Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5883–5887, June 1994
Biochemistry

Characterization of nucleoside-diphosphate kinase from *Pseudomonas aeruginosa*: Complex formation with succinyl-CoA synthetase

(transition to mucoidy/cellular development/energy metabolism)

ANDREW KAVANAUGH-BLACK, DENNIS M. CONNOLLY, SUDHA A. CHUGANI, AND A. M. CHAKRABARTY*

Department of Microbiology and Immunology (M/C 790), University of Illinois College of Medicine, 835 South Wolcott Avenue, Chicago, IL 60612

Communicated by I. C. Gunsalus, March 4, 1994

ABSTRACT **The enzyme nucleoside-diphosphate kinase (Ndk), responsible for the conversion of (deoxy)ribonucleoside diphosphates to their corresponding triphosphates, has been purified from *Pseudomonas aeruginosa*. The N-terminal 12 amino acid sequence of *P. aeruginosa* Ndk shows significant homology with that of *Myxococcus xanthus* and that of *Escherichia coli*. Ndk enzyme activity is also associated with succinyl-CoA synthetase activity in *P. aeruginosa*, whose α and β subunits show extensive sequence homology with those of *E. coli* and *Dictyostelium discoideum*. The 33-kDa α subunit of succinyl-CoA synthetase of *P. aeruginosa* appears to undergo autophosphorylation in the presence of either ATP or GTP, although the presence of small amounts of Ndk activity may influence the level of such phosphorylation.**

Nucleoside-diphosphate kinase (ATP:nucleoside-diphosphate phosphotransferase, EC 2.7.4.6; Ndk) is an essential enzyme for the maintenance of the correct cellular ratios of NTPs and dNTPs and is involved in regulation of cellular development. Ndk catalyzes the reversible transfer of the 5′-terminal phosphate from NTPs to NDPs by a ping-pong enzyme mechanism, summarized as follows: $N_1TP + N_2DP \rightleftharpoons N_1DP + N_2TP$ (1). The enzyme utilizes an autophosphorylated enzyme intermediate (2) with very little substrate specificity for either the base or the sugar utilized. The enzyme catalyzes the final step in NTP and dNTP synthesis, converting γ-phosphate bond energy (in the form of ATP) from oxidative phosphorylation into synthesis of DNA and RNA precursors and appears to be essential to growth of most types of cells under aerobic conditions (3). Although the enzyme pyruvate kinase of *Escherichia coli* can compensate for loss of Ndk activity in anaerobically grown cells (4), attempts to isolate deletion mutants of the *ndk* gene in *Myxococcus xanthus* have been unsuccessful, further substantiating the essential role of the enzyme in its cell growth (5).

Ndk has also been implicated in regulating or effecting developmental changes in eukaryotic cells. Reduced transcript levels for the human Ndk gene called nm23 were found to be associated with lowered metastatic potential in tumor cells (6, 7). Expression of nm23 from a constitutive promoter in highly metastatic murine tumor cells was found to actually suppress tumor metastasis (8). Null mutations in the Ndk gene of *Drosophila*, named *awd*, cause abnormalities in development of the larvae leading to tissue necrosis and death at the prepupal stage (7, 9). In the slime mold *Dictyostelium discoideum*, the Ndk gene is developmentally regulated with a sharp decrease in Ndk transcript levels coinciding with the onset of the starvation induced developmental cycle (10, 11). A gene encoding a DNA-binding protein, PuF, which is required for transcription of c-*myc in vitro*, is highly homologous to the human Ndk gene nm23-H2 (12). This implies that nm23-H2 may be involved in the regulation of c-*myc*.

Pseudomonas aeruginosa is a ubiquitous soil and waterborne bacterium which undergoes a transition to mucoidy under stress and/or starvation conditions (13, 14). We have purified Ndk from *P. aeruginosa* in order to study its involvement in these developmental changes and have found that Ndk is associated and copurifies with the ATP-utilizing prokaryotic succinyl-CoA synthetase (Scs) complex. These results indicate that aside from its role in DNA and RNA precursor synthesis, Ndk may be involved in regulating intracellular energy metabolism and that understanding its role in energy metabolism may be critical to understanding its involvement in regulation of development.

MATERIALS AND METHODS

Detection of [γ-^{32}P]ATP-Autophosphorylated Proteins. Samples were added to TMD buffer (50 mM Tris·HCl, pH 7.5/1 mM $MgCl_2$/0.2 mM dithiothreitol) in ≤19 μl and brought to a final volume of 20 μl with TMD buffer. [γ-^{32}P]ATP (1 μl; 74 MBq/ml) was added to the sample (final ATP concentration, 20 nM) and reactions were allowed to proceed for at least 6 sec. Reactions were terminated by addition of 4× SDS gel running buffer [0.2 M Tris·HCl, pH 6.8/0.62% dithiothreitol/8% SDS/0.01% bromophenol blue/40% (vol/vol) glycerol] and analyzed by electrophoresis in an SDS/polyacrylamide gel (3% stacking gel, 15% separating gel).

