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Mitoxantrone Binds to Nopp140, an Intrinsically Unstructured Protein, and Modulate its Interaction with Protein Kinase CK2

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Nopp140 is a highly phosphorylated protein that resides in the nucleolus of mammalian cell and is involved in the biogenesis of the nucleolus. It interacts with a variety of proteins related to the synthesis and assembly of the ribosome. It also can bind to a ubiquitous protein kinase CK2 that mediates cell growth and prevents apoptosis. We found that Nopp140 is an intrinsically unfolded protein (IUP) lacking stable secondary structures over its entire sequence of 709 residues. We discovered that mitoxantrone, an anticancer agent, was able to enhance the interaction between Nopp140 and CK2 and maintain suppressed activity of CK2. Surface plasma resonance studies on different domains of Nopp140 show that the C-terminal region of Nopp140 is responsible for binding with mitoxantrone. Our results present an interesting example where a small chemical compound binds to an intrinsically unfolded protein (IUP) and enhances protein-protein interactions.

Key Words : Nopp140, CK2, Mitoxantrone, Inositol-hexakisphosphate, Intrinsically unfolded protein

Introduction

Nopp140 is a nucleolus protein that is shuttled between the cytoplasm and nucleolus and is implicated in the nucleolus formation during cell division.[1] It interacts with RNA polymerase I,[2] p80 coilin,[3] NAP57,[4] snRNPs,[2] and protein kinase CK2.[5] In the nucleolus, Nopp140 participates in rDNA transcription[6] and preribosome particle assembly and transport.[7] Overexpression of Nopp140 results in altered nucleoli structure, incorrect localization of nucleolus proteins, inhibition of rRNA gene transcription, and improper formation of the nucleolus.[3,6] The knock down of Nopp140 by RNA interference (RNAi) results in a phenotype that resembles *Minute* syndrome in *Drosophila*.[8] These observations suggest that Nopp140 is crucial for normal cell growth and development.

Structurally, Nopp140 is an intrinsically unfolded protein (IUP) and exhibits typical features of IUPs such as having very little secondary structure and being highly sensitive to proteolysis.[9] Like many other IUPs, Nopp140 can be extensively phosphorylated as it contains more than 80 potential phosphorylation sites.[7] The concentration and degree of phosphorylation in Nopp140 fluctuates during the cell cycle. For example, the level of Nopp140 is much lower during mitosis than in interphase cells, although Nopp140 is more highly phosphorylated in mitotic cells than interphase cells.[10] Interestingly, the C-terminal half of Nopp140 displayed on the surface of T7 phage showed a high affinity to an anticancer agent doxorubicin which lowers the level of phosphorylation by CK2.[9,11] Several kinases such as PKA,[12] PKC,[13] cdc2 kinase,[14] and casein kinase 2 (CK2)[5] are able to phosphorylate Nopp140. Among them, CK2 was the most responsible for the extensive phosphorylation of Nopp140.[14] In fact, CK2 is a ubiquitous protein kinase that can phosphorylate more than 300 different proteins.[15] CK2 is highly conserved in eukaryotes and implicated in cell proliferation, apoptosis and differentiation.[16] Genetic studies have shown that CK2 is essential for survival in yeast[17] or embryonic development in mice.[18,19] In addition, elevated CK2 activity has been associated with many types of cancers including the kidney,[20] lung,[21] head and neck[22] and prostate cancers.[23] Targeted expression of CK2 in transgenic mice promotes tumorigenesis.[24,25] CK2 is regarded as a constitutively active enzyme since there are no secondary messengers that affect its cellular activity.[26]

Recently, it was shown that the activity of CK2 could be negatively regulated by interactions with APC.[27] Also, the activity of purified CK2 was repressed in the presence of cellular extract, which however could be recovered in the presence of inositol-hexakisphosphate ($InsP_6$). This observation insinuated the existence of potential $InsP_6$-dependent negative regulator(s) of CK2. Previously, we demonstrated that the C-terminus half of Nopp140 is responsible for the interaction with the catalytic subunit of CK2 (CK2α) and that $InsP_6$ specifically interacts with CK2α preventing the formation of Nopp140-CK2 complex.[28] We also showed that only the phosphorylated form of Nopp140 preferentially binds to CK2α, suppressing the catalytic activity of CK2.[29] These observations on Nopp140-CK2 interactions raised several questions regarding the structure characteristics of

Nopp140 that might be responsible for CK2 interactions. What would be the structural motif of Nopp140 that is critical for its interaction with CK2? Can one regulate the interactions between Nopp140 and CK2 by small molecules? Here, we report that interaction of intrinsically unstructured Nopp140 with CK2 is positively regulated by mitoxantrone.

