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Intrinsic nucleoside diphosphate kinase-like activity as a novel function of 14-3-3 proteins

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Abstract 14-3-3 proteins play a role in many cellular functions as molecular chaperone and adapter proteins: they bind to and modulate several proteins involved in cell proliferation and differentiation, and also function ATP-dependently in targeting of precursors to mitochondria. We show here that 14-3-3 purified from a human lymphoblastoma and also its recombinant τ isoform exhibited intrinsic nucleoside diphosphate (NDP) kinase-like activity. 14-3-3 proteins preferentially catalyzed the transfer of the γ-phosphate group from ATP, dATP or dGTP to all nucleoside diphosphates and this transfer involved acid-labile phosphoenzyme intermediates. They also simultaneously catalyzed the reverse reaction of ATP hydrolysis. These properties of 14-3-3 are similar to those of NDP kinase, but not to those of adenylate kinase.

Key words: 14-3-3; Nucleoside diphosphate kinase; ATP/ADP exchange; Molecular chaperone; ATPase

1. Introduction

14-3-3 proteins, first discovered as abundant, acidic proteins in the brain, are ubiquitously distributed (reviewed in [1,2]). At least five isoforms and two of their phosphorylated forms have been identified in mammals [3]. Although the exact function of 14-3-3 proteins is not known, numerous biological activities have been attributed to them. They were first implicated as activators of neuronal tyrosine and tryptophan hydroxylases [4] and of Ca^{2+}-dependent exocytosis from chromaffin cells [5], and as regulators of protein kinase C [6,7]. More recently, with development of the yeast two-hybrid system, 14-3-3 proteins were shown to bind to a number of important signaling proteins with phosphorylated serine, such as Raf-1, Bcr-Abl, Bcr, polyoma middle T antigen, PI-3 kinase (reviewed in [8,9]) and glycoprotein Ib-IX [10] and cell-cycle control proteins such as Cdc25 phosphatases [11]. Furthermore 14-3-3 proteins have also been identified as cytosolic chaperone-like proteins with ATPase activity as principal stimulators of mitochondrial import [12–14].

Though the known functions of 14-3-3 are very varied, most of them share the feature of modulating activity by mediating protein-protein interaction [14–16]. Hsp70 family proteins, molecular chaperones, that exhibit weak ATPase activity, bind to ATP and ADP, and the resulting change in nucleotide exchange regulates substrate protein binding and its dissociation [17–21]. In view of the mechanisms for molecular chaperone-protein interaction, 14-3-3 proteins, which act as both chaperones and adapter molecules [14–16], may also require ATP/ADP exchange for their function in a cycle of substrate binding and release.

In this paper, we report intrinsic ATP/ADP exchange activity as a novel activity of 14-3-3. The characteristics of the enzyme activity are similar to those of nucleoside diphosphate (NDP) kinases, but not to those of adenylate kinase.

2. Materials and methods

2.1. Materials

14-3-3 was purified from human lymphoblastoma T-cell line (Molt-4, Clone 8 cells) as described [6]. BSA, NDP kinase from bovine liver, various ribo- and deoxyribo-nucleoside tri-, di-, and monophosphates, AMP-PNP, and ATPγS were purchased from Sigma Chemical Co. (St. Louis, MO). [8-^{14}C]ADP, [2-^{14}C]CDP, [8-^{14}C]ATP, and [γ-^{32}P]ATP were obtained from Du Pont-New England Nuclear. A monoclonal antibody against human nm23-H1 protein (human NDP kinase-A) was from Novocastra Laboratories, UK.

2.2. Expression and purification of recombinant 14-3-3 fusion proteins

Recombinant human 14-3-3 τ fusion protein with a hexahistidine tag at the amino terminus was created by generating *Bam*HI and *Eco*RI restriction sites at the 5′ and 3′ end, respectively, of human 14-3-3 cDNAs by the polymerase chain reaction. The cDNA products were subcloned into the vector pET-21a^{+} (Novagen), transformed into *E. coli* BL21(DE3) and purified by TALON metal affinity resin (Clontech Laboratories, Inc.) chromatography according to the manufacturer's instructions. Homogeneity was confirmed by SDS-PAGE followed by Coomassie Brilliant Blue staining.

