Inferences Concerning the ATPase Properties of DnaK and Other HSP70s Are Affected by the ADP Kinase Activity of Copurifying Nucleoside-diphosphate Kinase*

(Received for publication, June 25, 1999, and in revised form, September 13, 1999)

Thomas K. Barthel and Graham C. Walker‡
From the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Preparations of Escherichia coli DnaK from our lab as well as preparations of DnaK and other HSP70 proteins from several major labs in the field produce a stoichiometric initial burst of [α-32P]ADP when incubated with [α-32P]ATP and contain an ADP kinase activity. We determined that the initial burst activity results from the transfer of γ-phosphate from the radiolabeled substrate [α-32P]ATP to unlabeled ADP bound by DnaK and is the same activity that results in ADP phosphorylation. The purification of DnaK from E. coli cells that carry a disrupted ndk gene, ndk::km, results in preparations with greatly reduced ADP kinase activities compared with preparations of DnaK purified from ndk+ cells. The reduction in the amount of ADP kinase activity in preparations of DnaK purified from ndk::km cells shows that nucleoside-diphosphate kinase (NDP kinase) is responsible for most of the ADP kinase activity present in DnaK preparations isolated from ndk+ cells. The remaining ADP kinase activity in preparations from ndk::km cells, which varies between preparations, is also a property of NDP kinase, which is most likely expressed because of a low frequency reversion of the disrupted ndk gene. A weak, but measurable physical interaction exists between DnaK and NDP kinase and may be at least partially responsible for the co-purification of NDP kinase with DnaK. The presence of contaminating NDP kinase can explain the range of $k_{cat}$ values reported for the ATPase activity of DnaK as well as recent reports of initial burst kinetics by DnaK (Banecki, B., and Zylicz, M. (1996) J. Biol. Chem. 271, 6137–6143) and an ATP-ATP exchange activity of DnaK (Hiromura, M., Yano, M., Mori, H., Inoue, M., and Kido, H. (1998) J. Biol. Chem. 273, 5435–5438).

DnaK is the principal Escherichia coli member of the highly conserved and ubiquitous family of HSP70 proteins. In addition to functioning in response to heat shock and other forms of stress, many HSP70 proteins, including DnaK, function in a variety of normal metabolic processes, including the folding of nascent polypeptides (3, 4), intracellular protein trafficking, membrane translocation (5), protein degradation (6), and disassembly of native protein complexes (7). HSP70 proteins function as molecular chaperones in all of these processes, undergoing multiple cycles of binding and release of the polypeptide substrate to facilitate its folding or to protect unfolded states from aggregation and denaturation (8–12). DnaK acts as a molecular chaperone in E. coli. In vitro, DnaK blocks the aggregation and allows the reactivation of heat-inactivated RNA polymerase (13), rhodanese (14), and firefly luciferase (15). DnaK dissociates dimeric RepA protein to allow P1 phage replication (16) and promotes the disassembly of the protein complex at ori-λ, allowing replication (17). DnaK is required for growth at high temperature (18) and is required for the negative regulation of the heat shock response at normal growth temperatures (19). DnaK represses the expression of heat shock genes by negatively regulating the synthesis and stability of α23 (6, 20), and this repression is transiently lifted upon heat shock (21).

Limited proteolysis of HSP70 proteins separates them into two stable domains. The COOH-terminal domain binds peptides in an extended conformation (22, 23) in a manner believed to mimic the binding of physiological substrates. The NH2-terminal domain possesses the weak intrinsic ATPase activity associated with every HSP70 protein (24, 25). The two domains interact, and the peptide binding and release cycle is tightly coupled to the ATP binding and hydrolysis cycle (8, 9, 11, 26). The ATPase activity of HSP70 proteins is therefore crucial for their biological function.

A range of quantitative values and functional descriptions of the ATPase activity of DnaK and other HSP70 proteins has been reported. The ATPase activity of unstimulated DnaK has been assigned $k_{cat}$ values ranging from 0.02 to 1.0 min⁻¹ (27–29) and $K_m$ values ranging from 20 nM to 4 μM (30, 31). The binding of ATP, but not nonhydrolyzable analogs of ATP, results in a conformational change of DnaK and in the release of its bound peptide substrate (32), which initially led to the conclusion that ATP hydrolysis results in a global conformational change of DnaK that in turn results in peptide release. However, because mutants of DnaK that have a defective ATPase activity release peptide (33) and undergo a conformational change (34) upon ATP binding, it was concluded that ATP binding, and not hydrolysis, causes a conformational change of DnaK that results in peptide release. Measurements of the amounts of ATP and ADP bound to DnaK during ATP hydrolysis (35) and of single ATP turnover rates (36, 37) led to the conclusion that the hydrolysis of ATP is the rate-limiting step in the ATPase reaction cycle of DnaK and cannot be required for the conformational change of DnaK and the consequent peptide release, both of which occur rapidly when ATP is added to DnaK (29, 37–39).

We found that a variety of DnaK and HSP70 preparations display initial burst kinetics in ATPase assays carried out with [α-32P]ATP, rapidly producing a stoichiometrically equal amount of [α-32P]ADP when incubated with ATP. We also found that these preparations contain an ADP kinase activity, phosphorylating ADP to ATP in the presence of ATP. However,
we subsequently found that the initial burst and ADP kinase activities are associated with a copurifying protein present in amounts that we could not detect by SDS-PAGE\(^1\) in the DnaK and HSP70 preparations. DnaK purified from cells with a disruption in the ndk gene, which encodes nucleoside-diphosphate kinase, had greatly reduced levels of the ADP kinase activity, showing that the activity is due primarily to the presence of copurifying nucleoside-diphosphate kinase in DnaK and HSP70 preparations. The presence of this protein likely accounts for results of two papers published while this study was in progress that report initial burst and ADP phosphorylation activities in DnaK preparations similar to those described in this work (1, 2).

**EXPERIMENTAL PROCEDURES**

**Reagents and Media—** ATP and ADP were purchased from Sigma. \(\alpha\)-[\(\gamma\)-32P]ATP, \(\gamma\)-[\(\gamma\)-32P]ATP, [\(\beta\)-32P]ADP, and \([\gamma\)-32P]ADP were purchased from NEN Life Science Products. AMP-PNP was purchased from Roche Molecular Biochemicals. LB liquid medium was as described in Ref. 40. Antibiotics were used at the following concentrations: ampicillin (100 \(\mu\)g/ml), tetracycline (12.5 \(\mu\)g/ml), chloramphenicol (30 \(\mu\)g/ml), and kanamycin (50 \(\mu\)g/ml).