Materials and Methods

Materials. Protein kinase CK2 and the dephosphorylated form of α-casein were purchased from New England Biolabs (England) and Sigma (USA), respectively. D-myo-Inositol 1,2,3,4,5,6-hexakisphosphate ($InsP_6$) was obtained from Calbiochem (Germany). Polyclonal antibodies against Nopp140 were prepared from mice immunized with purified Nopp140. Mitoxantrone dihydrochloride was obtained from Sigma. The NINDS custom collection II library consisting of 1,040 biologically active compounds was purchased from MicroSource Discoversy System (USA). All other consumables were of reagent grade.

Preparation of Proteins. For the preparation of the Nopp140 expression vectors and its fragments, DNA fragments expressing N-Nopp140 (amino acids 6-352), C1-Nopp140 (amino acids 528-704), C2-Nopp140 (amino acids 353-704) were amplified by polymerase chain reaction using primers containing extra *Bam*HI and *Sal*I restriction sequences.[29] The amplified DNAs were inserted into pET-28a (Merck Biosciences, Germany), which was designed to express the designated Nopp140 region with a N-terminus His-tag sequence. Wild-type or deletion mutants of Nopp140 were expressed in *E. coli* BL21(DE3) cells. The expression of Nopp140 was induced by the addition of 0.5 mM of isopropyl-β-D-thiogalactopyranoside (IPTG) in LB medium containing 50 μg/mL kanamycin when the optical density of culture at 600 nm reached 0.6. The cells were then harvested after incubation for 12 hr at 18 °C. After the cells were lysed using an M-110P microfludizer, (Microfluidics Co., USA), Nopp140 was purified by Ni-NTA affinity (Elpisbiotech, Korea) and SP-sepharose ion exchange chromatography (GE Healthcare, USA). The catalytic subunit of CK2 (CK2α) was prepared as a fusion protein of glutathione-S-transferase (GST-CK2α) as described previously.[28] The phosphorylated form of Nopp140 was prepared by extensive phosphorylation of the purified recombinant Nopp140 using CK2 as described previously.[30]

Protein Database Analysis. The secondary structure of Nopp140 was estimated by Jpred 3 methods.[31] The intrinsically unstructured region of Nopp140 was analyzed using intrinsically the unstructured/disordered protein Predictor (IUPRED) database.[32]

Circular Dichroism Spectroscopy. Circular dichroism spectra of Nopp140 and fragments of Nopp140 were obtained using a JASCO J-715 (Japan) spectropolarimeter equipped with a temperature-controlling unit, using a 0.1 cm path length quartz cuvette with a 1 nm bandwidth and 4 sec. response time. The standard far-UV CD spectra were collected at room temperature at a scan speed of 20 nm/min with a 0.1 nm step resolution. Six accumulations taken from 260 to 190 nm were added and averaged, followed by the subtraction of the solvent CD signal. The Nopp140 samples had a concentration of 0.2 mg/mL. An estimation of the secondary structure of the protein sample was calculated according to Chen *et al*..[33]

Measurement of the Interaction Between Nopp140 and CK2α. The interaction between Nopp140 and CK2α was examined by measuring the amount of phosphorylated Nopp140 that was bound to immobilized GST-CK2α. Each well of a glutathione coated 96-well plate (Pierce, USA) was incubated with 1 μg of GST-CK2α in 100 μL of PBS for 1 hr at room temperature. After washing the plate with 0.1% Tween-20 solution in PBS buffer (PBST) and blocking with 5% (w/v) skim milk in PBST, the phosphorylated form of Nopp140 (500 ng), 30 μM $InsP_6$ or another chemical in PBS was added to the wells and incubated for 1 hr at room temperature. The amount of His-tag labeled Nopp140 bound on to immobilized GST-CK2α was measured using HRP-labeled His-tag antibodies as described previously.[29]

Surface Plasmon Resonance. Surface plasmon resonance (SPR) analysis of the interaction between mitoxantrone and Nopp140 or CK2α was carried out using a SPR LAB (K-MAC, Korea) and ProteOn XPR36 (Bio-Rad, USA). Nopp140 or GST-CK2α (25 μg) was immobilized on the gold surface of a BK7 sensor chip by incubation in 100 μL of reaction buffer (50 mM sodium phosphate, pH 7.4, 200 mM NaCl) for 3 hr at 4 °C. After immobilization, mitoxantrone in the reaction buffer was injected at a flow rate of 30 μL/min, and the sensorgram was monitored at the angle which showed the largest slope during immobilization using SPR LAB. For SPR analysis using ProteOn XPR36, proteins were coupled to the carboxylmethylated dextran surface of a General Layer Medium (GLM) capacity chip (Bio-Rad, USA) following the protocol described in the Bio-Rad ProteOn XPR36 system instruction manual. Sensorgrams for all binding interactions were recorded in real time and analyzed after subtracting that from the blank channel. After each measurement, the surface was regenerated with regeneration buffer (0.5 M NaCl, 0.05 M NaOH).