Purification of Ndk from *P. aeruginosa* strain 8822. Strain 8822 was grown overnight in Luria broth containing 0.1% glucose, and a crude cell extract was prepared from 4 liters of culture by French press disruption and ultracentrifugation at 300,000 × *g* for 1 hr. The supernatant was subjected to $(NH_4)_2SO_4$ fractionation at 45% and 65% saturation (2), and precipitates at 65% were suspended in 9.6 ml of TMD buffer containing 1 M $(NH_4)_2SO_4$ (pH 7.5) and injected into a Bio-Gel TSK-phenyl-5-PW column (Bio-Rad). Wash-through fractions were collected, dialyzed extensively against MMD buffer (25 mM Mops, pH 8.0/1 mM $MgCl_2$/0.2 mM dithiothreitol), and loaded onto an Econo-Pak S cation-exchange column (Bio-Rad). Wash-through fractions containing the 16-kDa autophosphorylatable protein were loaded onto a Q Sepharose column (Pharmacia) and eluted with a 0–1 M NaCl gradient at 120–165 mM. These fractions were concentrated and injected onto a Superose 12 gel filtration column (Pharmacia) running with TMD buffer. Fractions containing the 16-kDa protein (Ndk) were collected and pooled. For N-ter-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ndk, nucleoside-diphosphate kinase; Scs, succinyl-CoA synthetase.

*To whom reprint requests should be addressed.

minal sequence determination, purified Ndk was isolated by SDS/15% polyacrylamide gel electrophoresis and electroblotted to Problott membranes (Applied Biosystems) with a Bio-Rad Trans-Blot unit. The N-terminal sequence was determined independently by both Ka-Leung Ngai (University of Illinois, Urbana) and Joe Leykam (Michigan State University Protein Sequence Facility, East Lansing).

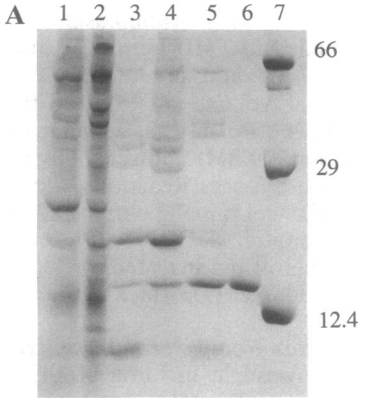
Biochemical Characterization of Ndk Activity. Ndk enzyme activity was measured as described (2). In brief, 76 ng of purified Ndk was added to 10 μCi (37 kBq) of [γ-^{32}P]ATP or [γ-^{32}P]GTP. An equimolar quantity of nonradioactive ADP, GDP, or UDP was added along with Ndk. Reactions were terminated after 30 sec by the addition of EDTA to 5 mM. Samples were analyzed on PEI-cellulose thin-layer chromatography plates (Sigma) with 0.75 M potassium phosphate (pH 3.65) in the mobile phase; autoradiography was used to visualize the radioactive NTPs.

Purification of Scs from *P. aeruginosa* 8822. Sixty-five percent $(NH_4)_2SO_4$ precipitates obtained from the cell-free extracts were resuspended in TMD buffer containing 20% glycerol (TMDG) and 1 M $(NH_4)_2SO_4$ (pH 7.5) and then loaded onto the TSK-phenyl column. Fractions containing the Scs fraction, unlike the Ndk fractions, bound to the column and were eluted with a gradient of 0–100% TMDG over 100 ml. These fractions were collected, dialyzed against MMDG (MMD buffer containing 20% glycerol), and loaded onto the Q-Sepharose anion-exchange column. Fractions containing Scs were eluted with a 0–1 M NaCl gradient at approximately 120–220 mM, pooled, exchanged into P_{10}MDG buffer (10 mM sodium phosphate, pH 7.2/1 mM $MgCl_2$, 0.2 mM dithiothreitol/20% glycerol), and loaded onto a Bio-Rad Econo-Pak HTP column. Bound Scs protein was eluted with 0–100% P_{500}MDG buffer (500 mM sodium phosphate, pH 6.8/1 mM $MgCl_2$/0.2 mM dithiothreitol/20% glycerol) over 100 ml. Fractions which contained the Scs activity were concentrated and injected into a Superose 12 gel filtration column equilibrated with TMDG. Fractions containing active Scs were eluted at ≈150 kDa. These were pooled, and the N-terminal sequence was determined as described for purified Ndk.