**Construction of ndk::km Strains—** The E. coli strains NA7623 and JC7623 were generously provided to us by the laboratory of Masayori Inouye. Strain NA7623 has the gene encoding nucleoside-diphosphate kinase (ndk) disrupted and is otherwise isogenic to JC7623. The ndk gene of NA7623 is disrupted by a kanamycin-resistance gene, which is inserted into its EcoRI site as described (41). The \(\Delta\)ndk52 allele was transduced into both NA7623 and JC7623 by using P1(GW8306) lysate as described (42). The resulting strains were TB3200 (ndk::km \(\Delta\)ndk) and TB3500 (ndk \(\Delta\)ndk) and were transformed with the DnaK overexpression plasmid pJM6 (43) by the standard CaCl\(_2\)/heat shock procedure (40) to create strains TB3220 (JC7623, ndk::km \(\Delta\)ndk, pJM6) and TB3520 (JC7623, ndk \(\Delta\)ndk, pJM6).

**HSP70 Proteins—** HSP70 proteins were generously supplied to us from the following sources with references to the method of purification: E. coli DnaK was provided to us by the laboratories of Bernd Bukau (35), Carol Gross (44), and Roger McMacken (45), 44-kDa NH\(_2\)-terminal recombinant Thermus thermophilus DnaK was provided to us by the laboratory of Andreae Joachimkai (46), recombinant human HSP70 was provided to us by the laboratory of Richard Morimoto (47), and recombinant hamster BiP was provided to us by the laboratory of Linda Hendershot (48). Bovine Hsc70, purified from bovine brain cells, was purchased from StressGen Biotechnologies Corp. (Victoria, British Columbia, Canada).

**DnaK Purification—** The standard protocol for the purification of DnaK was similar to the method previously described (43) with some modifications and was as follows. GW8320 (MC4100, \(\Delta\)ndk52 sidB1) cells (44) carrying the DnaK overexpressing plasmid pJM6 (pBS-P lac) (\(\Delta\)ndk) (43) were grown at 30 °C to late log phase (A\(_{600}\) \(\approx\) 0.6) in 2 liters of LB medium with ampicillin, tetracycline, and chloramphenicol. For experiments comparing DnaK purified from ndk\(^1\) cells to DnaK purified from ndk::km cells, TB3520 was used to produce DnaK instead of GW8320 pJM6 and was grown in LB with ampicillin and chloramphenicol. TB3220 was used to produce DnaK in an ndk::km background and was grown in LB with ampicillin, kanamycin, and chloramphenicol. Expression of DnaK was induced by adding isopropyl-1-thio-\(\beta\)-D-galactopyranoside (1 \(\mu\)M), and the culture was shifted to 37 °C to incubate for another 1 h. The cells were pelleted by centrifugation (5000 \(\times\) g, 30 min, 4 °C). The cell pellet was resuspended in 6 ml of Buffer L (10% sucrose, 50 mM Tris-HCl, pH 8.0) plus 24 ml of buffer L2 (20 mM spermidine, 5 mM diethiothreitol, 100 mM ammonium sulfate, 5 mM EDTA). The cell suspension was transferred into Oak Ridge centrifuge tubes and lysed by sonication (Heat Systems Sonicator XL, Farmingdale, NY). Following ultracentrifugation (30,000 rpm, 30 min, 4 °C), the supernatant was collected, and protein was precipitated with ammonium sulfate (380 g/liter). The protein was pelleted by ultracentrifugation (30,000 rpm, 30 min, 4 °C), resuspended in 5 ml of Buffer B (25 mM Hepes-KOH, pH 7.6, 50 mM KCl, 2.5 mM MgCl\(_2\), 1 mM EDTA, 10 mM 2-mercaptoethanol, 10% glycerol), and dialyzed in a Spectra/Por Membrane molecular weight cut-off = 12,000–14,000 Da (Spectrum Medical Industries, Inc., Los Angeles, CA) against Buffer B (300 ml) two times. The sample was applied to a DEAE-Sepharose FF (Amersham Pharmacia Biotech) column (250 ml) and resolved by running a linear 50 mM-550 mM KCl gradient over 500 ml of Buffer B. The column was washed with 20 ml of Buffer B plus 500 mM NaCl followed by 10 ml of Buffer B and eluted with 20 ml of Buffer B + 5 mM ATP. The ATP-agarose eluent was applied to a MonoQ HR 5/5 column (Amersham Pharmacia Biotech). The DnaK was eluted from the column with Buffer B as judged by the return of the A\(_{280}\) trace to baseline. DnaK was resolved by running a linear gradient of 50 mM-750 mM KCl over 30 ml in Buffer B. Fractions (1 ml) were collected, quick frozen on liquid nitrogen, and stored at −80 °C. The DEAE-Sepharose FF and MonoQ columns were run on an Amersham Pharmacia Biotech fast protein liquid chromatography system. All steps were carried out at 4 °C. DnaK was judged to be >99% pure by the absence of any visible bands other than DnaK on a Coomassie Brilliant Blue-stained SDS-PAGE gel. The concentration of DnaK samples was determined using Bio-Rad protein assay solution following the standard protocol. A DnaK sample whose concentration had been precisely determined by amino acid analysis (performed by the Biopolymers Laboratory at the M.I.T. Center for Cancer Research) was used as a standard in all DnaK concentration assays.