NMR Spectroscopy. NMR spectra were acquired using a Varian Unity INOVA 600 equipped with pulsed field gradient triple-resonance probe. Pulsed field gradients were used in all ^{1}H-detected heteronuclear experiments in order to minimize spectral artifacts as well as to select desired coherences using an enhanced-sensitivity approach. Also, water-selective pulses were employed to achieve minimal solvent saturation. The interaction of Nopp140 and MIX was monitored by using 2D ^{1}H-^{15}N heteronuclear single quantum coherence spectroscopy (HSQC) experiment at 5 °C in a buffer containing 50 mM sodium phosphate (pH 6.14) and 50 mM NaCl. Briefly, 0.323 mM ^{15}N-labeled Nopp140 (amino acids 528-704) was titrated with unlabeled mitoxantrone up to a 1:5 molar ratio. All data were processed and analyzed on a Sun SPARCstation using Varian Vnmr, nmrPipe/nmrDraw, and Sparky software.

***In vitro* Kinase Assays.** The kinase activity of CK2 was measured using P^{32}-labeled ATP and α-casein as substrates. Briefly, 6 μg of α-casein was incubated with 15 U of CK2 in 20 μL of reaction buffer (20 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 2.5 mM ATP) containing 1 μM [γ-^{32}P]-ATP (100 cpm pmole^{-1}) at 30 °C for 20 min. The effect of Nopp140, $InsP_6$ or mitoxantrone was examined by pre-incubation with CK2 before mixing with [γ-^{32}P]-ATP. After the reaction was complete, proteins were separated by SDS-PAGE, and the level of phosphorylation in α-casein was detected by autoradiography using a FUJIX BAS 2000 Phosphoimager (Fuji, Japan).

Results

Nopp140 is Intrinsically Unstructured. To investigate the structural features of Nopp140, different domains of Nopp140 including a full-length protein were expressed in *E. coli* and purified to homogeneity (Fig. S1). These recombinant proteins, the entire Nopp140, the N-terminal half (N-Nopp140; residues 6-352), C1-Nopp40 (residues 353-704) and C2-Nopp140 (residues 528-704), were examined by circular dichroism (CD) spectropolarimetry for their secondary structure contents. The CD spectrum of full-length Nopp140 showed an absorption minimum at 195 nm and little absorption at 220 nm (Fig. S2A) as previously reported.[29] This is a typical CD spectrum of an IUP. The other three short domains (N-Nopp140, C1-Nopp140 and C2-Nopp140) showed CD spectra typical of IUPs (Fig. S2B, C, D) with low α-helical content or β-strand structure.[31] When the proteins were phosphorylated their CD spectra (Fig. S2, solid lines) looked similar to those of the un-phosphorylated forms (Fig. S2, dotted lines). These results indicate that almost the entire Nopp140 protein is in an unstructured conformation, and phosphorylation hardly affects the overall disordered nature of Nopp140.

The unstructured nature of Nopp140 observed by CD agrees with structural prediction by a knowledge-based prediction algorithm such as IUPRED.[32] This program predicted a long non-globular region spanning the entire sequence of Nopp140 with short stretches of ordered regions at the N- and C-termini (Fig. S3). The disordered region was estimated to constitute about 94% of the entire sequence of Nopp140. Prediction of secondary structure contents using JPred3[34] showed that short stretches located at the N-terminus (residues 15-23, 28-37 and 49-58), central region (residues 285-292, 321-325) and the C-terminus (residues 539-542, 577-585, 665-672) of Nopp140 may contain helical structures. The CD results together with structural predictions indicate that Nopp140 is a typical IUP whose entire sequence, except a few stretches at the N- and C-terminus, is disordered.

