Journal of Bioenergetics and Biomembranes, Vol. 32, No. 3, 2000

The Binding Mode of Human Nucleoside Diphosphate Kinase B to Single-Strand DNA

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Received March 8, 2000; accepted May 12, 2000

In this paper, we studied the interaction of the human isoform B of nucleoside diphosphate kinase (NDP kinase B) with the *n*uclease *h*ypersensitive *e*lement (NHE) present in the promoter element of the c-*myc* oncogene. The DNA-binding properties of NDP kinase B and other NDP kinases are compared and the nucleotide requirement for binding are discussed. Using quantitative methods, we identified the DNA-binding sites on the protein and we proposed a structural model for a complex of one hexameric NDP kinase B with an oligonucleotide.

KEY WORDS: NM23; NDP kinases; *c-myc* transcription factor; single-strand DNA-binding proteins; architectural transcription factor; metastasis.

INTRODUCTION

The catalytic activity carried by nucleoside diphosphate kinase (NDP kinase) is to exchange a phosphate from a nucleoside triphosphate (NTP) to a nucleoside diphosphate (NDP). This ping-pong reaction occurs in two steps with formation of a phosphohistidine intermediate (Gilles *et al.*, 1991). Because of the ubiquitous importance of NTPs and dNTPs in cell function, including the regulatory role played by GTP and other nucleotides, NDP kinase could participate through its enzymatic activity in many cellular processes. In addition, through a number of genetical, biochemical, and structural evidence, it appeared that the role of NDP kinase may not be restricted to its enzymatic activity, at least in higher eukaryotes (Chiadmi *et al.*, 1993; Véron *et al.*, 1995; Xu *et al.*, 1996; Zimmermann *et al.*, 1999). In 1993, Postel *et al.* identified the isoform B of human NDP kinase (NDP kinase B) as a transcription factor for a regulatory element in the promoter of the c-*myc* oncogene called *n*uclease *h*ypersensitive *e*lement (NHE). It was later demonstrated that his property is fully conserved in a catalytically inactive mutant (Postel and Ferrone, 1994) and that NDP kinase B also stimulates *c-myc* transcription *ex vivo* (Berberich and Postel, 1995; Ji *et al.*, 1995). Subcellular localization of NDP kinase B in the nucleus is in agreement with a putative role of NDP kinase B in the control of gene expression (Kraeft *et al.*, 1996; Phung-Ba Pinon *et al.*, 1999).

In this paper, we review our recent results on the study of the interaction of purified recombinant human NDP kinase B with oligonucleotides representing the NHE element of the c-*myc* promoter. We describe the physical characteristics of this interaction and propose a molecular model for the binding of an oligonucleotide to hexameric NDP kinase B, which takes into account all of the available biochemical data.

NDP KINASE B SPECIFICALLY RECOGNIZES DNA SEQUENCES FORMED BY SINGLE-STRAND WITHIN THE NHE ELEMENT

NHE is a positive regulatory element for the c-*myc* oncogene (Hay *et al.*, 1987; Cooney *et al.*, 1988; Postel *et al.*, 1989). It corresponds to the sequence

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located −160 to −100 bp upstream from the initiation site of the P1 promoter (Berberich and Postel, 1995) (Fig. 1). The NHE is mostly composed of pyrimidines on the coding strand (mainly cytosines) and carries a DNase I cleavage site (site III_1, Siebenlist *et al.*, 1984). It was proposed that this polypurine/polypyrimidine sequence causes a major change in the B conformation of the DNA duplex (Mirkin *et al.*, 1987; Wells, 1988; Mirkin and Frank-Kamenitskii, 1994; Simonsson *et al.*, 1998). The c-*myc* NHE element was first identified as a site for interaction with a protein named PuF on the basis of footprints with dimethyl sulfate (DMS) and of EMSA experiment using NHE derive probes (Postel *et al.*, 1989; Ji *et al.*, 1995). The PuF-binding site (also called CT element) corresponds to the sequence −142 to −115 upstream from the P1 promoter of the c-*myc* oncogene (Fig. 1).