**ATPase Assays—** Reaction mixtures (25 \(\mu\)l) contained ATPase Buffer (40 mM Hepes-KOH, pH 7.6, 50 mM KCl, 11 mM Mg(OAc)\(_2\)) (\(\alpha\)-[\(\gamma\)-32P]ATP, or \(\gamma\)-[\(\gamma\)-32P]ATP and DnaK. Radiolabeled ATP stocks were made by adding radiolabeled ATP to unlabeled ATP to give a final activity of 82 \(\mu\)Ci/ml. The total concentration of ATP was recalculated following the addition of the radiolabeled ATP. Unless otherwise indicated, the ATP and ATPase Buffer were mixed and preincubated at 30 °C for 5 min, and hydrolysis was initiated by the addition of DnaK, marked as t = 0 in the assays. When present in the reaction mixture, ADP and ovalbumin were preincubated with the ATP prior to the addition of DnaK. The reaction was incubated in a water bath at 30 °C and was stopped at various times by spotting 2 ml of the reaction onto a polyethyleneimine-cellulose TLC plate (J. T. Baker Inc., Phillipsburg, NJ). Spotting the reaction on the TLC plate stopped the reaction immediately, as reactions quenched with 1 N HCl prior to spotting and reactions spotted without chemical quenching show the same extent of hydrolysis. The TLC plate was developed in 1 \(\mu\)l formic acid, 0.5 \(\mu\)l LiCl, dried, and exposed to a Molecular Dynamics storage phosphor screen. Data was obtained using a Molecular Dynamics PhosphorImager 445 Si and MacBAS Image Quant (Molecular Dynamics). The amount of radioactivity in the species present was determined by volume integration. The data were corrected for the level of background hydrolysis (typically 1% or less). The extent of hydrolysis was expressed as the fraction of ADP or P, to total nucleotide, or further calculations were made to determine the velocity of the reaction. The velocity of the ATPase reaction in terms of pmol of ATP hydrolyzed/min was determined by multiplying the measured (A\(_{280}\) + P) ratio by the starting amount of ATP in the reaction (indicated in the corresponding figure for each reaction) and dividing by time.

**ADP Kinase Assays—** Reaction mixtures (25 \(\mu\)l) contained ATPase Buffer, [\(\gamma\)-32P]ATP, ADP, and DnaK. Radiolabeled ATP stocks were made by adding radiolabeled ATP to unlabeled ATP to give a final activity of 4.00 \(\mu\)Ci/ml. The total concentration of ADP was recalculated following the addition of the radiolabeled ATP. The ATP, ADP, and ATPase Buffer were mixed and preincubated at 30 °C for 5 min, and ADP phosphorylation was initiated by the addition of the DnaK sample, marked as t = 0 in the assays. When ADP was used as a phosphate acceptor, the procedure used was exactly the same as above, except that [\(\gamma\)-32P]ADF was mixed with unlabeled CDP to make the radiolabeled CDP stock. The reaction was incubated in a water bath at 30 °C and was stopped at various times by spotting 2 \(\mu\)l of the reaction onto a polyethyleneimine-cellulose TLC plate. The TLC plate was developed and analyzed exactly as for the ATPase assays described above. The extent of ADP phosphorylation was expressed as the fraction of ATP to total nucleotide, or further calculations were made to determine the velocity of the reaction. The velocity of the ADP kinase reaction in terms of pmol of ADP phosphorylated/min was determined by dividing the measured (A\(_{280}\) + ADP) ratio by the starting amount of ADP in the reaction (indicated in the corresponding figure for each reaction) and dividing by time.

**Nucleotide Removal Treatment—** Nucleotide was removed from DnaK by a method similar to that previously described for Hsc70 (49). DnaK
was saturated with AMP-PNP (5 mM) in a total volume of 2 ml and incubated at 25 °C for 1 h. To remove free AMP-PNP, the sample was applied to a G-25M desalting column (Amersham Pharmacia Biotech PD-10 column) equilibrated with Buffer B and eluted with 3.5 ml of Buffer B. The sample was dialyzed in a Spectra/Por Membrane molecular weight cut-off ~12,000~14,000 Da against Buffer B (700 ml) three times. The dialyzed sample was applied to a MonoQ column and eluted with a double linear gradient of 50~225 mM KCl over 7.5 ml followed by 225~435 mM KCl over 45 ml in Buffer B.

Production and Purification of His-tagged Nucleoside-diphosphate Kinase (NDP Kinase)—A plasmid was constructed to express NDP kinase with an amino-terminal tag of 6 histidine residues. Two 3-mL primers were purchased from Life Technologies, Inc. designed to amplify the ndk gene from genomic E. coli DNA based on the known sequence of the gene (50) and include a 5′-NdeI and a 3′-BamHI restriction site. The NdeI-ndk-BamHI product was amplified by polymerase chain reaction using the GeneAmp kit (Perkin-Elmer Corp.). The amplification product was purified by subjecting it to agarose gel electrophoresis and by extracting its band using a QIAquick polymerase chain reaction purification kit (Qiagen). The purified product was digested with NdeI and BamHI and ligated into NdeI- and BamHI-linearized pET-16b plasmid (Novagen) to create the plasmid pET-16b/ndk.

The pET-16b/ndk plasmid was transformed into BL21 (DE3) pLysS E. coli cells to create the strain TB2000. The TB2000 cells were grown in 800 ml of LB with ampicillin and chloramphenicol at 37 °C to mid-log phase (OD600 ~ 0.6) and induced with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside to overexpress His-tagged NDP kinase (NdK-N-His). Following incubation at 37 °C for 1 h, the cells were pelleted by centrifugation (5000 × g, 20 min, 4 °C), resuspended in 1× binding buffer, low salt (5 mM imidazole, 50 mM NaCl, 20 mM Tris-HCl, pH 7.9), and lysed by sonication. Debris was removed from the lysed cells by ultracentrifugation (30,000 rpm, 20 min, 4 °C), and the supernatant was applied to a 2.5 ml His-Bind immobilized metal-ion affinity chromatography (IMAC) column (Novagen) equilibrated with 1× binding buffer, low salt. For co-purification experiments, the column was washed with 10 ml of 1× binding buffer, low salt followed by 10 ml of 0.5× wash buffer, low salt (30 mM imidazole, 50 mM NaCl, 20 mM Tris-HCl, pH 7.9). The gel bed was then resuspended in 0.5× wash buffer, low salt, and the gel suspension was transferred into an Amersham Pharmacia Biotech XK16 column. The XK16 column was attached to a Amersham Pharmacia Biotech fast protein liquid chromatography system, and the gel bed was settled with 0.5× wash buffer, low salt at a flow rate of 0.5 ml/min. A linear gradient of 30~500 mM imidazole was then run over 30 ml in 0.5× wash buffer, low salt, and 1-ml fractions were collected. All purification steps were performed at 4 °C.

Immunoblot Analyses—Fractions from the Ndk-N-His purification were collected, and a 10-μl aliquot of each sample was mixed with SDS-PAGE loading buffer (40), incubated in a boiling water bath for 3 min, and subjected to SDS-PAGE as described (40). The proteins were transferred from the gels to polyvinylidene difluoride membranes, and antibody reactions and chemiluminescent detection were performed using a “Western Lights” kit (Tropix, Bedford, MA) as described in the manual. The antibody used was affinity purified rabbit a-DnaK (1:5000 dilution).