2.3. Assay of ATP hydrolysis and ATP synthesis

The ATP hydrolysis and ATP synthesis activities of 14-3-3 were analyzed by measuring the conversion of [^{14}C]ATP to [^{14}C]ADP, and that of [^{14}C]ADP to [^{14}C]ATP, respectively, as described [22,23]. The reactions were carried out at 37°C for 6 h in Buffer A (100 mM HEPES-KOH, pH 8, 5 mM ATP, 0.5 mM ADP, 6 mM $MgCl_2$) with 0–3.3 μg of 14-3-3, and 0.05 μCi of [8-^{14}C]ATP or 0.02 μCi of [8-^{14}C]ADP in assays of ATP hydrolysis and ATP synthesis activity, respectively, in a total volume of 20 μl. After incubation, samples of the reaction mixtures were promptly spotted onto polyethylene-imino-cellulose TLC plates (Macherey-Nagel). ADP and ATP were separated by ascending chromatography in 1 M formic acid containing 0.7 M LiCl and the radioactivities of the resolved spots were quantitated with a Bio Imaging Analyzer BAS 1500 (Fuji Photo Film Co., Japan).

2.4. Nucleotide specificity of 14-3-3 as phosphate acceptor

The nucleotide specificity of 14-3-3 proteins was examined by incubation at 37°C for 6 h in Buffer B (100 mM HEPES-KOH, pH 8, 1 μCi of [γ-^{32}P]ATP, 6 mM $MgCl_2$) with 33 pmol (calculated as a monomer with a mean molecular mass of 30 kDa) of 14-3-3 and 0.5 mM concentrations of various ribo- or deoxyribo-nucleoside diphosphates as phosphate acceptors in 20 μl of reaction mixture [24]. After incubation, the nucleotides were resolved by polyethylene-imi-

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Abbreviations: NDP, nucleoside diphosphate; *E. coli*, *Escherichia coli*; Hsp, heat shock protein; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; ATPγS, adenosine 5′-3-*O*-(thio)triphosphate; TLC, thin-layer chromatography; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

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no-cellulose TLC in 0.75 M KH_2PO_4, pH 3.5, and quantified with an imaging analyzer.

2.5. Detection of autophosphorylated 14-3-3 intermediates

Autophosphorylation and CDP-dependent dephosphorylation of 14-3-3 proteins were analyzed using 1–3 μg of 14-3-3 proteins or its recombinant τ isoform, 10 μCi of [γ-^{32}P]ATP (6000 Ci/mmol), 100 μM ATP and 6 mM $MgCl_2$ in 100 mM HEPES-KOH, pH 8, in the presence and absence of 5 mM CDP, respectively, in a total volume of 20 μl. After incubation at 37°C for 6 h, both reactions were quenched by addition of 6 mM EDTA and cooling on ice, and half of each sample was then treated with SDS sample buffer without boiling and subjected to 15% SDS-PAGE. After electrophoresis, the gel was dried without acid fixation and analyzed with an imaging analyzer. The other halves of the samples were analyzed by TLC and then imaging analysis. The acid and base stability of phosphorylated 14-3-3 proteins was analyzed as described [25].