Phosphorylation of Scs. Six hundred twenty-six nanograms of the purified Scs complex was incubated for 5 min with 500 nM succinyl-CoA and inorganic [^{32}P]phosphate ranging from 55 to 550 mM. Reactions were terminated with 4× SDS gel running buffer and the reaction products were loaded onto an SDS/polyacrylamide gel (3% stacking gel, 15% resolving gel). ^{32}P labeling of p33 (α subunit of Scs) was quantitated on an AMBIS radioanalytic imaging system (AMBIS Systems).

Dephosphorylation of Scs. Three hundred thirty-six nanograms of purified Scs complex was phosphorylated by incubation for 2 min with 2 μCi of [γ-^{32}P]ATP in TMD buffer, followed by incubation for 2 min with equimolar concentrations of succinate and coenzyme A. Reactions were terminated with 4× SDS gel running buffer, and ^{32}P remaining on p33 (α subunit of Scs) was quantitated as described above.

Biochemical Characterization of Ndk Activity Associated with Purified Scs Complex. Either 182 ng of purified Ndk or 626 ng of purified Scs complex was added to [γ-^{32}P]ATP at 50 μCi/ml. GDP, CDP, or UDP was added to 5 mM in 4-μl reaction mixtures, and the reaction was terminated by addition of 2 μl of 50 mM EDTA after 5 min for Ndk and 4 hr for Scs complex. One microliter from each reaction was then spotted onto thin-layer chromatography plates and ^{32}P-labeled species were separated as described above for characterization of Ndk activity.



B

P. aeruginosa	M	A	L	Q	R	T	L	S	I	I	K	P	
	:	:	.	:	:	:	:	:	:	:	:	:	
M. xanthus	M	A	I	E	R	T	L	S	I	I	K	P	
	:	:	:	:	:	:	.	:	:	:	:	:	
E. coli	M	A	I	E	R	T	F	S	I	I	K	P	

FIG. 1. Purification of Ndk from *P. aeruginosa* and comparison of its N-terminal sequence with Ndk isolated from *M. xanthus* and *E. coli*. (*A*) Ndk was purified from *P. aeruginosa* 8822 as described in *Materials and Methods*. Thirty-six micrograms of total protein was taken following each purification step and analyzed by SDS/15% polyacrylamide gel electrophoresis followed by staining with Coomasie brilliant blue R-250 for detection of total proteins present. Lane 1, crude cell extract; lane 2, 65% $(NH_4)_2SO_4$ precipitation; lane 3, hydrophobic interaction chromatography; lane 4, cation-exchange chromatography; lane 5, anion-exchange chromatography; lane 6, gel filtration chromatography; lane 7, protein markers of indicated sizes in kilodaltons. (*B*) The *N*-terminal sequence of the isolated 16-kDa protein is identical at 10 out of 12 and 9 out of 12 residues to the Ndk proteins reported from *M. xanthus* (5) and *E. coli* (15), respectively.

RESULTS

p16 Shares a High Degree of Protein Sequence Homology with Ndk Proteins of *M. xanthus* and *E. coli*. *P. aeruginosa* undergoes a developmental change to a mucoid form induced by conditions of stress and/or starvation (13, 14). It has been observed that in several mutant lines of mucoid *P. aeruginosa*, there is a decrease in autophosphorylation levels for a 16-kDa protein (p16) which is concurrent with a loss in the mucoid phenotype of the bacterium. To investigate the nature of this p16 protein, we purified the protein from the nonmu-

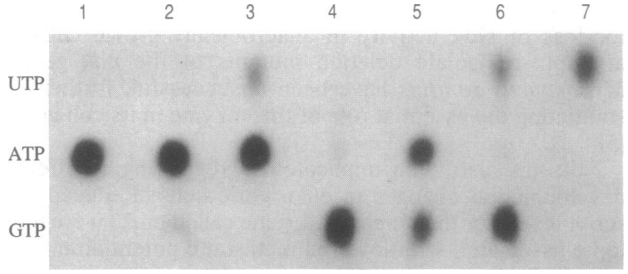


FIG. 2. The 16-kDa protein is a Ndk based upon its biochemical properties. The purified 16-kDa protein when phosphorylated by either ATP or GTP can transfer the γ-terminal phosphate to either GDP, UDP, or ADP. Ndk was added to [γ-^{32}P]ATP (lanes 1–3) or [γ-^{32}P]GTP (lanes 4–6). After addition of an equimolar concentration of GDP (lane 2), UDP (lanes 3 and 6), or ADP (lane 5), reactions were terminated and samples were analyzed by thin-layer chromatography followed by autoradiography to show the formation of ^{32}P-labeled GTP (lane 2), UTP (lanes 3 and 6), or ATP (lane 5) from the nonradioactive NDPs. As a control for the migration of UTP, [α-^{32}P]UTP was loaded alone in lane 7, while control [γ-^{32}P]ATP and [γ-^{32}P]GTP are shown in lanes 1 and 4. The labeled GTP band in lane 2 is somewhat faint and is not clearly visible in the photoreproduction.