Mitoxantrone Stabilizes the Interaction between Nopp140 and CK2α. An early study showed that an un-phosphorylated form of Nopp140 bound strongly to doxorubicin.[14] More recently, we have shown that the phosphorylated form of Nopp140 could bind to the catalytic subunit of CK2 (CK2α) and inhibit the kinase activity of CK2.[28] These observations suggested that the interaction between Nopp140 and CK2α could be regulated by small molecules. Thus, we screened a commercial library that consisted of bioactive or drug compounds in order to identify small molecules capable of stabilizing the interaction between phosphorylated Nopp140 and GST-CK2α in the presence of

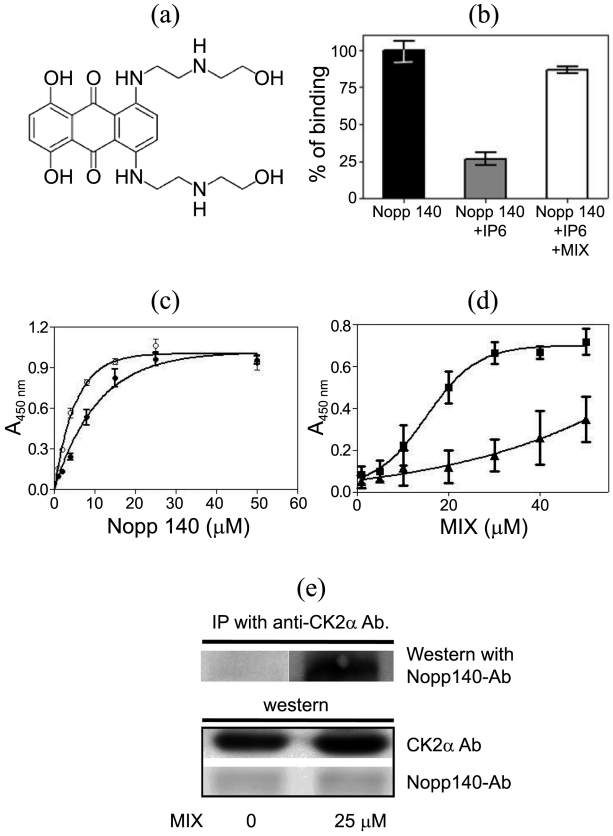

Figure 1. Stabilization of the interaction between Nopp140 and CK2α by mitoxantrone. (a) The structure of mitoxantrone (MIX). (b) The effect of $InsP_6$ and mitoxantrone on the binding of Nopp140 to CK2α. The amount of bound Nopp140 to GST-CK2α (black column) in the presence of $InsP_6$ (gray column), or $InsP_6$ and mitoxantrone (white column) was measured using an anti-His tag antibody. (c) Concentration dependent binding of Nopp140 to CK2α in the presence (open circle) or absence (filled circle) of 20 mM of mitoxantrone was measured using an anti-His tag antibody. The apparent dissociation constant for the interaction between Nopp140 and CK2α was obtained from a nonlinear regression fitting using GraphPad Prism version 5. (d) The concentration dependent effect of mitoxantrone on the binding of un-phosphorylated Nopp140 (filled triangle) or phosphorylated Nopp140 (filled square) with CK2α in the presence of 30 μM $InsP_6$ was measured using an anti-His tag antibody. (e) Effect of mitoxantrone on the interaction between Nopp140 and CK2α in cell was examined. HEK293T cells were treated with DMSO or 25 mM mitoxantrone for 2 hrs. Anti-CK2α antibody conjugated agarose was mixed with cell lysate, and proteins which bound to anti-CK2α antibody were separated by SDS-PAGE, and the amount of Nopp140 bound to CK2α was measured with anti-Nopp140 antibody (top panel). The amount of CK2α or Nopp140 in the lysate of cells treated with DMSO or mitoxantrone was treated cells was measured with anti-CK2α or anti-Nopp140 antibodies (bottom panel).

$InsP_6$. The native state of CK2α in GST-CK2α was confirmed by measuring its kinase activity (data not shown). From this screening (Fig. S4), mitoxantrone was identified to have such activity which is a derivative of anthraquinone (Fig. 1(a)) that has been used for the treatment of certain types of cancer as well as multiple sclerosis. It was shown earlier that Nopp140 bound specifically to immobilized CK2α with a high affinity, and this interaction was interrupted by 30 μM or higher concentrations of $InsP_6$ (Fig. 1(b)).[28] However, in the presence of 20 μM mitoxantrone, $InsP_6$ failed to interrupt the interaction (Fig. 1(b)). The effect of mitoxantrone on the interaction was further examined by measuring the binding affinity of Nopp140 to CK2α in the presence of different concentrations of mitoxantrone. As shown in Figure 1(c), the binding affinity of Nopp140 to CK2α increased in the presence of 20 mM mitoxantrone, and the K_D values decreased from 8.3 nM to 3.6 nM, indicating that the affinity of Nopp140 to CK2α was significantly enhanced in the presence of mitoxantrone. The concentration-dependent effect of mitoxantrone on the interaction between Nopp140 and CK2α was further examined by measuring the amount of bound Nopp140 to CK2α in the presence of 30 μM $InsP_6$. The affinity of Nopp140 to CK2α increased as the concentration of mitoxantrone increased from 0-20 μM (Fig. 1(d)). These results indicate that the interaction between Nopp140 and CK2α is stabilized by mitoxantrone and becomes insensitive to $InsP_6$. To further examine the effect of mitoxantrone on the interaction between Nopp140 and CK2α in cells, we analyzed the formation of Nopp140-CK2α complex by co-immunoprecipitation in HEK293T cells which were grown in the presence or absence of mitoxantrone. The amount of Nopp140 precipitated along with CK2α by anti-CK2α antibodies significantly increased when the cells were grown in the presence of mitoxantrone (Fig. 1(e), top panel). On the contrary, the treatment of mitoxantone was not affected the expression level of CK2α or Nopp140 (Fig. 1(e), bottom panel). These results indicate that the interaction between Nopp140 and CK2α is significantly stabilized by mitoxantrone, and their interaction becomes insensitive to $InsP_6$.