Using purified recombinant NDP kinase B and a series of oligonucleotides, we performed *in vitro* experiments to characterize the formation of the ADN/protein complex. The complex was quantified by gel retardation (EMSA) (Hildebrandt *et al.* 1995), filtration on nitrocellulose filters (Agou *et al.* 1999), and by fluorescence spectroscopy (Agou *et al.* 1999). It should be noted that, contrary to fluorescence methods, gel retardation or nitrocellulose filtration experiments are not performed at equilibrium, since in an EMSA, the uncomplexed protein and DNA are separated away from the complex during the electrophoresis. Similarly, in a nitrocellulose filter-binding assay, free DNA concentration decreases during the time of the filtration resulting in a bias in the measure of the binding constant. Thus, these methods only allow the measurement of the apparent affinity constant of the ADN/protein complex while fluorescence, which is performed at equilibrium, yields the true dissociation constant.

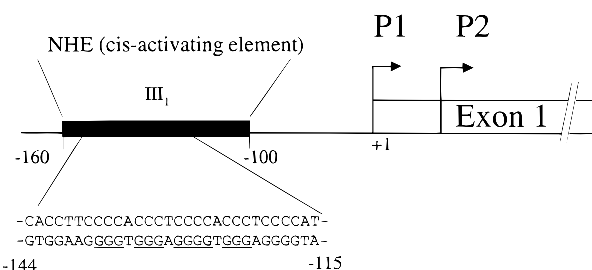

Fig. 1. Upstream region of the human *c-myc* gene. The *cis*-activating element of the c-*myc* gene called NHE element (−160 to −100 bp relative to the transcription start site P1) is indicated. P1 and P2 are the two major *c-myc* promoters. The sequence of the interaction with human NDP kinase B inside the NHE element, resulting from DNA footprintings are underlined (−144 to −115; Postel *et al.*, 1989; Ji *et al.*, 1995).

We have measured the binding affinity of NDP kinase B for a double-strand oligonucleotides corresponding to the PuF binding site. We first showed by denaturing urea gel electrophoresis that the double-strand oligonucleotide was free of any trace of [γ-P^{32}]ATP that is used for oligonucleotide labeling, since even traces of free ATP would allow the autophosphorylation of NDP kinase B, which obviously would blur the results in a filter-binding assay by enzyme phosphorylation. We also verified, by native gel electrophoresis, that the duplex, formed from the stoichiometric mixing of polypurine and polypyrimidine oligonucleotides, was free from any detectable single-strand or tetraplex-type structures. This is important since purine-rich strands are able to form highly stable G quartets (Simonsson and Sjoback 1999), which could indirectly reinforce the presence of the complementary pyrimidine single strand.

Using the nitrocellulose-binding assay under low stringent conditions, i.e, low ionic strength, we were unable to detect any complex between NDP kinase B and the double-strand *c-myc* element corresponding to bp −144/−115 (*c-myc*/ds(−144/−115), indicating a very poor binding of NDP kinase B to a double-strand oligonucleotide. This result was confirmed by EMSA using 5 n*M* radiolabeled DNA and a high protein concentration (4 μM). Only a small fraction of DNA (5%) was bound to the protein and a rough estimate of the amount of complex formed yielded an apparent dissociation constant of about 40 μM (Hildebrandt *et al.*, 1995). The affinity of NDP kinase B for a double-stranded oligonucleotide corresponding to the *c-myc* NHE element was estimated in a more precise way using fluorescence spectroscopy methods. These methods are based either on a change of intrinsic protein fluorescence (Agou *et al.*, 1999) or on the change of fluorescence polarization of a fluorophore coupled to the oligonucleotide upon binding of the oligonucleotide to the protein. The results obtained by these two methods were very similar. Figure 2 shows a titration curve following the change in fluorescence polarization of fluorescein coupled to the 5′-end of a purine-rich oligonucleotide. The insert shows the absence of direct association of the fluorophore with NDP kinase B in absence of DNA. The curve shown in Fig. 2 allows the calculation of a K_d of 80 μM, again showing the very weak affinity of NDP kinase B for the *c-myc* oligonucleotide when it is in a double-stranded form.