Protein Sequencing—Protein sequencing was performed at the M.I.T. Biopolymers Laboratory. The sample was loaded onto an Applied Biosystems PreSorb sample preparation cartridge with a polyvinylidene difluoride membrane. The sample was then sequenced with an Applied Biosystems Procise 494 protein sequencer.

RESULTS

Preparations of DnaK and Other HSP70 Proteins Hydrolyze [α-32P]ATP with Biphasic Kinetics—In the experiments described below, we used DnaK that we had purified from DnaK-overproducing E. coli cells by a method that involves three primary chromatographic steps: DEAE-Sepharose anion exchange, ATP-agarose affinity, and MonoQ anion exchange columns. This method is basically the same one used by other groups in the field (e.g. 35, 44, 45). Our preparations of DnaK were determined to be >99% pure by SDS-PAGE analysis. ATPase assays using our preparations of DnaK showed that the rate of [α-32P]ADP production appeared constant over time when the molar amount of [α-32P]ATP in the assay was 100-fold or more greater than the molar amount of DnaK. However, when the molar amount of [α-32P]ATP was less than 100-fold greater than the molar amount of DnaK, an initial burst of [α-32P]ADP formation became apparent. When the amount of [α-32P]ATP was only severalfold greater than the amount of DnaK in the assay, the rate of [α-32P]ADP formation clearly showed biphasic kinetics: an initial burst of [α-32P]ADP formation within the first min followed by a much slower steady-state rate of ADP formation. [α-32P]ATP was incubated with several different concentrations of DnaK at 30 °C, and the amount of ADP produced was measured over time (Fig. 1A).

For our preparations of DnaK, the magnitude of the initial burst of ADP production increased directly with the amount of DnaK in the assay. From the results presented in Fig. 1A, the sizes of the initial bursts were determined by interpolation to be 1.39 μM, 2.87 μM, and 5.13 μM of ADP for 1.44 μM, 2.88 μM, and 5.77 μM of DnaK, respectively. Thus, the molar size of the initial burst of ADP was approximately equal to the molar amount of DnaK present in the reaction or 1 ADP:1 DnaK. Because the size of the initial burst was stoichiometrically equal to the amount of DnaK, the burst was not detectable when the concentration of ATP approached or exceeded 100 times the concentration of DnaK, as the background amount of [α-32P]ADP in the [α-32P]ATP used in our assays was typically about 1% of the total nucleotide.

The possibility that the initial burst reflected the true steady-state rate of ATP hydrolysis by DnaK while the slower rate resulted from inactivation or loss of DnaK from solution was addressed. Previously, we had observed a significant decrease in the rate of ATP hydrolysis over time when DnaK was present at nanomolar concentrations. This loss of activity over time was determined to be because of the loss of DnaK from solution, and the addition of 2.0 μg/μl ovalbumin to the assay buffer to act as a nonspecific carrier for DnaK corrected this problem. The addition of ovalbumin to assays with DnaK present at micromolar concentration had little effect on the kinetic profile of the ATPase reaction (Fig. 1B), and therefore there was no significant loss of DnaK from solution at these concentrations. DnaK was also preincubated at 30 °C for 30 min prior

![Fig. 1. DnaK preparations produce a stoichiometric burst of [α-32P]ADP when incubated with [α-32P]ATP which proceeds steady-state hydrolysis. A, DnaK (■, 1.44 μM; ■, 2.88 μM; □, 5.77 μM) was added to [α-32P]ATP (19.8 μM) in ATPase Buffer and incubated at 30 °C. The reaction was stopped following various incubation times, and the amount of ADP and ATP was determined. B, DnaK (2.88 μM) either with no preincubation or following a 30 °C preincubation for 30 min was added to [α-32P]ATP (19.8 μM) in ATPase Buffer with (●) or without (□) 2.0 μg/μl ovalbumin. The sample was incubated at 30 °C. The reaction was stopped following various incubation times, and the amount of ADP and ATP was determined.](http://www.jbc.org/content/24/10/36572/F1)

Downloaded from http://www.jbc.org by guest on September 24, 2017
to the addition of ATP to determine if there was any thermal or time-dependent loss of DnaK ATPase activity (Fig. 1B). The kinetic profiles of DNAK ATPase with and without preincubation and with and without ovalbumin were all nearly identical and thus demonstrated that the initial burst of ADP formation was a true characteristic of our DNAK preparations and was not because of any loss of enzymatic activity.

Several different preparations of DNAK from our lab were similarly assayed, and all showed a stoichiometric burst of (α-32P)ADP production. We obtained DNAK and other HSP70 proteins from other labs and various commercial sources. Samples of DNAK from three major labs in the field, the 44-kDa amino-terminal ATPase fragment of DNAK from *T. thermophilus*, HSP70 from one lab, and BiP from one lab all displayed similar initial burst activities in ATPase assays. Hsc70 purified from bovine brain purchased from a commercial source also showed an initial burst of ADP production (data not shown).

**DNAK Preparations Phosphorylate ADP to ATP in the Presence of ATP**—We obtained results not described here that led us to consider the possibility that the DNAK samples we were analyzing might be able to phosphorylate ADP in the presence of ATP. DNAK was incubated with ATP and [8-14C]ADP at 30 °C, and the radiolabeled nucleotide content of the mixture was analyzed at various times. Fig. 2A shows that [8-14C]ATP was produced under these conditions. A quantitative kinetic analysis of the results (Fig. 2B) shows that the amount of [8-14C]ATP rapidly accumulated to the level that would be predicted for equal sharing of γ-phosphates by the amounts of labeled and unlabeled adenosine nucleotide in the reaction mixture. The amount of [8-14C]ATP then decreased at a slow, constant rate. This decrease in the amount of [8-14C]ATP was presumably because of the steady-state hydrolysis of [8-14C]ATP to [8-14C]ADP by DNAK.

The same DNAK, HSP70, Hsc70, and BiP preparations discussed previously were assayed. DNAK preparations from three different labs, the amino-terminal ATPase fragment of DNAK from *T. thermophilus*, a HSP70 preparation, a BiP preparation, and Hsc70 purified from bovine brain were all found to possess an ADP kinase activity with kinetics similar to those of our DNAK preparations (data not shown).