Mitoxantrone Specifically Binds to Nopp140. The stabilization the interaction between Nopp140 and CK2α by mitoxantrone suggests that at least one of the two proteins should directly bind to mitoxantrone. In order to determine which of the two binds with mitoxantrone, we examined the binding kinetics of mitoxantrone to immobilized Nopp140 or CK2α using surface plasma resonance analysis.[14,35] Mitoxantrone displayed a typical association sensorgram to the gold surface containing immobilized Nopp140. The apparent dissociation constant between Nopp140 and mitoxantrone was calculated to be 11.9×10^{-6} M from the sensorgrams obtained at different concentrations of mitoxantrone (Fig. 2(a)). In contrast, mitoxantrone failed to show any significant binding to CK2α (Fig. 2(b), solid and dotted lines). The failure of mixtoantrone-CK2α binding was not due the denaturation of CK2α during immobilization to the gold surface since Nopp140 had a strong binding affinity to CK2α (Fig. 2(b), broken line), as shown in the plate-binding measurement (Fig. 2(b), (c)). The binding region of Nopp140 to mitoxantrone was further examined by measuring the binding of mitoxantrone to N-Nopp140, C1-Nopp140, or C2-Nopp140 by SPR. The amount of bound mitoxantrone to

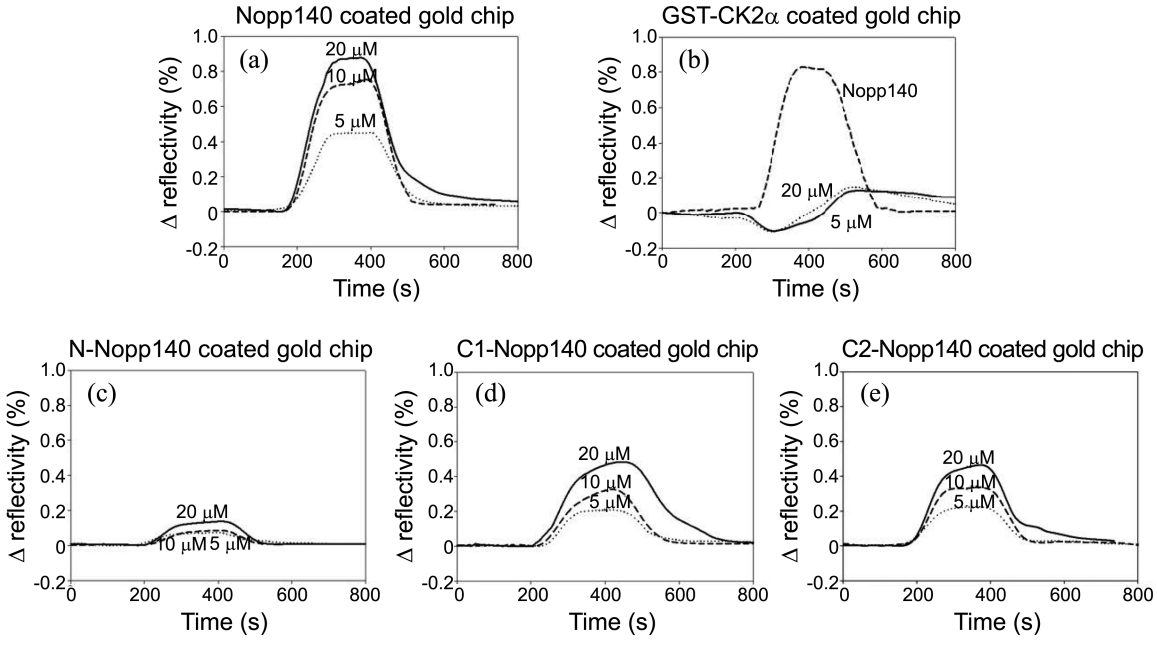

Figure 2. SPR sensorgram of the proteins on mitoxantrone. (a). Sensorgrams of binding between 5 μM, 10 μM and 20 μM mitoxantrone and Nopp140 immobilized to a gold chip. (b) Sensorgrams of binding between phosphorylated Nopp140 (1 μg/mL, broken line) and GST-CK2α immobilized on the surface of the sensor chip at 5 μM (solid line) and 20 μM (dotted line) mitoxantrone. Sensorgrams of binding between 5 μM (dotted line), 10 μM (broken line) and 20 μM (solid line) of mitoxantrone and N-Nopp140 (6-352) (c), C1-Nopp140 (528-704) (d), or C2-Nopp140 (353-704) (e) immobilized to a gold chip.