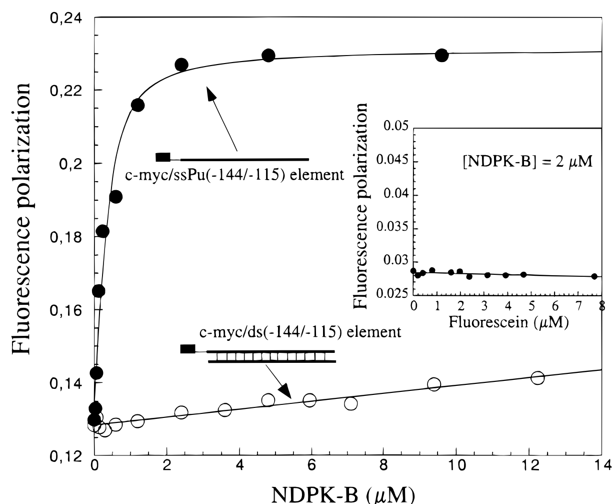

Fig. 2. Specific binding of human NDP kinase B to the NHE. The affinities of NDP kinase B for double-strand element *c-myc*/ds-30 (0.2 μM) or for the purine-rich single-strand element *c-myc*/ssPu-30 (0.2 μM) were measured by monitoring the change of fluorescence polarization of the fluorescein attached to the 5′-end of the purine-rich strand. Experimental conditions were performed at 20°C in 20 m*M* potassium phosphate buffer at pH 7. 0 containing 75 m*M* potassium chloride, 5 m*M* magnesium chloride, and 1 m*M* dithioerythreitol. Insert: Titration of NDP kinase B (2 μ*M*) by fluorescein alone in the same buffer. Dissociation constants, calculated as described (Agou *et al.*, 1999), were 0.11 and 80 μM for single- and double-strand NHE element, respectively.

When similar binding experiments using the fluorescence polarization were performed using a single-strand oligonucleotide representing the purine-rich element [*c-myc*/ssPu(−144/−115)], a strong binding of the oligonucleotide to NDP kinase B could be seen, which allows the calculation of an affinity of K_d = 0.11 ± 0.01 μ*M* (Fig. 2). Therefore, this difference in binding affinity (more than 700 times) for the same oligonucleotide, whether under a single or a double-strand form, shows that NDP kinase B can be considered single-strand specific. The high affinity of NDP kinase B for single-strand DNA raises questions about the significance of the dissociation constant values measured with double-stranded DNA (see above). Indeed, the DNA–protein complex formed in the presence of added double-strand DNA may result from the association with a minor fraction of single-strand DNA resulting from the dissociation of the double strand. Using polyacrylamide gel electrophoresis under native conditions, Hildebrandt *et al.* (1995) showed that a DNA duplex, formed with a 18-bp oligonucleotide containing 51% of GC, is fully dissociated into single strand upon incubation with NDP kinase B. Using fluorescence spectroscopy, we were unable to measure the dissociation constant for short DNA duplexes (10-mer) by fluorescence because the DNA was immediately denatured after protein addition. It is, therefore, important in binding experiments to take into account the conditions determining the melting point of the oligonucleotide used, i.e., temperature, ionic strength, number, and type of base pairing. The formation of single-strand from a double-strand oligonucleotide in presence of NDP kinase B may explain why footprinting experiments with DMS could be performed with the double-stranded NHE (Postel *et al.*, 1989; Ji *et al.*, 1995) while we were unable to demonstrate significant double-strand binding to this element.

After demonstrating that NDP kinase B can form a high-affinity complex (K_d = 0.11 μ*M*) with the NHE element, we next examined whether this interaction was dependent on the base composition of the bound oligonucleotide.

NDP KINASE B BINDS TO SINGLE-STRAND DNA WITH NO SEQUENCE SPECIFICITY

We measured the dissociation constants for a series of oligonucleotides varying in length and sequence (Table I). The oligonucleotide used corresponded either to the c-*myc* promoter (region −144/−115) or to the adenovirus major late promoter (AML), which also has a purine-rich sequence (Table I). In our hands, the nitrocellulose-binding assay could not be used to measure the formation of single strand–protein complexes between NDP kinase B and pyrimidine-rich oligonucleotides. Indeed, cytosine-rich oligonucleotides corresponding to the c-*myc* or to the AML promoter did not form any complex with the protein. This is probably due to the fact that the washing buffer used in the filter-binding assay to stabilize the DNA/protein complex is acidic (pH 5.5), and increases the global positive charge of the protein. This also results in artifacts since, at this pH, the cytosines are protonated (10%) and a pyrimidine strand is more positively charged than the G-rich strand. To precisely compare the binding affinity of purine-rich to pyrimidine-rich oligonucleotides, we used the quenching of intrinsic protein fluorescence upon binding of the oligonucleotide. The intrinsic fluorescence emission of NDP kinase B, due mainly to the three tryptophan contained within the protein, is decreased by 5% in the presence of the oligonucleotide (Agou *et al.*, 1999) (Table I). Using this method, we showed that the affin-