NDP Kinase Affects the ATPase Activity of DNAK Preparations

---

**Fig. 2. DNAK preparations phosphorylate ADP to ATP in the presence of ATP.** A, DNAK (3.04 μM) was added to a mixture of ATP (20 μM) and [8-14C]ADP (61.2 μM) in ATPase Buffer and incubated at 30 °C. As a control, [8-14C]ADP and ATP were incubated with no DNAK. Following various incubation times, each incubation was spotted on a polyethyleneimine-cellulose TLC plate, developed in 1 M formic acid, 0.5 M LiCl, and exposed to a phosphorimaging screen. B, the amount of radiolabeled ATP and ADP was determined for each time point and expressed as a ratio of ATP to total radiolabeled nucleotide.

NDP kinase catalyzes the transfer of γ-phosphate from a nucleoside triphosphate to a nucleoside diphosphate with a turnover rate of approximately 1000 s⁻¹ under conditions similar to those used in our ATPase assays (51). This turnover rate is 600,000–6,000,000 times higher than most published values of the turnover rate of the ATPase activity of DNAK, and therefore a relatively tiny amount of NDP kinase could significantly affect the kinetic properties of the ATPase activity of a preparation of DNAK. Thus, we were concerned that an amount of NDP kinase, undetectable by standard SDS-PAGE analysis, might have been present in all of the DNAK/HSP70 preparations we had analyzed and could have been responsible for some of the activities that we had measured.

NDP kinase is substrate-nonspecific and catalyzes the transfer of phosphate from any nucleoside triphosphate to any nucleoside diphosphate with nearly equal efficiency (2). DNAK, on the other hand, while reported to hydrolyze GTP, CTP, and UTP to some extent, hydrolyzes ATP with the greatest efficiency (32), and ATP is believed to be required for proper DNAK function (32, 33). Therefore, the ability of a DNAK/HSP70 preparation to transfer the γ-phosphate from ATP and no other nucleoside triphosphate to ADP and no other nucleoside diphosphate would have provided compelling evidence that the DNAK/HSP70 protein itself is responsible for the ADP kinase activity. On the other hand, the ability of a DNAK/HSP70 preparation to transfer the γ-phosphate from any nucleoside triphosphate to any nucleoside diphosphate with equal efficiency would have increased the likelihood that the activity is because of some other protein. All DNAK and Hsc70 preparations discussed above were therefore incubated with ATP and either radiolabeled ADP or radiolabeled CDP. When radiolabeled ADP was provided as a phosphate acceptor, radiolabeled ATP was produced, whereas when radiolabeled CDP was provided as a phosphate acceptor, radiolabeled CTP was produced with similar kinetics (data not shown). Thus, the DNAK and HSP70 preparations were nonspecific as to which nucleoside diphosphate serves as a phosphate acceptor.

The presence of NDP kinase in a DNAK preparation would provide an alternative means for an initial burst of (α-32P)ADP formation during the incubation of the preparation with (α-32P)ATP. HSP70 proteins bind ADP with very high affinity (for Hsc70, Kd = 1.8 × 10⁻⁸ M), and ADP cannot be removed from HSP70 by simple dialysis (49). Our preparations of DNAK as well as the DNAK and HSP70 preparations from other labs described in this section were not treated to remove ADP and are therefore likely to have some ADP bound. Thus, NDP kinase would be able to use this HSP70-bound ADP as a phos-
incubated with ATP (20 μM) in ATPase Buffer at 30 °C for 10 min. Free nucleotide was removed from the DnaK saturated with ATP. A preparation of the 44-kDa amino-terminal fragment of DnaK from the thermophilic bacterium _T. thermophilus_ was used as a substrate, which phosphatase acceptor and rapidly transfer the γ-phosphate from some of the ATP in the incubation mixture onto the ADP, at the same time converting the substrate ATP into ADP. If [γ-32P]ATP was used as a substrate, this phosphate transfer would create a burst of [γ-32P]ADP formation that would mimic the burst of [α-32P]ADP formation expected from a single, rapid round of ATP hydrolysis. However, [γ-32P]ATP was used as a substrate, the transfer of radiolabeled γ-phosphate to ADP would only reform [γ-32P]ATP, which is indistinguishable from the original substrate, and there would be no initial burst of product formation. A single, rapid round of ATP hydrolysis, by contrast, would result in a initial burst of 32Pi formation. To test this hypothesis, DnaK was purified from _E. coli_ cells with a disruption in the _ndk_ gene. A _ndk::km_ DnaK strain was constructed by transducing the ΔdnaK52 allele into the _E. coli_ strain NA7623. The _ndk_ gene of NA7623 is disrupted by a kanamycin-resistance gene insert (41). The isogenic _ndk::ΔdnaK_ strain was constructed by transducing the ΔdnaK52 allele into the _ndk^+_ parent strain of NA7623,

![Burst of [γ-32P]ADP produced by DnaK preparations](image-url)

**A** Burst of [γ-32P]ADP produced by DnaK preparations is because of phosphorylation of bound ADP. A, DnaK (2.89 μM) was added to either [α-32P]ATP or [γ-32P]ATP (19.8 μM) with or without ADP (20 μM) in ATPase Buffer and incubated at 30 °C. The reaction was stopped following various incubation times, and the amount of radiolabeled ATP and radiolabeled ADP or radiolabeled Pi was determined. The results were plotted as follows: •, [α-32P]ATP; □, [α-32P]ATP + ADP; ○, [γ-32P]ATP; ●, [γ-32P]ATP + ADP. B, DnaK (3.01 μM) was incubated with a saturating concentration of ATP (20 μM) in ATPase Buffer at 30 °C for 10 min. Free nucleotide was removed from the DnaK by applying the sample to a Bio-Rad BioSpin 30 column. DnaK saturated with ATP (●) or not saturated with ATP (□) was added to [α-32P]ATP in ATPase Buffer and incubated at 30 °C. The reaction was stopped following various incubation times, and the amount of ADP and ATP was determined.