coid strain 8822. The purification scheme is presented in Fig. 1*A*. The N-terminal sequence of the purified protein was determined and compared with other protein sequences reported in the ATLAS database through FASTA (16). p16 was found to match at 10 out of 12 amino acids the Ndk of *M. xanthus* (5, 17) and at 9 out of 12 amino acids the Ndk isolated from *E. coli* (3, 15) (Fig. 1*B*).

Biochemical Characterization of p16 as a NdK. The homology of p16 to these Ndk proteins suggested that it might be a Ndk. To confirm this, the biochemical activity of p16 was determined. Ndk enzymes are biochemically defined by their ability to catalyze the transfer of the γ phosphate from any NTP to any other NDP (1). Purified p16 was shown to be able to catalyze the transfer of the γ phosphate from either [γ-^{32}P]ATP or [γ-^{32}P]GTP to either GDP, UDP, or ADP (Fig. 2), which confirms that p16 is a nucleoside diphosphate kinase, and will hereafter be referred to as the Ndk from *P. aeruginosa*.

Ndk Copurifies with a Phosphorylated 33-kDa (p33) Protein. During purification of Ndk it was noted that p16 was copurifying with a 33-kDa protein which could be phosphorylated by both [γ-^{32}P]ATP and [γ-^{32}P]GTP. Fractions were collected from both the washthrough and bound eluates (Fig. 3*B*) from a TSK-phenyl hydrophobic interaction HPLC column loaded with a partially purified crude cell extract from *P. aeruginosa*

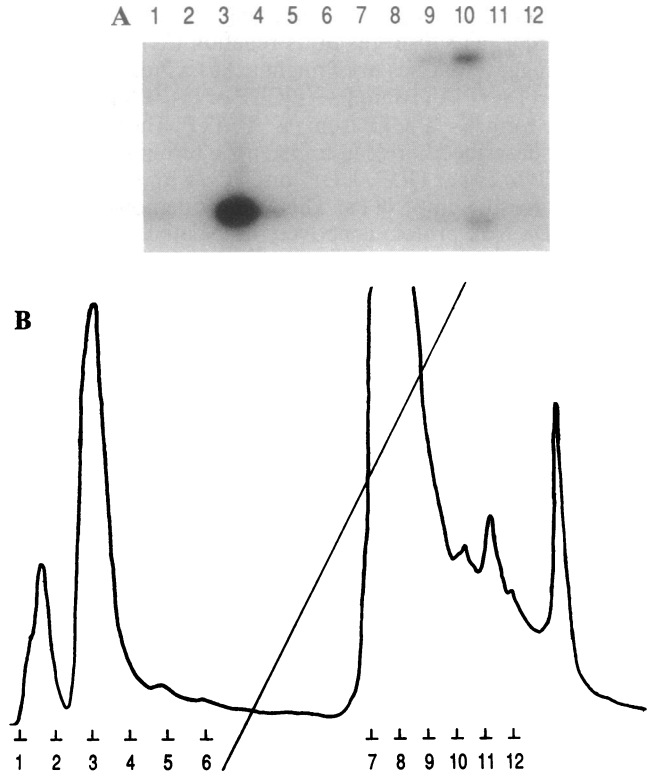
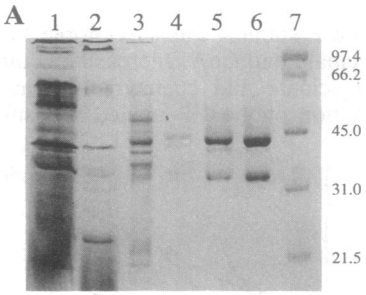


FIG. 3. Ndk copurifies with a 33-kDa [γ-^{32}P]ATP-phosphorylated protein. (*A*) Partially purified cell extracts from *P. aeruginosa* 8822 were loaded onto a TSK-phenyl column (as described in *Materials and Methods*), and fractions were collected during wash through and elution of protein. A phosphorylation assay was performed on each fraction collected. Samples from each fraction were incubated for 6 sec with 20 nM of [γ-^{32}P]ATP, and the reaction mixture was analyzed by SDS/15% PAGE followed by autoradiography. The sizes of the phosphorylated protein bands are 16 kDa (lower) and 33 kDa (upper), respectively. Proteins that did not bind to the TSK-phenyl column are present in fractions 1–6, while bound proteins were eluted during the continuous gradient of 0–100% elution buffer in fractions 7–12. (*B*) The protein profile (absorbance at 280 nm) is represented with numbers below to represent the fraction numbers assayed, with the elution gradient beginning at fraction 6.