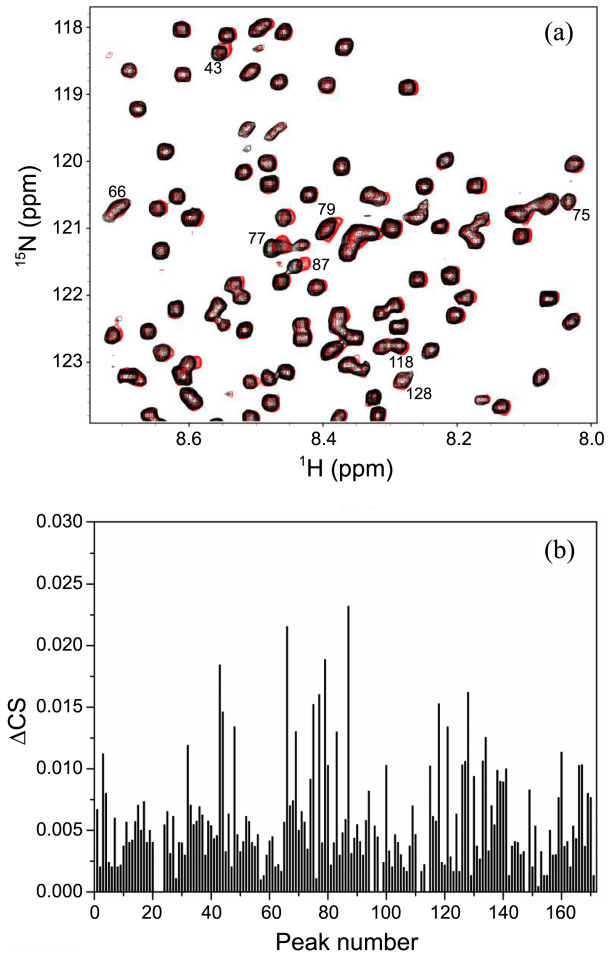

Figure 3. Chemical shift perturbation of Nopp140 by mitoxantrone. (a) Two ^{1}H-^{15}N HSQC spectra of the C-terminal region (528-704) of Nopp140, one in a ligand-free state (black) and the other in complex with mitoxantrone (red). For the bound state, the molar ratio of Nopp140 to mitoxantrone is 1:5. The Nopp140 resonances showing chemical shift changes of ΔCS > 0.015 ppm are labeled with peak numbers. (b) Chemical shift perturbation in Nopp140 (528-704) upon binding to mitoxantrone. The weighted ΔCS values were calculated as previously described (44) and plotted against the peak number of Nopp140.

N-Nopp140 was less than 10% of the amount bound to full-length Nopp140 (Fig. 2(c)). In contrast, mitoxantrone showed significant binding to C1-Nopp140 as well as C2-Nopp140 (Fig. 2(d), (e)), indicating that mitoxantrone interacts with the C-terminal region (residues 528-704) of Nopp140. It is worth noting that CK2α also binds to the C-terminus region of Nopp140.[28] These results suggest that mitoxantrone binds to the C-terminal region of Nopp140 and converts it into a high-affinity conformation for CK2α binding. The interaction between mitoxantrone and Nopp140 is also confirmed by 2D ^{1}H-^{15}N heteronuclear single quantum coherence spectroscopy (HSQC) experiment using ^{15}N-labeled C2-Nopp140 and unlabeled mitoxantrone. When C2-Nopp140 is titrated with mitoxantrone, several resonances were found to experience noticeable chemical shift perturbation (Fig. 3(a)), indicating that several residues of C2-Nopp140 participate direct contact with mitoxantrone.