3. Results

3.1. Characterization of 14-3-3 proteins purified from a human lymphoblastoma and its recombinant 14-3-3 τ isoform

14-3-3 proteins purified from a human lymphoblastoma and the recombinant 14-3-3 τ isoform showed two bands with molecular masses of 28 and 32 kDa and a band of 28 kDa, respectively, without visible contaminating proteins on SDS-PAGE (Fig. 1). 14-3-3 proteins from a human lymphoblastoma were a mixture of at least the 6 isoforms, such as the τ, ζ, η, β and γ isoforms of 28 kDa and the ε isoform of 32 kDa, which were identified by Western immunoblotting analysis with isoform specific antibodies (data not shown). We used the recombinant 14-3-3 τ isoform for experiments because of its abundant expression in human T-cells [26,27]. To confirm its identity and purity, we digested the recombinant τ isoform with lysyl endopeptidase, separated the peptide fragments by HPLC and sequenced their amino acids in a Perkin Elmer Applied Biosystems 492 protein sequencer. All peptides derived from the purified recombinant protein had identical sequences to the corresponding sequences deduced from the nucleotide sequence of the human τ isoform (data not shown).

3.2. Intrinsic nucleoside diphosphate kinase-like activity of 14-3-3

The ATPase activity of 14-3-3 proteins was first implicated

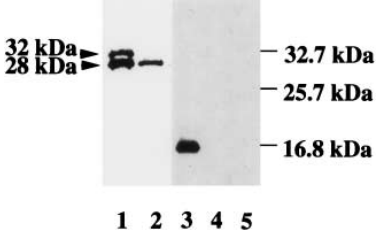

Fig. 1. SDS-PAGE and Western blot analyses of 14-3-3 from a human lymphoblastoma and the recombinant 14-3-3 τ isoform. 14-3-3 (2 μg) purified from a human lymphoblastoma (lane 1) and the recombinant 14-3-3 τ isoform (1 μg) purified (lane 2) were analyzed by SDS-PAGE (15–25% gradient) under denaturing and reducing conditions followed by Coomassie Brilliant Blue staining. Western blot immunoanalyses of 14-3-3 proteins from the human lymphoblastoma (lane 4), its recombinant 14-3-3 τ isoform (lane 5) and NDP kinase as a positive control (lane 3) (0.1 μg of each) probed with a monoclonal antibody against human nm23-H1 protein (human NDP kinase-A) followed by detection with ECL Western blotting reagents.

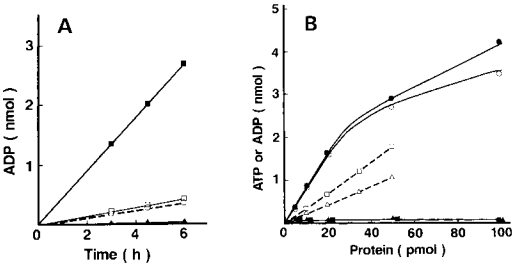

Fig. 2. Characterization of the activities of 14-3-3 proteins for ATP hydrolysis and ATP synthesis. A: Time course of ATP hydrolysis of 14-3-3 proteins purified from a human lymphoblastoma. ATP hydrolysis was analyzed under the conditions given in Section 2 using 30 pmoles of 14-3-3, 5 mM ATP, 0.05 μCi of [8-^{14}C]ATP and 6 mM $MgCl_2$ in the presence (■) or absence (□) of 0.5 mM ADP. The activity in the presence of 0.5 mM AMP instead of ADP (○) was also analyzed. After 6 h reaction at pH 8, samples were analyzed by TLC and ADP formation was calculated with an imaging analyzer. As a negative control, BSA (30 pmol) (▲) was analyzed instead of 14-3-3. B: Dose-response curves of the effect of 14-3-3 (0–100 pmol) on activity for ATP synthesis with 5 mM ATP, 0.02 μCi of [8-^{14}C]ADP and 6 mM $MgCl_2$ in the presence (●) or absence (■) of 0.5 mM ADP. The ATP synthesis activity was also analyzed with 0.5 mM AMP instead of ADP (▲). The ATP synthesis activity of the recombinant τ isoform (0–50 pmol) (□) was also analyzed. Dose-response curves of 14-3-3 (0–100 pmol) on the activities of ATP hydrolysis (○) and the recombinant τ isoform (△) (0–50 pmoles) were analyzed with 5 mM ATP, 0.5 mM ADP, 0.05 μCi of [8-^{14}C]ATP and 6 mM $MgCl_2$ in the reaction mixture.