8822, and a [γ-^{32}P]ATP phosphorylation assay was performed on each fraction. As seen in the autoradiogram of [γ-^{32}P]ATP-phosphorylated proteins isolated from an SDS/15% polyacrylamide gel (Fig. 3*A*), phosphorylated Ndk was present in two sets of eluted fractions. The first peak of active Ndk (16 kDa) appeared in the washthrough fraction from the column, while a second peak of active Ndk bound to the column and eluted along with the 33 kDa [γ-^{32}P]ATP phosphorylated protein. The second Ndk peak appeared after ≈120 ml of binding and elution buffer had washed through the column and was therefore not due to a tail from the first Ndk peak. Ndk was never found bound to the TSK-phenyl column without being associated with the p33 protein peak. We decided to purify this p33 protein in order to identify its N-terminal amino acid sequence and any potential enzymatic activity.

p33 Is Homologous at its N Terminus to the Scs Proteins of *D. discoideum* and *E. coli*. The purification profile for the Ndk-associated complex is presented in Fig. 4*A*. Final purification revealed a two-subunit complex composed of 33- and 43-kDa subunits as measured by SDS/PAGE. The N-terminal amino acid sequence was determined for the two subunits and compared with the amino acid sequences reported in the ATLAS database as described for Ndk. As shown in Fig. 4*B*, the N-terminal sequence of the isolated 33-kDa protein subunit was found to be identical at 11 out of 12 residues to the α subunit of Scs isolated from *D. discoideum* (18) and at 9 out of 12 residues to the α subunit of Scs isolated from *E.*



B

α Subunits

P. aeruginosa					S	V	L	I	N	K	D	T	K	V	I	I
D. discoideum	D	T	K	P	S	V	L	I	N	K	X	T	K	V	I	I
E. coli				M	S	I	L	I	D	K	N	T	K	V	I	I

β Subunits

P. aeruginosa	M	N	L	H	E	Y	Q	G	K	Q	L	F	A	E	Y
E. coli	M	N	L	H	E	Y	Q	A	K	Q	L	F	A	R	Y

FIG. 4. Purification of Scs complex from *P. aeruginosa* and comparison of its N-terminal sequence with Scs from *D. discoideum* and *E. coli*. (*A*) The Scs complex was purified from *P. aeruginosa 8822*. Ten micrograms of total protein was taken following each purification step and analyzed by SDS/15% PAGE followed by staining with Coomasie brilliant blue R-250 for detection of total proteins present. Lane 1, crude cell extract; lane 2, 65% $(NH_4)_2SO_4$ precipitation; lane 3, hydrophobic interaction chromatography; lane 4, cation-exchange chromatography; lane 5, anion-exchange chromatography; lane 6, gel filtration chromatography; lane 7, markers of indicated sizes in kilodaltons. (*B*) The N-terminal sequence of the isolated 33-kDa protein is identical at 11 out of 12 and 9 out of 12 residues to the α subunit of Scs proteins isolated from *D. discoideum* (18) and *E. coli* (19), respectively. The N-terminal sequence of the isolated 43-kDa protein is identical at 13 out of 15 residues to the β subunit of the Scs protein isolated from *E. coli* (19).

coli (19). The 43-kDa subunit (Fig. 4*B*) was identical at 13 out of 15 residues to the β subunit of Scs isolated from *E. coli* (19).

The Isolated 33- and 43-kDa Proteins Are the α and β Subunits of Scs. The Scs complex catalyzes the following overall reaction (20) in three partial reactions.

$$\text{Scs} + \text{ATP} \rightleftharpoons \text{Scs}\text{–}P + \text{ADP}$$

$$\text{Scs}\text{–}P + \text{succinate} \rightleftharpoons \text{Scs–succinyl-}P$$

$$\text{Scs–succinyl-}P + \text{CoA} \rightleftharpoons \text{succinyl-CoA} + P_i + \text{Scs}$$

$$\text{ATP} + \text{succinate} + \text{CoA} \rightleftharpoons \text{ADP} + P_i + \text{succinyl-CoA}$$

The *E. coli* enzyme (succinate–CoA ligase, EC 6.2.1.5; Scs) has an $(\alpha\beta)_2$ tetramer structure, with molecular masses of 29,500 Da for the α subunit and 38,500 Da for the β subunit. The α subunit contains an autophosphorylatable histidine residue, whereas the β subunit contains the binding sites for succinate and CoA (20). To confirm that p33 is the α subunit of Scs, we phosphorylated the purified $\alpha\beta$ complex by incubating it with [^{32}P]P_i and succinyl-CoA. After 5 min, reactions were terminated, the products were loaded onto SDS/polyacrylamide gels, and the extent of ^{32}P labeling of p33 was determined. Increasing concentrations of [^{32}P]P_i resulted in increased incorporation of ^{32}P into p33 (Fig. 5*A*). The p33 protein was not phosphorylated by incubation with [^{32}P]P_i in the absence of succinyl-CoA or in the presence of malonyl-CoA or acetyl-CoA.