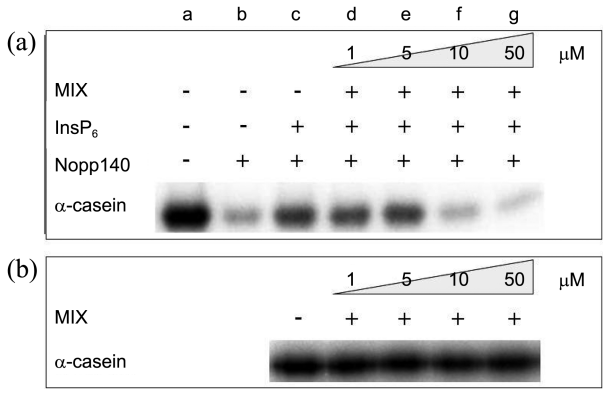

Figure 4. Inhibition of CK2-dependent phosphorylation of the Nopp140 by mitoxantrone. (a) The effect of mitoxantrone on the phosphorylation of α-casein by CK2 was examined by isotope labeling. α-casein was incubated with CK2 in the presence of [γ-^{32}P] ATP, $InsP_6$ and phosphorylated Nopp140, and an increasing concentration of mitoxantrone for 20 min at 30 °C. After the reaction was complete, proteins were separated by SDS-PAGE, and the degree of isotope labeling on α-casein was measured by autoradiography. (b) The phosphorylation of α-casein by CK2 in the presence of [γ-^{32}P] ATP and different concentrations of mitoxantrone was performed for 20 min at 30 °C. After the reaction was complete, isotope-labeled α-casein was detected by autoradiography.

Although mitoxantorne binds directly to Nopp140, this interaction seems not induce dramatic conformational change of Nopp140 since the CD spectra of C2-Nopp140 in the absence or presence of mitoxantorne (up to 50 μM) are almost identical (Fig. S5).

Mitoxantrone Enhanced the Inhibitory Activity of Nopp140 against CK2. In order to address if the inhibitory activity of Nopp140 against CK2α might be sustained by mitoxantrone even in the presence of $InsP_6$ phosphorylation of α-casein by CK2 was measured in the presence of Nopp140, $InsP_6$ or mitoxantrone. The phosphorylation of α-casein by CK2 was reduced to 20% by Nopp140 (Fig. 4(a), lane a, b). In the presence of 30 μM $InsP_6$, which interrupts the interaction between Nopp140 and CK2α,[9,36] the anti-phosphorylative activity of Nopp140 against CK2 was reduced, and more α-casein was phosphorylated (Fig. 4(a), lane c). When mitoxantrone was added along with $InsP_6$, the activity of CK2 was significantly reduced (Fig. 4(a), lane d to g), indicating that the interaction between CK2 and Nopp140 was stabilized by mitoxantrone, and the suppressed activity of CK2α by Nopp140 was maintained. The catalytic activity of CK2α was not affected by mitoxantrone up to 50 μM (Fig. 4(b)). These results indicate that mitoxantrone bound to Nopp140 and stabilized the Nopp140-CK2α complex.

Discussion

Genome analysis of higher organisms revealed that more than 30% of proteins contain partially or fully unstructured regions and are referred to as intrinsically unstructured/unfolded proteins or IUPs. Particularly, IUPs were observed

frequently in transcription factors or key regulators of cell growth, such as the transactivation domain of p53.[37] In general, IUPs function by interacting with target proteins and modulating their biological activities of target proteins rather than acting as catalysts. The sequence of Nopp140 with repetition of serine, threonine and hydrophilic residues is a characteristic of intrinsically unfolded proteins. Nopp140 is predicted to have more than 94% of unstructured regions, which is consistent with the results of CD experiments (Fig. S2). A small portion of helical regions was predicted for short stretches of the Nopp140 sequence: the N-terminal end (residues 15-23, 28-37 and 49-58), middle region (residues 285-292, 321-325) and the C-terminus regions (residues 539-542, 577-585, 665-672). Clusters of hydrophobic amino acids are present near these helical regions. These regions most likely interact with other proteins, since the hydrophobic stretches of IUPs mainly serve as binding motifs for target binding.[37-40] Previously, we demonstrated that the C-terminus half of Nopp140 was important for binding with CK2.[29] In this work, we showed that mitoxantrone interacted with the C-terminal region of Nopp140 (Fig. 2), which increases the likelihood that the hydrophobic regions at the C-terminus of Nopp140 may serve as binding sites for CK2α and mitoxantrone.