in the function of ATP-dependent conformational modulators which stimulate the import of precursor proteins into mitochondria [12–14]. We found that the ATPase activity of 14-3-3 proteins was stimulated up to 6.7-fold by 0.5 mM ADP, but not by AMP (Fig. 2A), although ADP is a product inhibitor of common ATPase. The enzyme activities with and without 0.5 mM ADP were linear with time for 6 h. Furthermore, the activity was linear at concentrations of up to about 20–30 pmol, and was then curvilinear (Fig. 2B). The rates of ATP hydrolysis with 30 pmol of 14-3-3 proteins were determined in reaction mixture in the presence of increasing concentrations of ATP and 0.5 mM ADP and the K_m for ATP was estimated to be 1.41 mM (data not shown). Slight inhibition was observed with concentrations of above 2 mM ADP (data not shown).

Then we analyzed the reverse reaction of the enzyme, the activity for ATP synthesis. The enzyme exhibited activity for ATP synthesis in the presence of 5 mM ATP, 0.5 mM ADP and 6 mM $MgCl_2$, but not in the presence of 0.5 mM AMP instead of 0.5 mM ADP. ATP synthesis activity of this enzyme was not detected in the presence of ADP without nucleoside triphosphate, such as ATP, in the reaction mixture (Fig. 2B). These results suggest that the activity was similar to that of NDP kinase, but not adenylate kinase. The activity for ATP synthesis was linear with up to about 20–30 pmol of enzyme and the rate was similar to that for ATP hydrolysis. The K_m value of the activity for ATP synthesis from ADP was estimated to be 0.2 mM (data not shown). Since both K_m values are slightly lower than the concentrations of ATP (≈5 mM) and ADP (≈0.5 mM) in the cytosol, changes in substrate availability would be expected to affect the in vivo enzyme reaction significantly. The purified recombinant 14-3-3 τ isoform also exhibited both ATP hydrolysis and ATP synthesis activities, as shown in Fig. 2B, although the activities for ATP hydrolysis and ATP synthesis were 32% and 48%,

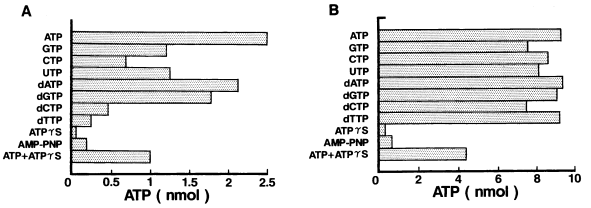

Fig. 3. Comparison of various ribo- and deoxyribo-nucleoside triphosphates used as phosphate donors for the conversion of ADP to ATP by 14-3-3 and NDP kinase. The ATP synthesis activities of 14-3-3 proteins (30 pmoles) (A) and NDP kinase (10 pmoles) (B) were analyzed after incubation for 6 h and 30 min, respectively, under the conditions given in Section 2 using 0.5 mM ADP, 0.02 μCi of [8-^{14}C]ADP, 6 mM $MgCl_2$ and 5 mM concentrations of various ribo-, deoxyribo-nucleoside triphosphates, ATPγS, and AMP-PNP in 100 mM HEPES-KOH buffer, pH 8. After reactions, samples were analyzed by TLC and ATP formation was calculated with an imaging analyzer.

respectively, of those of 14-3-3 proteins from a human lymphoblastoma. Broad pH optima of 7–9 were observed for ATPase and ATP synthesis (data not shown).