Next it was determined whether the [γ-^{32}P]ATP-phosphorylated α subunit was susceptible to dephosphorylation by succinate and CoA. Increasing equimolar concentrations of succinate and coenzyme A caused increased dephosphorylation of the ^{32}P-labeled p33 subunit (Fig. 5).

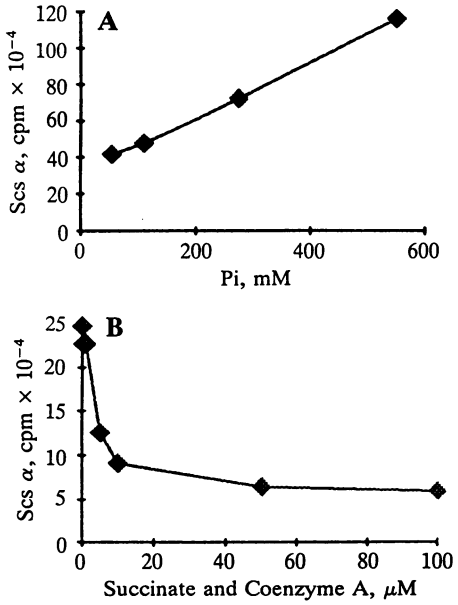


FIG. 5. p33 is a Scs. (*A*) The 33-kDa/43-kDa complex is autophosphorylated by incubation with [^{32}P]P_i and succinyl-CoA. Reactions mixtures containing 626 ng of the complex and 500 nM succinyl-CoA were incubated with increasing concentrations of [^{32}P]P_i for 5 min, terminated, and analyzed by SDS/PAGE followed by quantitation of ^{32}P incorporation into p33 as described in *Materials and Methods*. (*B*) ^{32}P-labeled p33 is dephosphorylated by incubation with equimolar concentrations of succinate and CoA. The 33-kDa/43-kDa complex was autophosphorylated with 2 μCi of [γ-^{32}P]ATP for 2 min and then incubated with increasing concentrations of succinate and CoA for 2 min. Reactions were terminated and analyzed for ^{32}P incorporation into p33 as described above.

Incubation of the ^{32}P-labeled p33 subunit with either succinate or coenzyme A alone was less effective in dephosphorylation. The phosphorylated p33 protein (α subunit of Scs) was completely stable over the time period of the assay in the absence of either succinate or CoA.

Biochemical Activity of Ndk Is Present in the Purified Scs Complex. During purification of the Scs complex, the Ndk protein continued to be associated with the Scs complex, as observed by [γ-^{32}P]ATP phosphorylation assays. A further proof of the association of Ndk in a complex with succinyl-CoA synthetase is that purified Ndk, when mixed with purified succinyl-CoA synthetase, was eluted in a broad peak at a higher apparent molecular weight from a Superose 12 FPLC column than was Ndk alone. This indicates that Ndk must form a complex with Scs. Since the Ndk/Scs complex is unstable, it is eventually disrupted; still, the temporal complex formed between Ndk and Scs causes Ndk to migrate with the larger Scs, which thus increases its apparent molecular weight during elution from the column. In the final purified Scs complex preparation, the level of Ndk present was not enough to observe by Coomassie blue staining or by autophosphorylation with [γ-^{32}P]ATP. However, the presence of Ndk in even this very pure preparation is confirmed (Fig. 6) by its ability to transfer the γ-[^{32}P] phosphate from ATP to either GDP, CDP, or UDP. Succinyl-CoA synthetase has been variously described as utilizing ATP alone as a substrate in catalysis (21) or as utilizing ATP, GTP, and ITP, in that order, as substrates in its reaction (22). These latter reports suggest that Scs itself might act as a Ndk in its ability to convert [γ-^{32}P]ATP into [γ-^{32}P]GTP or [γ-^{32}P]ITP, yet was unable to form [γ-^{32}P]CTP from [γ-^{32}P]ATP. The purified Scs complex described here can transfer the terminal phosphate from ATP to either GDP, UDP, or CDP, with the formation of the corresponding NTPs. This clearly demonstrates that the 16-kDa Ndk protein is actively associated with Scs in a