![Burst of [γ-32P]ADP produced by DnaK preparations](image-url)

**B** Burst of [γ-32P]ADP produced by DnaK preparations is because of phosphorylation of bound ADP. A, DnaK (2.89 μM) was added to either [α-32P]ATP or [γ-32P]ATP (19.8 μM) with or without ADP (20 μM) in ATPase Buffer and incubated at 30 °C. The reaction was stopped following various incubation times, and the amount of radiolabeled ATP and radiolabeled ADP or radiolabeled Pi was determined. The results were plotted as follows: •, [α-32P]ATP; □, [α-32P]ATP + ADP; ○, [γ-32P]ATP; ●, [γ-32P]ATP + ADP. B, DnaK (3.01 μM) was incubated with a saturating concentration of ATP (20 μM) in ATPase Buffer at 30 °C for 10 min. Free nucleotide was removed from the DnaK by applying the sample to a Bio-Rad BioSpin 30 column. DnaK saturated with ATP (●) or not saturated with ATP (□) was added to [α-32P]ATP in ATPase Buffer and incubated at 30 °C. The reaction was stopped following various incubation times, and the amount of ADP and ATP was determined.
NDP Kinase Affects the ATPase Activity of DnaK Preparations

Fig. 4. Chromatograms of protein concentration and ADP kinase activity from MonoQ separation of DnaK. DnaK overexpressed in either ndk<sup>-</sup> (A) or ndk::km (B) cells was purified according to the standard purification protocol. Fractions from the final chromatographic step (MonoQ anion-exchange column) were collected and assayed for total protein concentration (□) and ADP kinase activity (●). Note the difference in the scales of the right y axis between the two panels.

 Jamaican. The DnaK overexpressing plasmid pJM6 was then transfected into both the ndk<sup>-</sup> ΔdnaK strain and the ndk::km ΔdnaK strain to make strains TB3520 and TB3220, respectively. DnaK was overexpressed in and purified from both strains following the standard purification protocol.

Fractions were collected from the final purification step, MonoQ anion-exchange chromatography, and assayed for ADP kinase activity and total protein concentration. Fig. 4 shows overlaid chromatograms of the rate of ADP phosphorylation and the protein concentration of MonoQ fractions of DnaK purified from ndk<sup>-</sup> cells and ndk::km cells. In both cases, the protein and ADP kinase activity peaks overlap, although the ADP kinase activity reaches its highest level about 3 ml before the protein concentration reaches its highest level. Most significantly, whereas ADP kinase activity is present in both DnaK preparations, the amount of activity is approximately 500-fold lower in the ndk::km preparation than in the ndk<sup>-</sup> preparation. Thus we concluded that most, but not all, of the ADP kinase activity in DnaK preparations was because of the presence of NDP kinase. It did not seem likely that the NDP kinase-independent ADP kinase activity was an intrinsic property of DnaK, because the activity and protein peaks, while overlapping, do not coincide exactly (Fig. 4B). As is the case for DnaK purified from ndk<sup>-</sup> cells, the only bands visible on Coomassie Brilliant Blue-stained SDS-PAGE gels of the MonoQ fractions of DnaK purified from ndk::km cells are the 70-kDa bands of DnaK, even in the fractions with the highest ADP kinase activity (data not shown).

Treatment to Remove Nucleotide Results in One Predominant Form of DnaK, Which Has a Lowered NDP Kinase Activity—In our preparations, DnaK elutes from a MonoQ anion-exchange column in multiple peaks (Fig. 5A), all of which are positively identified as DnaK by SDS-PAGE and immunoblotting analysis. Pooled MonoQ fractions of DnaK (from the MonoQ separation shown in Fig. 5A) were treated to remove bound ADP by saturating the DnaK with nonhydrolyzable AMP-PNP followed by extensive dialysis as described previously (49). When the nucleotide-free DnaK was reinjected onto a MonoQ column, it eluted as one major peak followed by several much smaller broad peaks (Fig. 5B). To determine if the presence of nucleotide itself results in the multiple forms of DnaK, which lead to the multiple peaks during MonoQ separation, the nucleotide-free DnaK peaks were collected from the MonoQ separation shown in Fig. 5B and saturated with 2 mM ATP. Following a 15-min incubation at 25 °C, the DnaK was immediately loaded onto a MonoQ column. The DnaK does not separate into multiple peaks following saturation with ATP (data not shown), indicating that the presence of different nucleotide-bound states of DnaK is not the cause of the multiple peaks of DnaK on a MonoQ column. Some other change occurs to the DnaK during the nucleotide removal treatment that converts the DnaK from several forms into one predominant form. One possible explanation for this conversion is that the treatment removes peptide fragments that are bound by DnaK.

ADP phosphorylation assays were performed on the MonoQ fractions of DnaK preceding and following treatment to remove bound nucleotide. MonoQ fraction 13 of the nucleotide-free DnaK preparation, which contains very little DnaK, has the highest ADP kinase activity of all the MonoQ fractions (Fig. 5B). The absolute level of the ADP kinase activity that co-elutes with the single peak, nucleotide-free DnaK is significantly lower than the ADP kinase activity that co-elutes with the multiple peak, untreated DnaK (Fig. 5, A and B). The ADP kinase activity in MonoQ fraction 15, which contains the highest amount of nucleotide-free DnaK, is less than 0.2 pmol of ADP phosphorylated/min/5 μl, nearly 50-fold lower than the 8.98 pmol of ADP phosphorylated/min/5 μl for the MonoQ fraction containing the highest amount of untreated DnaK (Fig. 5, A and B).

To confirm that nucleoside-diphosphate kinase is responsible for the initial burst of [α<sup>-32</sup>P]ADP produced when certain DnaK preparations are incubated with [α<sup>-32</sup>P]ATP, the rate of [α<sup>-32</sup>P]ADP produced by single peak, nucleotide-free DnaK prepared from ndk::km cells (fraction 15 in Fig. 5B) incubated with [α<sup>-32</sup>P]ATP was compared with the rate of [α<sup>-32</sup>P]ADP produced by DnaK prepared from ndk<sup>-</sup> cells incubated with

![Image](http://www.jbc.org/)
NDP Kinase Affects the ATPase Activity of DnaK Preparations

[α-32P]ATP (Fig. 6). No initial burst of [α-32P]ADP was produced by the DnaK preparation with the greatly reduced ADP kinase activity, even when a molar equivalent of unlabeled ADP was added to the incubation mixture (Fig. 6).