3.3. Nucleotide specificity of NDP kinase-like activity of 14-3-3

NDP kinase catalyzes the transfer of the γ-phosphate group from any nucleoside triphosphate to any nucleoside diphosphate. As shown in Fig. 3B, NDP kinase used all ribo- and deoxyribo-nucleoside triphosphates tested as phosphate donors efficiently, but failed to utilize the non-hydrolyzable ATP analogs AMP-PNP and ATPγS. In the presence of an equal concentration of ATPγS to ATP, this transfer by NDP kinase was competitively inhibited about 52% on incubation for 30 min. On the other hand, 14-3-3 preferentially utilized ATP, dATP and dGTP, and transferred the γ-phosphate group to ADP, as shown in Fig. 3A. Of the nucleoside triphosphates, dTTP, dCTP and CTP were poor phosphate donors. Significant inhibition of the activity of 14-3-3 was also observed with equal concentrations of ATPγS to ATP. From the differences in the reaction times and doses of enzymes used in these assays, it is evident that the specific activity for ATP synthesis of NDP kinase was approximately 36 times that of 14-3-3.

Next we tested the specificities of nucleotides as acceptors in the transfer of the γ-^{32}P-phosphate of ATP as catalyzed by 14-3-3 proteins. As shown in Fig. 4A, 14-3-3 proteins converted nucleoside diphosphates (GDP, CDP, UDP, dGDP, dCDP and dTDP) to the corresponding nucleoside triphosphates with almost similar efficiencies. Similar results were observed in the substrate specificity of NDP kinase (Fig. 4B), although the conversion efficiency of the NDP kinase was much higher than that of 14-3-3. In these assay conditions, the conversions of ADP and dADP to ATP and dATP, respectively, were not clearly analyzed, because the newly formed products overlapped the phosphate donor, [γ-^{32}P]ATP. Nevertheless, we confirmed that the rates of conversion of [^{14}C]ADP and [^{14}C]CDP to the corresponding nucleoside triphosphates by 14-3-3 proteins were similar (data not shown).

Since NDP kinase requires Mg^{2+} or Mn^{2+} for activity, the effects of divalent cations on the ATPase and ATP synthesis activities of 14-3-3 were analyzed. Both activities of 14-3-3 were strongly stimulated by 6 mM Mg^{2+} and Mn^{2+}, and moderately by Co^{2+} and Ni^{2+} (data not shown).

3.4. Formation of autophosphorylated 14-3-3 intermediates and their CDP-dependent dephosphorylation.

The NDP kinase-like activity of 14-3-3 proteins might be due to a contaminant, since the activity is similar to that of NDP kinase. However, this possibility is unlikely for the following reasons. First, 14-3-3 proteins purified from a lymphoblastoma and the recombinant τ isoform were >97% and >99% pure, respectively, as determined by SDS-PAGE and no NDP kinase-like protein bands corresponding to molecular masses of about 16 kDa were observed (Fig. 1). Second, no immunoreactivity of the samples of 14-3-3 and the recombinant τ isoform against anti-NDP kinase-A antibodies was also observed as shown in Fig. 1.

NDP kinase autophosphorylates the active site histidine of the intermediate in the process of catalytic phosphate transfer reaction [28,29]. 14-3-3 proteins (3 μg) exhibited autophosphorylated intermediate bands corresponding to proteins with molecular masses of 32 and 28 kDa (Fig. 5A, lane 1) and the recombinant τ isoform (1 μg) gave a 28 kDa autophosphorylated intermediate band (Fig. 5A, lane 3) in the absence of nucleoside diphosphate as an acceptor in the reaction mixture, which contained 10 μCi of [γ-^{32}P]ATP, 100 μM ATP and 6 mM $MgCl_2$ in HEPES-KOH buffer, pH 8. Under the conditions used, no conversion of CDP to CTP by 14-3-3 proteins and the recombinant τ isoform was observed (Fig. 5B, lanes 1 and 3, respectively). In the presence of 5 mM CDP, however, the radioactivities of these phosphorylated intermediates of 14-3-3 and the recombinant 14-3-3 τ isoform decreased with concomitant formation of ^{32}P-labeled CTP from CDP (Fig. 5A, lane 2 and 4, and Fig. 5B, lane 2 and 4, respectively). Furthermore, decreases in the radioactivities of the autophosphorylated intermediates of 14-3-3 proteins