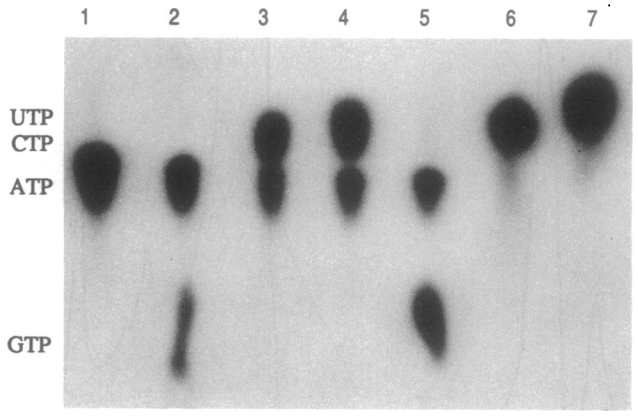


FIG. 6. Ndk activity remains associated with the purified Scs complex. The biochemical activity characteristic of Ndk is present in the purified preparations of Scs complex. Six hundred twenty-six nanograms of purified Scs complex (lanes 2–4) was added to [γ-^{32}P]ATP (lanes 1–7) at 50 μCi/ml. GDP (lanes 2 and 5), CDP (lanes 3 and 6), or UDP (lanes 4 and 7) was added to 5 mM and reactions were terminated after 4 hr by the addition of EDTA to 5 mM. Samples were then analyzed by thin-layer chromatography followed by autoradiography to show the formation of γ-^{32}P-labeled GTP, CTP, or UTP from the nonradioactive NDPs. As a control for the migration of either ATP, GTP, CTP, or UTP, lane 1 contained [γ-^{32}P]ATP alone, while lanes 5–7 contained [γ-^{32}P]ATP along with 182 ng of highly purified Ndk added to the respective NDP in order to transfer the γ-phosphate to either GDP (lane 5), CDP (lane 6), or UDP (lane 7), as already described (5). After 5 min, reactions were terminated with EDTA, and samples were analyzed as above. The partial conversion of [γ-^{32}P]ATP to [^{32}P]GTP, [^{32}P]CTP, and [^{32}P]UTP in lanes 2, 3, and 4 appears to demonstrate the presence of small amounts of Ndk in the 626 ng of purified Scs complex.

complex and is responsible for the Ndk activity reported for this purified protein complex.

DISCUSSION

The binding of Ndk to the Scs complex shown here indicates an *in vivo* association between these proteins, forming an Ndk/Scs metabolon (23) which can facilitate the channeling of γ-phosphate bond energy from NTPs into the tricarboxylic acid cycle, as well as facilitating the reverse reaction. This is to our knowledge the first report of such an association existing between Ndk and Scs in a prokaryotic organism. In eukaryotes, rabbit mitochondrial Ndk has been found to copurify with the mitochondrial Scs within glycerol gradients. Addition of purified rabbit cytosolic Ndk, along with catalytic quantities of GDP, was found to increase the activity of the Scs complex, possibly by channeling activated γ-phosphate from ATP into the eukaryotic GTP-requiring Scs enzyme (24). Also, Ndk was found to be associated with the purified Scs preparations from *D. discoideum* and is thought to be responsible for the stimulation of [γ-^{32}P]ATP labeling of mitochondrial Scs in the presence of small quantities of GDP (18). Since the *E. coli* Scs complex preferentially uses ATP, the presence of an *in vivo* Ndk/Scs complex in *P. aeruginosa* raises the question of whether Ndk might not function by directly activating the Scs complex by γ-phosphate bond energy from any NTP.

If this is true, then the involvement of Ndk in energy metabolism and the developmental regulation of *ndk* found in eukaryotes may be related. The presence of Ndk within rat liver mitochondria is known to enhance the formation of ATP during oxidative respiration (25), while the level of [γ-^{32}P]ATP or [γ-^{32}P]GTP phosphorylation of the succinyl-CoA synthetase from *D. discoideum* has been found to decrease during starvation-induced developmental changes (18). These starvation conditions are also cited as inducing a sharp decrease in *ndk* transcript levels in *D. discoideum* (10, 11). It may be that regulation of *ndk* during developmental changes is part of a common prokaryotic–eukaryotic stress/starvation regulatory mechanism which induces fundamental changes in function of the tricarboxylic acid cycle during various stress or starvation conditions.