The Protein Responsible for the ADP Kinase Activity in DnaK Preparations Purified from ndk::km Cells Is NDP Kinase—We noticed that some preparations of DnaK purified from ndk::km cells had an ADP kinase activity much higher than most of the other ndk::km DnaK preparations. The level of ADP kinase activity in the MonoQ fraction with the highest activity from the preparation with the highest activity was 80,000 pmol of ADP phosphorylated/min/μl sample or nearly as high as the level of activity in the MonoQ fraction with the highest level of activity of DnaK purified from ndk+ cells. Following the treatment to remove nucleotide from the DnaK and MonoQ chromatography, the MonoQ fraction with the highest level of ADP kinase activity was free of DnaK. The fraction with the highest amount of ADP kinase activity, MonoQ fraction 13, phosphorylated ADP at a rate of 15,000 pmol/min/μl sample. A sample of this MonoQ fraction (10 μl) was subjected to SDS-PAGE, and the silver-stained gel revealed a single band of approximately 15 kDa. This sample was subjected to 10 rounds of amino-terminal peptide sequencing. A search of the E. coli genome database revealed that the only DNA sequence in E. coli matching the protein sequence obtained (AIERTFSIIK) is the sequence spanning codons 2–11 of the ndk gene. Therefore, the protein responsible for the residual ADP kinase activity in DnaK preparations purified from ndk::km E. coli cells is NDN kinase. The concentration of NDP kinase in MonoQ fraction 13 was determined by the protein sequencing analysis to be 0.1 μg or 1.6 ng/μl. Assuming that the relationship between ADP kinase activity and the amount of NDP kinase protein is linear, the amount of NDP kinase in nucleotide-free, single MonoQ peak DnaK fractions with low ADP kinase activity (0.05 pmol of ADP phosphorylated/min/μl sample) is approximately 0.03 pg/μl. The kanamycin gene disruption of the ndk gene was created by inserting an EcoRI fragment into the single EcoRI site of the ndk gene and did not include the removal of any segment of the ndk gene (41). Assuming the NDP kinase was present in the ndk::km cells themselves, some sort of genetic rearrangement must have occurred during the growth of the culture for overproduction of DnaK that resulted in the restoration of the ndk gene. Apparently, this event occurred randomly and results in the production of vastly different amounts of NDP kinase between various independent cultures. The problem could presumably be avoided by employing a nonrevertible allele of ndk.

DnaK Has a Weak Physical Interaction with NDP Kinase—A recent publication (52) reports the co-purification of a 16-kDa NDP kinase protein with Hsc70 from eukaryotic cells. The authors also describe a physical interaction, co-immunoprecipitation, and functional interaction, and stimulation of peptide release from Hsc70 between the two proteins (52). We investigated whether or not a physical interaction also exists between E. coli DnaK and NDP kinase.

Affinity chromatography was used to look for evidence of a physical interaction between the two proteins. The ndk gene was cloned by polymerase chain reaction and inserted into the plasmid PET-16b so that it would express NDP kinase with an amino-terminal tag of 10 histidine residues (Ndk-N-His). The pPET-16b/ndk plasmid was transferred into BL21(DE3)pLysS E. coli cells to create the strain TB2000. Proteins containing a stretch of histidine residues bind to Ni2+ ions and can be purified in a single step by IMAC. TB2000 cells were induced to overexpress Ndk-N-His and lysed by sonication. The cell lysate supernatant was applied to a His-Bind IMAC column. The column was washed and eluted with a 30–500 mM imidazole gradient. Shown are Coomassie Brilliant Blue-stained SDS-PAGE gels (top) and Western blots of SDS-PAGE gels identical to the Coomassie Brilliant Blue-stained gels above probed with α-DnaK antibody (bottom). The lanes are labeled as follows: S, molecular mass standards; C, crude lysate supernatant; F, IMAC flow through; W, IMAC wash; numbers, eluent fraction number (fraction 1–30 mxi imidazole to fraction 23–390 mxi imidazole over a linear gradient).

NDP kinase was eluted (Fig. 7) with a 200–500 mM imidazole gradient. Fractions (1 ml each) eluting from the column were collected. Samples of the TB2000 crude lysate supernatant, IMAC flow-through, IMAC wash, and the eluted fractions were run on SDS-PAGE, and proteins were visualized by Coomassie Brilliant Blue staining (Fig. 7). A large amount of Ndk-N-His protein, running at about 16 kDa on SDS-PAGE, is present in eluted fractions 8–23. Another protein, running at about 25 kDa, elutes at a slightly lower imidazole concentration than Ndk-N-His. This protein is likely the α-subunit of succinyl-CoA synthetase, which has been shown to physically interact with NDP kinase (53). A strong physical interaction between DnaK and Ndk-N-His would be indicated by the co-elution of DnaK with Ndk-N-His from the IMAC column. Careful inspection of the Coomassie Brilliant Blue-stained SDS-PAGE gels in Fig. 7 reveals very faint bands at about 70-kDa in fractions 12–16. SDS-PAGE gels identical to those shown in Fig. 7 were run and blotted onto polyvinylidene difluoride membranes for Western blotting. The membranes were probed with α-DnaK antibody. The α-DnaK Western blots are shown in Fig. 7.
small fraction of the DnaK present in the cells co-elutes with Ndk-N-His, as much larger amounts of DnaK appear in the column flow through and column wash lanes of the Western blot (Fig. 7). No DnaK, or any other protein, was detected in the eluent of a IMAC column loaded with the lysate of BL21(DE3)pLysS cells carrying only the vector pET-16b, showing that the DnaK does not interact with the IMAC column, and the DnaK co-elutes with Ndk-N-His bound to the IMAC column because of an interaction with Ndk-N-His itself. However, because of the small fraction of DnaK that binds to Ndk-N-His, the significance of this interaction is uncertain.

**DISCUSSION**

Preparations of *E. coli* DnaK from our lab, which have been purified by a procedure very similar to those employed by numerous other labs, were found to contain an ADP kinase activity that is because of the presence of small amounts of co-purifying NDP kinase. The NDP kinase that co-purifies with DnaK and is responsible for this activity is not unique to our preparations, as we detected the same kinase activity in *E. coli* DnaK samples from several other labs, the 44-kDa amino-terminal ATPase domain of *T. thermophilus*, human HSP70, bovine Hsc70, and hamster BiP. In all, we found ADP kinase activity in preparations from six different laboratories and one commercial source. DnaK purified from *E. coli* cells containing a disruption of the *ndk* gene typically has approximately a 500-fold reduction in the level of co-purifying ADP kinase activity when compared with DnaK purified from *ndk*+ cells. This observation confirms that the ADP kinase activity found in preparations of DnaK is because of the presence of a very small amount of co-purifying NDP kinase.

