2 autophosphorylation activity as an indicator for its enzymatic properties since Pkn2 expressed in *E. coli* effectively autophosphorylates both at serine and threonine residues and its natural substrates in *M. xanthus* have not yet been identified. The present results demonstrate that Pkn2 has similar optimal conditions for pH, temperature and ionic strength as eukaryotic kinases. Pkn2 was effectively inhibited by staurosporine while genistein failed to inhibit, supporting a notion that Pkn2 is a protein Ser/Thr kinase. The V_{max} value of Pkn2 for ATP was found to be almost comparable to PKA even though K_m value for ATP was relatively high. It should be noted that Pkn2 used in the present work was refolded from inclusion bodies produced in *E. coli*. The refolded Pkn2 was found to be soluble in spite of the fact that Pkn2 is a transmembrane protein. At present, it is not known how the transmembrane domain of Pkn2 is folded in the soluble preparation.

Interestingly, Pkn2 phosphorylation was stimulated significantly in the presence of manganese, 28 times higher compared with magnesium. In eukaryotic protein kinases known to utilize manganese, their enzyme activities in the presence of manganese was similar to that in the presence of magnesium. Therefore, Pkn2 is very unique in terms of its dependence on manganese.

Manganese is known to be essential for some enzymes such as superoxide dismutase [31] and pyruvate carboxylase [32]. Manganese has been shown to have an effect on the protein phosphorylation patterns both in prokaryotes and eukaryotes [33–35]. *M. xanthus* is a soil-dwelling bacterium and manganese, which is reasonably abundant in soil, may function as the physiological activator for Pkn2. In the presence of 10 mM $MnCl_2$, *M. xanthus* strain overproducing Pkn2 showed a significantly delayed development since Pkn2 functions as a negative regulator (unpublished results). In contrast, the development of *Streptomyces*, a Gram-positive bacterium, can be induced by the addition of manganese [36]. At this point,

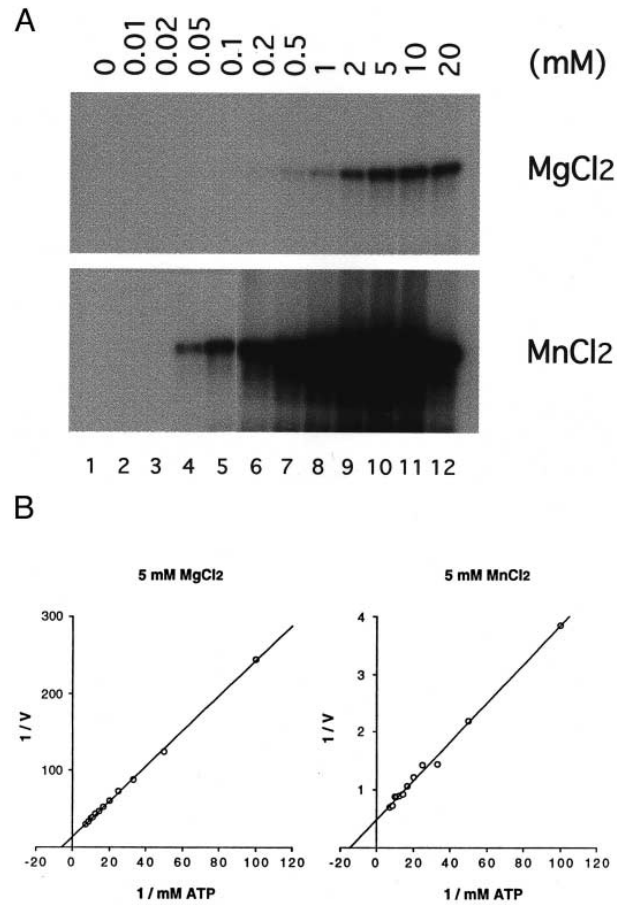

Fig. 4. Comparison of the effect of Mg^{2+} and Mn^{2+} ions on Pkn2 autophosphorylation activity. A: The optimal concentrations of Mg^{2+} and Mn^{2+} ions for the Pkn2 activity. Pkn2 was incubated in the presence of increasing concentrations of $MgCl_2$ or $MnCl_2$ (from 0 to 20 mM). Each sample contains 10 nM EDTA. Enzyme activities are plotted relative to the value in 10 mM $MgCl_2$. B: Lineweaver-Burk plot of Pkn2 autophosphorylation. The reaction was carried out in different concentrations of [γ-^{32}P]ATP in the presence of 5 mM $MgCl_2$ or $MnCl_2$.

however, the physiological function of Pkn2 autophosphorylation in *M. xanthus* remains to be addressed.