The association of small amounts of Ndk even in the highly purified Scs complex raises interesting questions about the nature of phosphorylation of the α subunit (p33) of succinyl-CoA synthetase. While this subunit is believed to be autophosphorylated (20–22), it is equally possible that Ndk may act as a protein kinase, directly phosphorylating the α subunit of Scs in *P. aeruginosa*, or by activating the Scs complex by channeling ATP into the active site of the *P. aeruginosa* Scs enzyme. Since we have a mutant of *P. aeruginosa* which shows a much reduced level of phosphorylation of the p16 Ndk, it would be of interest to see how Ndk levels are modulated in the cells and how this affects the efficiency of the tricarboxylic acid cycle, thereby affecting *P. aeruginosa*'s transition to mucoidy.

This work was supported by U.S. Public Health Service Grant AI31546 and in part by Grant AI16790-14 from the National Institutes of Health. A.K.-B. and D.M.C. were supported by fellowships from the Cystic Fibrosis Foundation (F508 and F234, respectively).

1. Parks, R. E. & Agarwal, R. P. (1973) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), pp. 307–333.
2. Muñoz-Dorado, J., Almaula, N., Inouye, S. & Inouye, M. (1993) *J. Bacteriol.* **175**, 1176–1181.
3. Ray, N. B. & Mathews, C. K. (1992) *Curr. Top. Cell Regul.* **33**, 343–357.
4. Saeki, T., Hori, M. & Umezawa, H. (1973) *J. Biochem. (Tokyo)* **76**, 631–637.
5. Muñoz-Dorado, J., Inouye, M. & Inouye, S. (1990) *J. Biol. Chem.* **265**, 2702–2706.
6. Steeg, P. S., Bevilacqua, G., Kopper, L., Thorgeirsson, U. P., Talmadge, J. E., Liotta, L. A. & Sobel, M. E. (1988) *J. Natl. Cancer Inst.* **80**, 200–204.
7. Rosengard, A. M., Krutzsch, H. C., Shearn, A., Biggs, J. R., Barker, E., Margulies, I. M. K., King, R. C., Liotta, L. A. & Steeg, P. S. (1989) *Nature (London)* **342**, 177–180.
8. Leone, A., Flatow, U., King, R. C., Sandeen, M. A., Margulies, I. M. K., Liotta, L. A. & Steeg, P. S. (1991) *Cell* **65**, 25–35.
9. Biggs, J., Hersperger, E., Steeg, P. S., Liotta, L. A. & Shearn, A. (1990) *Cell* **63**, 933–940.
10. Wallet, V., Mutzel, R., Troll, H., Barzu, O., Wurster, B., Veron, M. & Lacombe, M. L. (1990) *J. Natl. Cancer Inst.* **82**, 1199–1202.
11. Lacombe, M. L., Wallet, V., Troll, H. & Veron, M. (1990) *J. Biol. Chem.* **265**, 10012–10018.
12. Postel, E., Berberich, S. J., Flint, S. J. & Ferrone, C. A. (1993) *Science* **261**, 478–480.
13. May, T. B., Shinabarger, D., Maharaj, R., Kato, J., Chu, L., Devault, J. D., Roychoudhury, S., Zielinski, N. A., Berry, A., Rothmel, R. K., Misra, T. K. & Chakrabarty, A. M. (1991) *Clin. Microbiol. Rev.* **4**, 191–206.
14. Terry, J. M., Piña, S. E. & Mattingly, S. J. (1992) *Infect. Immun.* **60**, 1329–1335.
15. Hama, H., Almaula, N., Lerner, C. G., Inouye, S. & Inouye, M. (1991) *Gene* **105**, 31–36.
16. Pearson, W. R. (1990) *Methods Enzymol.* **193**, 63–98.
17. Muñoz-Dorado, J., Inouye, S. & Inouye, M. (1990) *J. Biol. Chem.* **265**, 2707–2712.
18. Anschutz, A. L., Um, H.-D., Siegel, N. R., Veron, M. & Klein, C. (1993) *Biochim. Biophys. Acta* **1162**, 40–46.
19. Buck, D., Spencer, M. E. & Guest, J. R. (1985) *Biochemistry* **24**, 6245–6252.
20. Bridger, W. A. (1984) *Curr. Top. Cell Regul.* **24**, 345–355.
21. Gibson, J., Upper, C. D. & Gunsalus, I. C. (1967) *J. Biol. Chem.* **242**, 2474–2477.
22. Murakami, K., Mitchell, T. & Nishimura, J. S. (1972) *J. Biol. Chem.* **247**, 6247–6252.
23. Srivastava, D. K. & Bernhard, S. A. (1986) *Science* **234**, 1081–1086.
24. Kadrmas, E. F., Ray, P. D. & Lambeth, D. O. (1991) *Biochim. Biophys. Acta* **1074**, 339–346.
25. Pedersen, P. L. (1973) *J. Biol. Chem.* **248**, 3956–3962.