The implications of the presence of NDP kinase in DnaK preparations are significant. Many discrepancies regarding the characterization of the ATPase activity of DnaK and other HSP70 proteins exist in the literature. NDP kinase, in addition to a γ-phosphate transfer activity, has an ATPase activity with a *k*<sub>cat</sub> value much higher than that of DnaK (51). The rapid kinetics of ATP hydrolysis by NDP kinase makes accurate kinetic measurements of DnaK ATPase impossible in its presence. The presence of differing amounts of undetected NDP kinase in DnaK preparations from different labs could explain, at least in part, the wide range of *k*<sub>cat</sub> values reported for the ATPase activity of DnaK from 0.018 min<sup>-1</sup> (29) to 1.3 min<sup>-1</sup> (54, 55) to 1.0 min<sup>-1</sup> (28). Several labs have also described the existence of differing ATPase rates between different preparations of DnaK within their own lab and different fractions within the same preparation (36). NDP kinase is likely to be responsible for the report of initial burst kinetics by DnaK during ATPase assays (1), similar to our observations, and for the report of an ADP kinase activity by DnaK (2), also similar to our observations. Therefore, the conclusions drawn in these reports, that DnaK hydrolyzes ATP with initial burst kinetics and that DnaK has an ADP kinase or ADP-ATP exchange activity, are erroneous. The NTP hydrolysis activity of NDP kinase is also nucleotide nonspecific (51), and its presence could account for the previous report of GTP, CTP, and UTP hydrolysis by DnaK with rates lower than the rate of ATP hydrolysis (32). The wide range of values for the peptide-, DnaJ-, and GrpE-stimulated rates of DnaK ATPase could also be due, in part, to the presence of a very small amount of copurifying NDP kinase.

The identity of the protein responsible for the remaining ADP kinase activity in preparations of DnaK purified from *ndk::km* cells was initially unknown to us, as we had assumed that the kanamycin gene insertion in the *ndk* gene resulted in a complete knock-out of NDP kinase production. However, the presence of abnormally high levels of ADP kinase activity in some *ndk::km* preparations together with our ability to effectively resolve the ADP kinase activity from DnaK following the treatment of DnaK to remove nucleotide, allowed us to collect enough pure protein to allow amino-terminal protein sequencing. Surprisingly, the protein responsible for the residual ADP kinase activity was found to be NDP kinase. Southern blot analysis of the NA7623 strain, which contains the *ndk::km* disruption, showed that the *ndk* gene was effectively disrupted and no duplication of the *ndk* gene was present in the strain (41). We postulate that because the *ndk::km* disruption only involves an insertion into the *ndk* gene and no deletion within the gene itself some low frequency genetic event occurs randomly that results in the restoration of the *ndk*+ gene.

The protein sequencing analysis of the NDP kinase protein also provided a value for the absolute amount of the protein in a sample for which we had determined the level of ADP kinase activity and allowed us to calculate a value for the turnover rate of approximately 500 s<sup>-1</sup> using our assay conditions. This value corresponds well with previously reported *k*<sub>cat</sub> values for bacterial NDP kinases (51). It also allowed us to calculate a value of ~10 ng/μl for the amount of NDP kinase in the fraction of DnaK purified from *ndk*+ cells with the highest ADP kinase activity. This value was typically lowered to ~20 pg/μl when DnaK was purified from *ndk::km* cells and ~0.3 pg/μl following the nucleotide-removal treatment of DnaK purified from *ndk::km* cells. NDP kinase is able to use any NTP or dNTP as a phosphate donor substrate and any NDP or dNDP as a phosphate acceptor substrate. Its presence therefore explains the nonspecific nature with regard to phosphate acceptor substrate that we observed.

NDP kinase is ubiquitous and highly conserved. *E. coli* NDP kinase and human NDP kinase are 43% identical in their amino acid sequences (50). NDP kinases from different organisms are also remarkably similar in their tertiary protein structures (56). It is therefore likely that NDP kinases from different organisms would behave similarly during various chromatographic separations because of their similar physical properties; just as highly conserved HSP70 proteins from different organisms behave similarly during different chromatographic separations. NDP kinase binds ATP with high affinity (51) and would therefore be likely to co-elute with DnaK from an ATP-agarose column. NDP kinase has been shown to elute from a MonoQ anion-exchange column at a lower KCl concentration than HSP70 (2), but trailing of the NDP kinase peak into the HSP70 peak could compromise the success of this separation. *E. coli* NDP kinase forms stable tetramers with a molecular mass of 66 kDa (57), and thus gel filtration chromatography would also be ineffective at separating NDP kinase from the 69.1-kDa DnaK. During the course of this study, it was reported that a 16-kDa NDP kinase protein co-purifies and co-immunoprecipitates with Hsc70 purified from eukaryotic cells (52). Such a physical interaction, if it existed, between *E. coli* DnaK and NDP kinase would make a complete separation of the two proteins, even based upon significant physical differences, difficult.

DnaK has a weak physical interaction with His-tagged NDP kinase. The significance of this interaction is not known. DnaK, because it is a molecular chaperone, binds a wide range of substrate peptides (58). The weak interaction with His-tagged NDP kinase could simply result from the nonspecific binding of the chaperone to a second protein. Alternatively, it could be the result of a weak but specific interaction between the two proteins. It is also not known what effect the histidine tag on the NDP kinase protein has on this interaction and whether DnaK would bind unmodified NDP kinase with higher, lower, or equal affinity. However, DnaK is one of only two proteins that...
can be detected by a Coomassie Brilliant Blue-stained SDS-PAGE gel to co-elute with His-tagged NDP kinase. If the interaction of the proteins in the cytoplasm is specific and physiologically relevant, perhaps NDP kinase modulates the functioning of DnaK as a 16-kDa NDP kinase protein has been suggested to modulate Hac70 (52). An alternative hypothesis is suggested by the observation that NDP kinase and an ATPase from Mycobacterium bovis cells (59). E. coli DnaK forms a stable complex with NDP kinase from M. bovis,3 and this interaction could be related to the secretion of these two proteins. The physical interaction between E. coli DnaK and NDP kinase, regardless of its significance, may be responsible for the co-purification of small but significant amounts of NDP kinase in DnaK preparations.

Acknowledgments—We thank Bernd Bukau for discussing the problems that NDP kinase contamination has previously presented in assays of other ATPase proteins. We are grateful to colleagues in the field for generously providing DnaK, HSP70, and BiP preparations and to Masayori Inouye for providing E. coli strains NA7623 and JC7623 to us. We thank the members of our lab for helpful discussions.

REFERENCES


3 A. M. Chakrabarty, personal communication.