Table I. Binding Dissociation Constants for Oligonucleotides to NDP Kinase B[a]

Name of oligonucleotide	Sequence (5′ → 3′)	K_d^A (n*M*)	K_d^B (n*M*)
c-myc/ssPu(−144/−115)	ATGGGGAGGGTGGGGAGGGTGGGGAAGGTG	45 ± 7	100 ± 10
c-myc/ssPy(−144/−115)	CACCTTCCCCACCCTCCCCACCCTCCCCAT	85 ± 10	(n.d.)
c-myc/ssPu-10	TGGGGAGGGT	30 ± 6	(−)
c-myc/ssPy-10	ACCCTCCCCA	80 ± 8	(n.d.)
AML/ssPu-30	CGCAAGCAGGAGTGAGAGAAGGCGTAGCAC	70 ± 10	200 ± 30

[a] K_d^A and K_d^B are dissociation constants calculated by intrinsic fluorescence of protein and filter-binding assays, respectively. They were determined as described (Agou *et al.*, 1999). n.d., not detectable, −, not determined.

ity for the full-length purine-rich c-*myc* element (30-mer) is not significantly different from that of the complementary pyrimidine-rich strand (K_d = 45 ± 7 n*M* and 85 ± 10 n*M* respectively). Since the activator element is composed of a repetition of the same DNA motif, we also separately measured the affinity of a single purine-rich (*c-myc*/ssPyr-10) or pyrimidine-rich (*c-myc*/ssPyr-10) motif. Very similar values were again found for these two elements (30 and 80 n*M*, respectively) and with the AML purine-rich (K_d = 70 nM), although the latter differs in sequence from the *c-myc* element.

In contrast to the pyrimidine-rich oligonucleotide, the filter-binding assay can be used with purine-rich sequences. Using this assay, we studied the effect of ionic strength of pH on the stability of the ssDNA/NDP kinase B. The results show that two major salt bridges are formed within the complex. Since the stoichiometry of binding is of three oligonucleotides bound per hexameric, each monomer within the hexamer contributes to the oligonucleotide binding by the formation of one specific salt bridge (Agou *et al.*, 1999).

SINGLE-STRAND DNA BINDING IS SPECIFIC TO HUMAN NDP KINASE B

In humans, two major isoforms of NDP kinase are present, NDP kinase A and NDP kinase B, respectively, encoded by the genes *nm23-H*$_1$ and *nm23-H*$_2$. Although the proteins are 88% identical and have very similar specific activity, these two isozymes have very different *pIs*: NDP kinase A is an acidic protein (p*I* = 6.4) while NDP kinase B is very basic (p*I* = 8.7). Accumulating evidence indicates that human NDP kinase A and NDP kinase B have a different regulating function and the important difference in their total charge could be related to this observation. Indeed, the expression of one or the other of these two human isozymes into a *Drosophila* strain carrying a *null* mutation for the endogenous *Drosophila* NDP kinase gene (*awd*) results in nonidentical complemented phenotypes (Xu *et al.*, 1996). The two isozymes also appear to have a different cellular localization. NDP kinase B was shown to be present both in the cytosol and in the nucleus in fibroblast (Kraeft *et al.* 1996) and breast cancer cell lines (Phung-Ba Pinon *et al.*, 1999), while NDP kinase A was present only in the cytosol in high and low metastasic breast cancer cell lines (Phung-Ba Pinon *et al.*, 1999).

NDP kinase A has no significant DNA-binding activity, whether using double or single-strand oligonucleotides. Using the c-*myc*/ssPy(−144/−115) oligonucleotide and EMSA, a complex could barely be detected even under extreme conditions, i.e., in presence of 5 n*M* single-strand oligonucleotide and a very high concentration of NDP kinase A (200 μg/ml) (Hildebrandt *et al.*, 1995). We have now confirmed this result using fluorescence polarization (Fig. 3). While NDP kinase B easily forms a high-affinity complex with the single-strand purine-rich element (*c-myc*/ssPu(−144/−115); K_d = 0.11 ± 0.01 μ*M*), no complex could be detected with the same oligonucleotide and varying NDP kinase A concentrations (0.2–12 μ*M*).