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References

[1] Munoz-Dorado, J., Inouye, S. and Inouye, M. (1991) Cell 67, 995–1006.
[2] Zhang, W., Munoz-Dorado, J., Inouye, M. and Inouye, S. (1992) J. Bacteriol. 174, 5450–5453.
[3] Galyov, E.E., Hakansson, S., Forsberg, A. and Wolf-Wats, H. (1993) Nature 361, 730–732.
[4] Matsumoto, A., Hong, S.K., Ishizuka, H., Horinouchi, S. and Beppu, T. (1994) Gene 146, 47–56.
[5] Zhang, C.-C. (1993) Proc. Natl. Acad. Sci. USA 90, 11840–11844.
[6] Hanks, S.K. and Hunter, T. (1995) FASEB J. 9, 576–596.
[7] Zhang, C.-C. (1996) Mol. Microbiol. 20, 9–15.
[8] Udo, H., Inouye, M. and Inouye, S. (1995) Genes Dev. 9, 972–983.
[9] Vieira, J. and Messing, J. (1982) Gene 19, 259–268.
[10] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) Methods Enzymol. 185, 60–89.
[11] Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, New York.
[12] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[13] Tamaoki, T. (1991) Methods Enzymol. 201, 340–347.
[14] Hidaka, H., Inagaki, M., Kawamoto, S. and Sasaki, Y. (1984) Biochemistry 23, 5036–5041.
[15] Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. (1987) J. Biol. Chem. 262, 5592–5595.
[16] Reimann, E.M., Walsh, D.A. and Krebs, E.G. (1971) J. Biol. Chem. 246, 1986–1995.
[17] Suzuki, K., Terao, T. and Osawa, T. (1981) J. Biochem. 89, 1–11.
[18] Aswad, D.W. and Greengard, P. (1981) J. Biol. Chem. 256, 3494–3500.
[19] Walsh, M.P., Vallet, B., Autric, F. and Demaille, J.G. (1979) J. Biol. Chem. 254, 12136–12144.
[20] Richert, N.D., Blithe, D.L. and Pastan, I. (1982) J. Biol. Chem. 257, 7143–7150.
[21] Otani, M., Inouye, M. and Inouye, S. (1995) J. Bacteriol. 177, 4261–4265.
[22] Womack, B.J., Gilmore, D.F. and White, D. (1989) J. Bacteriol. 171, 6093–6096.
[23] Zhang, W., Inouye, M. and Inouye, S. (1996) Mol. Microbiol. 20, 435–447.
[24] Inoue, M., Kishimoto, A., Takai, Y. and Nishizuka, Y. (1977) J. Biol. Chem. 252, 7610–7616.
[25] Bennett, M.K., and Kennedy, M.B. (1987) Proc. Natl. Acad. Sci. USA 84, 1794–1798.
[26] Sellers, J.R. and Harvey, E.V. (1984) Biochem. 23, 5821–5826.
[27] Elias, L. and Davis, A. (1985) J. Biol. Chem. 260, 7023–7028.
[28] NG, T.I. and Grose, C. (1992) Virology 191, 9–18.
[29] Lin, C.R., Kapiloff, M.S., Durgerian, S., Tatemoto, K., Russo, A.F., Hanson, P., Schulman, H. and Rosenfeld, M.G. (1987) Proc. Natl. Acad. Sci. USA 84, 5962–5966.
[30] Lorenz, W., Inglese, J., Palczewski, K., Onorato, J.J., Caron, M.G. and Lefkowitz, R.J. (1991) Proc. Natl. Acad. Sci. USA 88, 8715–8719.
[31] Mazeaud, F., Maral, J. and Michelson, A.M. (1979) Biochem. Biophysic. Res. Commun. 86, 1161–1168.
[32] Scrutton, M.C., Utter, M.F., and Mildvan, A.S. (1966) J. Biol. Chem. 241, 3480–3487.
[33] Freestone, P., Grant, S., Toth, I. and Norris, V. (1995) Mol. Microbiol. 15, 573–580.
[34] Goldenring, J.R., Modlin, I.M., Tyshkov, M., Zdon, M.J., Schafer, D.E. and Ballantyne, G.H. (1986) Surgery 100, 181–187.
[35] Knight, S., Kohr, W. and Korc, M. (1991) Biochim. Biophys. Acta. 1092, 196–204.
[36] Hong, S.-K., Mastumoto, A., Horinouchi, S. and Beppu, T. (1993) Mol. Gen. Genet. 236, 347–354.