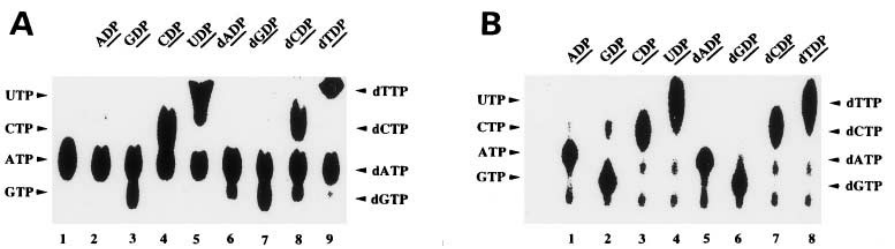

Fig. 4. Specificity of nucleoside diphosphates as phosphate acceptors for 14-3-3 proteins. The substrate specificities of 14-3-3 proteins (33 pmol) (A) and NDP kinase (3.3 pmol) (B) were assayed under the conditions given in Section 2 using 1 μCi of [γ-^{32}P]ATP (6000 Ci/mmol) as a phosphate donor, 6 mM $MgCl_2$ and 0.5 mM concentrations of various ribo- and deoxyribo-nucleoside diphosphates (lanes 2–9 in A and lanes 1–8 in B) in 100 mM HEPES-KOH buffer, pH 8, in a total volume of 20 μl. After incubation for 6 h for A and 30 min for B, samples were analyzed by TLC, followed by autoradiography with an imaging analyzer.

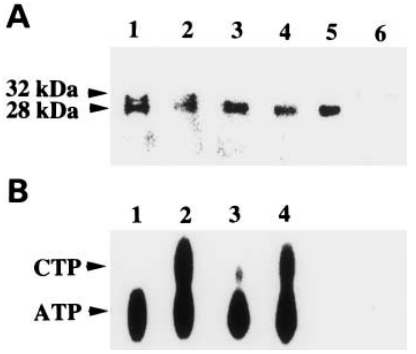

Fig. 5. Autophosphorylation and CDP-dependent dephosphorylation of 14-3-3 proteins and its 14-3-3 τ isoform. Autophosphorylation and CDP-dependent dephosphorylation of 14-3-3 proteins (3 μg) and its recombinant 14-3-3 τ isoform (1 μg) were analyzed as described in Section 2. In the absence of CDP as an acceptor in the reaction mixture, 14-3-3 proteins and its recombinant τ isoform gave two autophosphorylated bands of 28 and 32 kDa and one band of 28 kDa on SDS-PAGE, as shown in A, lanes 1 and 3, respectively. Under the conditions used, no conversion of CDP to CTP was observed in the analyses by TLC as shown in B, lanes 1 and 3, respectively. However, in the presence of 5 mM CDP in the reaction mixture, decrease in the autophosphorylated intermediates of 14-3-3 proteins (A, lane 2) and its recombinant τ isoform (A, lane 4) with concomitant formation of CTP from CDP (B, lanes 2 and 4, respectively) was observed. For characterization of alkali stability of the autophosphorylated 14-3-3, the phosphorylated τ isoform in SDS sample buffer at pH 8.5 without boiling was electrophoresed, and the gel was dried without acid fixation (A, lane 5) [25]. For acid stability, the autophosphorylated protein was boiled in SDS sample buffer at pH 6.8, electrophoresed and the gel was fixed in 20% trichloroacetic acid, followed by Coomassie staining, destaining in methanol/acetic acid, and drying (A, lane 6) [25].