The fact that the ability to bind single-strand DNA with a high affinity is restricted to NDP kinase B, may have important biological consequences since NDP kinase A and NDP kinase B subunits are able to form hybrid hexamers *in vivo*. Indeed, it has been shown that these two isozymes associate in varying ratios to form hybrid hexamers (Gilles *et al.*, 1991). While the presence of the two types of subunits within a single hexamer has probably no effect on the catalytic activity, the presence of NDP kinase A subunits in a heterohexamer may have a dominant inhibitor effect on the single-strand DNA binding properties of the oligomer.

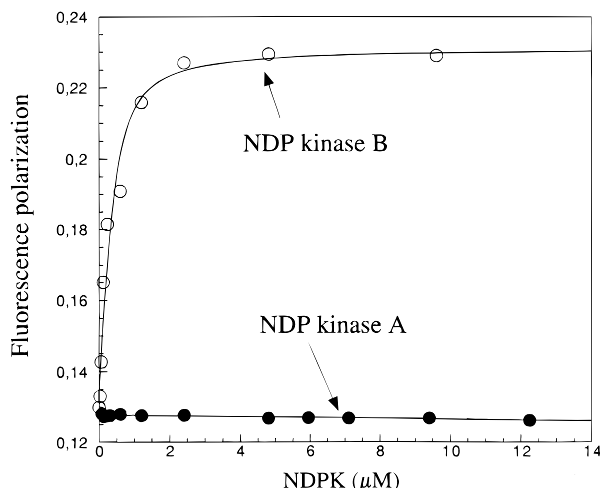

Fig. 3. Comparative study of the single-strand DNA binding to human NDP kinase A and B. The oligonucleotide *c-myc*/ssPu-30 (0.2 μ*M*) with fluorescein attached to the 5′-end was titrated either with NDP kinase B or with NDP kinase A under the experimental conditions described in Fig. 2.

THE ACTIVE SITE IS INVOLVED IN OLIGONUCLEOTIDE BINDING

A detailed review of NDP kinase structure is presented in Janin *et al.* (2000). For our purpose, we would like to emphasize only one particular aspect of the structure of the complex between NDP kinase and a nucleotide, i.e., its interaction with the base. In all known nucleotide-binding proteins including kinases, protein kinases, or GTP-binding proteins, the base of the nucleotide does hydrogen bonds to the protein. In NDP kinase, the nucleotide interacts with the protein only through hydrophobic contacts: a conserved phenylalanine 60 stacks to the base, while a valine (Val 112) hydrophobically interact on the other side. This mode of binding explains the slight specificity of NDP kinase for the base of the nucleotide substrate. Thus, one of the bases composing an oligonucleotide bound to NDP kinase B could interact in a similar way with the active site.

In order to test this hypothesis, we used the filter-binding assay to determine whether the binding of the signal strand *c-myc*/ssPu(−144/−115) was affected in the presence of deoxy- and nucleoside diphosphate (NDP) or nucleoside triphosphate (NTP) (Agou *et al.*, 1999). Addition of increasing concentrations of NDP or NTP inhibit the binding of the oligonucleotide with an apparent inhibition constant considerably higher than the K_d of the protein for single-strand DNA (about 700-fold for ADP). The inhibitory effect is depending on the nucleotide of the substrate used, with K_i values of 17, 70, 80, and 900 μ*M* for GDP, ADP, dTDP, and CDP, respectively. Although NDP kinase was long considered to be totally nonspecific for the nucleotide substrate, it was recently found that its catalytic efficiency is not identical for all NTPs. Indeed, a similar relative specificity was reported with the order G > T = A > C (Schaertl *et al.*, 1998; Schneider *et al.*, 1998). Inversely, we have examined whether the oligonucleotide has an effect on the enzymic reaction. We have shown that the *c-myc*/ssPu oligonucleotide is a competitive inhibitor with values of K_i close to those of the dissociation constant measured by fluorescence (Raveh *et al.*, 2000). These results indicate that several bases of the oligonucleotide binds to the same site in NDP kinase B, as the substrate of the catalytic reaction.

THE SURFACE CONTACTS BETWEEN NDP KINASE B AND SINGLE-STRAND DNA

In order to define the molecular contacts between NDP kinase B and a single-strand DNA, we performed *in vitro* cross-linking experiments (see Hockensmith *et al.*, 1991 for methodology). Briefly, the protein DNA complex was first irradiated with a pulse (5 ns) of a laser UV, resulting in activation of the bases and in the formation of covalent bonds between bases of the nucleotide (principally thymines) and amino acid(s) within the complex. We used 11- or 30-mer single-strand thymine homopolymers because NDP kinase B binds to single-strand DNA with no sequence specificity and that cross-linking is most efficient with thymines. After digestion of the cross-linked complex with endoproteinase Lys-C, the nucleopeptides were purified by HPLC and identified by mass spectrometry. Using this method, we were able to identify the same three distinct cross-linked nucleopeptides, indicating a similar binding mode, all localized at the surface of the hexamer around the active site (Raveh *et al.*, 2000).