and the τ isoform were also observed on addition of 5 mM CDP to the reaction mixture after the first reaction of formation of autophosphorylated intermediates (data not shown). These results indicate that 14-3-3 catalyzes transfer of the γ-phosphate group from ATP to CDP and that this transfer involves a phosphoenzyme intermediate. These properties are similar to those reported for NDP kinase [28,29]. For characterization of the phosphorylation of 14-3-3, the autophosphorylated 14-3-3 τ isoform was subjected to acid and basic treatments as described [25,29], which allow the evaluation of a high-energy phosphate on basic residues, especially on a histidine residue, in NDP kinase and histidine protein kinase. The phosphorylated intermediate of the 14-3-3 τ isoform was stable on basic treatment (Fig. 5A, lane 5), but labile on acid treatment, resulting in marked decrease in the autophosphorylation level, as shown in Fig. 5A, lane 6. Since the phosphorylations of serine, threonine and tyrosine are stable on acid treatment, this suggests that the acid-labile and alkaline-stable phosphorylated residue(s) in 14-3-3 may be a basic amino acid(s), such as histidine, lysine and arginine, although this residue(s) has not yet been identified. These results strongly indicate that 14-3-3 proteins have similar properties to NDP kinase and that the observed activity of our preparation was not due to contaminant proteins.

4. Discussion

Known functions of 14-3-3 are highly varied, but most of them share the features of those of chaperone and adapter molecules, mediating protein-protein interactions. Recent studies on the crystal structure of the human T-cell 14-3-3 τ isoform and recombinant ζ isoform revealed that each subunit of 14-3-3 consists of a bundle of nine antiparallel helices that form a palisade around the basic face of an amphipathic groove, a putative substrate protein-binding surface [30,31]. In the mechanisms of substrate protein binding to, and release from a molecular chaperone, such as Hsp70 or DnaK, ATP and ADP-dependent cycle of protein/peptide binding and release has been extensively studied [17–20]. The first step in the mechanism involves the binding of a protein/peptide to an Hsp70·ADP complex, resulting in a conformational change accelerating ADP-ATP exchange in the presence of ATP (step 2). The binding of ATP causes a conformational change which triggers substrate release from the complex (step 3). Finally, ATP is hydrolyzed to ADP to afford an Hsp70·ADP complex which then participates in a new cycle of binding (step 4). These results suggest that nucleotide exchange is rate-limiting in the cycle. The ATPase activity of 14-3-3 proteins was originally reported from studies on their function in import of precursor proteins into mitochondria [12–14]. The present work established the intrinsic NDP kinase-like activity of 14-3-3 proteins, catalyzing not only ATP/ADP exchange but also other nucleotide exchanges that may accelerate cycles of association and dissociation of substrate proteins with 14-3-3 and also modulate the functions of substrate proteins. The functional significance of the NDP kinase-like activity of 14-3-3 proteins is unknown, but perhaps it can regulate the association and dissociation of substrate proteins to 14-3-3 and also transfer of the γ-phosphate group from 14-3-3 to a serine/threonine residue(s) of substrate proteins involving a high-energy phosphoprotein intermediate, as reported for the 16 kDa NDP kinase [32], resulting in modulation of protein binding. The precise role of 14-3-3 in cellular regulation awaits further biochemical and genetic analyses.