These results allowed us to propose a structural model for the interaction between NDP kinase B and a 11-mer homo poly-T oligonucleotide (Fig. 4) (Raveh *et al.* 2000). This model is based on the known crystal structure of NDP kinase B with (Moréra *et al.*, 1995) and without GDP bound (Webb *et al.*, 1995). It also takes into account the mobility of helixes HA and H2, that have been demonstrated in the crystal structure (Janin *et al.*, 2000). The comparison of the sequence of the cross-linked peptides with the corresponding

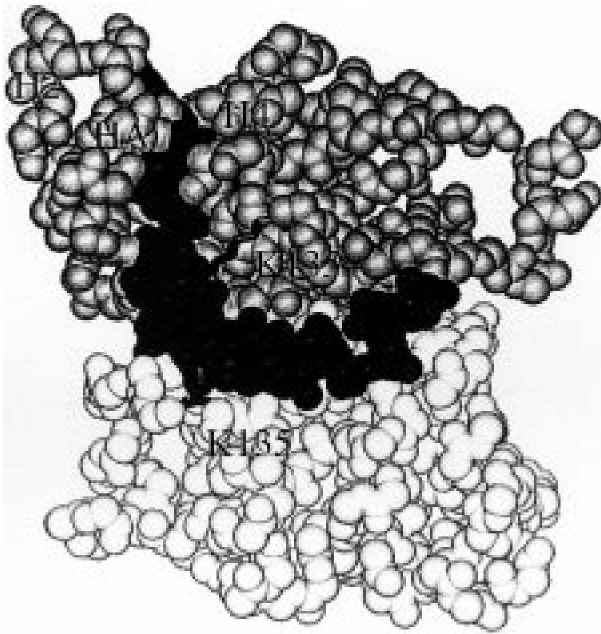

Fig. 4. Structural model of NDP kinase B binding to a single strand aptamer (11-mer). The model was built using InsightII software and builder module (Molecular Simulations) on the base of the crystal structure of NDP kinase B in the presence of GDP (Moréra *et al.*, 1995) and the biochemical data described in Raveh et al. (2000). For clarity, only dimeric unit within the hexamer is shown in CPK and each subunit is represented with different shades of grey. The two K135 from each subunit, which form specific salt bridges with the oligonucleotide (shown in the dark area), are represented.

peptides in NDP kinase A allowed us to identify Lys135 as a likely candidate for a cross-link event. This result is in agreement with a previous finding showing that mutation of this residue by site-directed mutagenesis modifies the protein–DNA association properties (Postel *et al.*, 1996). In the model shown in Fig. 4, the two Lys135 belonging to a dimeric unit within one hexamer form two specific salt bridges with the phosphate backbone of the oligonucleotide, in excellent agreement with our previous predictions (Agou *et al.*, 1999). This model is also in agreement with the previous determination of stoichiometry of three oligonucleotides bound per hexameric NDP kinase B molecule. It may explain why NDP kinase B discriminates between the single- and double-strand forms of DNA, as shown above. Indeed, duplex DNA is less "elastic" than its single-strand form and could not be bent or twisted, as shown for a single-strand bound in Fig. 4.

IS NDP KINASE B AN ARCHITECTURAL TRANSCRIPTION FACTOR?

The characteristics of the DNA-binding activity of NDP kinase B are different from those of conventional transcription factors: inspection of the structure of NDP kinase B shows that is composed of a single domain. No subdomains that could correspond to either a DNA-binding domain and/or to an activation domain can be identified (Janin *et al.*, 2000). In addition, no interaction with components of the transcription machinery has ever been demonstrated. By contrast, the DNA-binding properties of NDP kinase B are reminiscent of so called "architectural transcription factors," because NDP kinase B has little affinity for DNA duplexes but rather binds single-strand DNA with high affinity but without sequence specificity. An example of such a factor is the heterogeneous nuclear ribonucleoprotein K (hnRNP-K), which was first identified as a member of a group of at least 20 major proteins constituting hnRNP particles. It is presumably involved in the transport and/or processing of heterogeneous nuclear RNA and mature RNA (Pinol-Roma *et al.*, 1990). This protein has also been identified as a specific single-strand architectural transcription factor for the human c-*myc* gene (Levens *et al.*, 1997; Tomonaga *et al.*, 1998).

We propose that the specific binding to single-strand DNA allows NDP kinase B to provide for a local increased flexibility of DNA. The creation of a single-stranded hinge would increase the elastic properties of DNA in the NHE element. This increase of flexural (bending) and torsional (twisting) elasticity at this DNA site should drive transcription in promoting the interactions between a transcription factor, recognizing its cognate sequence and the basal apparatus. These interactions could not occur with the duplex DNA because of its rigidity and periodicity (10.4 bp/turn). Furthermore, analysis of the single-strand element *in vivo* by mapping the bases reactive to potassium permanganate using ligation-mediated polymerase chain reaction, showed different patterns in cell lines expressing or not the c-*myc* gene (Michelotti *et al.*, 1996), suggesting a structural transition of DNA during the activation of the promoter.

Double-strand cleavage activity of the NHE associated with NDP kinase B was recently reported and it was proposed that the regulatory role of NDP kinase B was linked to a change in DNA conformation due to topoisomerase activity carried by NDP kinase B

(Postel, 1999). However, using highly purified preparations of recombinant NDP kinase B, we were unable to detect any cleavage product of 30-mer oligonucleotides either by EMSA (Hildebrandt *et al.*, 1995), by gel filtration, or by MALDI-TOF mass spectrometry. We incubated the *c-myc*/ds-30 element with purified NDP kinase B under experimental conditions described by Postel (1999). The mixture was then directly analyzed by gel filtration or MALDI-TOF mass spectrometry. Using these two methods, we could not detect shorter oligonucleotides generated from a cleavage inside the double-strand element of the c-*myc* gene (Agou, unpublished results). Additional experiments are needed to resolve the contradictory results concerning the possible DNA cleavage activity linked to NDP kinase B.

A global comparison of prokaryotic and eukaryotic genomes indicates that the presence of homopurine/homopyrimidine sequences susceptible to form non-B DNA conformations is found only in eukaryotic genomes (Cox and Mirkin 1997). Structures similar to the NHE element found in the c-*myc* promoter seem to be present in the promoters of other oncogenes (Simonsson *et al.*, 1998). We hypothesize that the single-strand DNA binding activity of NDP kinase B could stimulate transcription by inducing a structural transition of the DNA within these promoters.

It should be noted that the affinity of NDP kinase B for single-strand oligonucleotide is considerably better than for any of the nucleoside triphosphates (350- and 1450-fold higher for GTP and ATP, respectively; Schaertl *et al.*, 1998; Agou *et al.*, 1999). On the other hand, the concentration of the single-strand target is likely to be considerably less than that of the nucleotides inside the cell. It is tempting to speculate that *in vivo* the protein is alternatively able to synthesize NTP or to bind single-strand DNA, depending on the local concentration of the NTP pool. The generation of single strand in the nucleus may target the NDP kinase B to maintain the NTP pool near the active site of several cellular events (transcription, replication, and reparation). In a study with *Dictyostelium* NDP kinase, we showed that single-strand binding activity and enzymatic activity strongly depends on the oligomeric state of the protein. Only the hexameric NDPK is fully active in the enzymic reaction, whereas the dimer is catalytically inactive (Mesnildrey *et al.*, 1998). Inversely, the hexamer of *Dictyostelium* has a poor single-strand binding activity, while the dimer forms a high-affinity complex (Mesnildrey *et al.*, 1997). Considering a similar behavior for human NDP kinase B and a better affinity of human hexamer compared to that of *Dictyostelium* (Agou, unpublished results), one could estimate the affinity of single-strand DNA for human dimeric form near 0.2 n*M*. In that way, this "double activity" could be modulated as a function of protein oligomerization, which itself could vary between normal and tumoral cells.

ACKNOWLEDGMENTS

This work was performed in part with grants from "Association pour la Recherche sur le Cancer," ARC n 99371 and "Agence Nationale de Recherches sur le Sida," A.N.R.S n 99004. We are indebted to A. Namane for mass spectrometry analysis. The help of Yuxing Sarah Fan and Avram Slovic during part of this work is gratefully acknowledged.

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