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References

[1] Aitken, A. (1995) Trends Biochem. Sci. 20, 95–97.
[2] Burbelo, P.D. and Hall, A. (1995) Curr. Biol. 5, 95–96.
[3] Aitken, A., Collinge, D.B., van Heusden, G.P.H., Roseboom, P.H., Isobe, T., Rosenfeld, G. and Soll, J. (1992) Trends Biochem. Sci. 17, 498–501.
[4] Yamauchi, Y., Nakata, H. and Fujisawa, H. (1981) J. Biol. Chem. 256, 5404–5409.
[5] Morgan, A. and Burgoyne, R.D. (1992) Nature 355, 833–836.
[6] Toker, A., Ellis, C.A., Sellers, L.A. and Aitken, A. (1990) Eur. J. Biochem. 191, 421–429.
[7] Isobe, T., Hiyane, Y., Ichimura, T., Okuyama, T., Takahashi, N., Nakajo, S. and Nakaya, K. (1992) FEBS Lett. 308, 121–124.
[8] Morrison, D.K. (1994) Science 266, 56–57.
[9] Muslin, A.J., Tanner, J.W., Allen, P.M. and Shaw, A.S. (1995) Cell 84, 889–897.
[10] Du, X., Harris, S.J., Tetaz, T.J., Ginsberg, M.H. and Berndt, M.C. (1994) J. Biol. Chem. 269, 18287–18290.
[11] Conklin, D.S., Galaktionov, K. and Beach, D. (1995) Proc. Natl. Acad. Sci. USA 92, 7892–7896.
[12] Komiya, T., Hachiya, N., Sakaguchi, M., Omura, T. and Mihara, K. (1994) J. Biol. Chem. 269, 30893–30897.
[13] Hachiya, N., Komiya, T., Alam, R., Iwahashi, J., Sakaguchi, M., Omura, T. and Mihara, K. (1994) EMBO J. 13, 5146–5154.
[14] Komiya, T., Sakaguchi, M. and Mihara, K. (1996) EMBO J. 15, 399–407.

[15] Jones, D.H., Ley, S. and Aitken, A. (1995) FEBS Lett. 368, 55–58.
[16] Vincenz, C. and Dixit, V.M. (1996) J. Biol. Chem. 271, 20029–20034.
[17] Welch, W.J. and Feramisco, J.R. (1985) Mol. Cell. Biol. 5, 1229–1237.
[18] Gao, B., Emoto, Y., Greene, L. and Eisenberg, E. (1993) J. Biol. Chem. 268, 8507–8513.
[19] McCarty, J.S. and Walker, G.C. (1991) Proc. Natl. Acad. Sci. USA 88, 9513–9517.
[20] Takenaka, I.M., Leung, S.-M., McAndrew, S.J., Brown, J.P. and Hightower, L.E. (1995) J. Biol. Chem. 270, 10839–19844.
[21] Hartl, F.U. (1997) Nature 381, 571–580.
[22] Flynn, G.C., Chappell, T.G. and Rothman, J.E. (1989) Science 245, 385–390.
[23] Braell, W.A., Schlossman, D.M., Schmid, S.L. and Rothman, J.E. (1984) J. Cell Biol. 99, 734–741.
[24] Lu, Q. and Inouye, M. (1996) Proc. Natl. Acad. Sci. USA 93, 5720–5725.
[25] Parks, R.E.J. and Agarwal, R.P. (1973) Enzymes 8, 307–334.
[26] Nielsen, P.J. (1991) Biochim. Biophys. Acta 1088, 425–428.
[27] Meller, N., Liu, Y.-C., Collins, T.L., Bonnefoy-Berard, N., Baier, G., Isakov, N. and Altman, A. (1996) Mol. Cell. Biol. 16, 5782–5791.
[28] Biondi, R.M., Walz, K., Issinger, O.-G., Engel, M. and Passeron, S. (1996) Anal. Biochem. 242, 165–171.
[29] Deville-Bonne, D., Sellam, O., Merola, F., Lascu, I., Desmadril, M. and Veron, M. (1996) Biochemistry 35, 14643–14650.
[30] Xiao, B., Smerdon, S.J., Jones, D.H., Dodson, G.G., Soneji, Y., Aitken, A. and Gamblin, S.J. (1995) Nature 367, 188–191.
[31] Liu, D., Bienkowska, J., Petosa, C., Collier, R.J., Fu, H. and Liddington, R. (1995) Nature 367, 191–194.
[32] Engel, M., Veron, M., Theisinger, B., Lacombe, M.-L., Seib, T., Dooley, S. and Welter, C. (1995) Eur. J. Biochem. 234, 200–207.