Several examples of the specific protein-protein interactions between IUPs and their target proteins have been reported.[41] Unlike the complexes formed between IUPs and their target proteins, the interaction between IUPs and small molecules has rarely been reported. Previously, we have shown that doxorubicin could bind to the C-terminal half of unphosphorylated form of Nopp140 with apparent K_D of 4.5×10^{-6} M,[14] and lowered its level of CK2-dependent phosphorylation of Nopp140.[9] The interaction between IUPs and small molecules that bind to IUP had also been reported by Follis *et al*.[42] The intrinsically disordered regions of c-Myc and Max interacted with each other forming a helical heterodimer. Small molecules that interrupted this interaction[43] were shown to interact with the intrinsically disordered region of c-Myc and induced helical conformation.[42] The specific binding of mitoxantrone to Nopp140 provides another example of an IUP-small molecule interaction. The C-terminal region of Nopp140, which consists of clustered regions of hydrophobic amino acids and potential helical region may form a pocket structure that could accommodate mitoxantrone.

Pre-existing transient secondary structures termed "pre-structured motifs (PreSMos)" have been identified in many IUPs[41] and were shown to serve as target-binding active sites in IUPs. It is worth noting that the interaction between Nopp140 and CK2α may be regulated by $InsP_6$ and mitoxantrone. These compounds have the opposite effect on the activity of CK2. $InsP_6$ binds to the positively charged surface of CK2α and interferes with the binding between Nopp140 and CK2α.[29] Hence, CK2α could not be repressed by Nopp140 in the presence of high concentration of $InsP_6$. In contrast, mitoxantrone enhanced the interaction between Nopp140 and CK2α (Fig. 1, Fig. S4) and maintained the suppressed state of CK2α. The atomic structure of CK2α-$InsP_6$, Nopp140-CK2α and Nopp140-mitoxantron will provide more details regarding the regulatory mechanism of CK2α by Nopp140, $InsP_6$ and mitoxantrone. Further analysis of the effect of mitoxantrone on the interaction between Nopp140 and CK2 in disease models would provide a better understanding the therapeutic activity of mitoxantrone.

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References

1. Meier, U. T.; Blobel, G. *J. Cell Biol.* **1990**, *111*, 2235-2245.
2. Yang, Y.; Isaac, C.; Wang, C.; Dragon, F.; Pogacic, V.; Meier, U. T. *Mol. Biol. Cell* **2000**, *11*, 567-577.
3. Isaac, C.; Yang, Y.; Meier, U. T. *J. Cell Biol.* **1998**, *142*, 319-329.
4. Meier, U. T.; Blobel, G. *J. Cell Biol*. **1994**, *127*, 1505-1514.
5. Li, D.; Meier, U. T.; Dobrowolska, G.; Krebs, E. G. *J. Biol. Chem*. **1997**, *272*, 3773-3779.
6. Chen, H. K.; Pai, C. Y.; Huang, J. Y.; Yeh, N. H. *Mol. Cell. Biol*. **1999**, *19*, 8536-8546.
7. Meier, U. T.; Blobel G. *Cell* **1992**, *70*, 127-138.
8. Cui, Z.; DiMario, P. J. *Mol. Biol. Cell* **2007**, *18*, 2179-2191.
9. Kim, Y. K.; Lee, W. K.; Jin, Y.; Lee, K. J.; Jeon, H.; Yu, Y. G. *J. Biochem. Mol. Biol*. **2006**, *39*, 774-781.
10. Pai, C. Y.; Yeh, N. H. *Biochem. Biophys. Res. Commun*. **1996**, *221*, 581-587.
11. Jin, Y.; Yu, J.; Yu, Y. G. *Chem. Biol*. **2002**, *9*, 157-162.
12. Hoffmann, J.; Schwoch, G. *Biochem. J*. **1989**, *263*, 785-793.
13. Wise, C. A.; Chiang, L. C.; Paznekas, W. A.; Sharma, M.; Musy, M. M.; Ashley, J. A.; Lovett, M.; Jabs, E. W. *Proc Natl. Acad. Sci. USA* **1997**, *94*, 3110-3115.
14. Pai, C. Y.; Chen, H. K.; Sheu, H. L.; Yeh, N. H. *J. Cell Sci*. **1995**, *108*(Pt 5) 1911-1920.
15. Meggio, F.; Pinna, L. A. *FASEB J*. **2003**, *17*, 349-368.
16. Tawfic, S.; Yu, S.; Wang, H.; Faust, R.; Davis, A.; Ahmed, K. *Histol. Histopathol*. **2001**, *16*, 573-582.
17. Glover, C. V. 3rd *Prog. Nucleic Acid Res. Mol. Biol*. **1998**, *59*, 95-133.
18. Buchou, T.; Vernet, M.; Blond, O.; Jensen, H. H.; Pointu, H.; Olsen, B. B.; Cochet, C.; Issinger, O. G.; Boldyreff, B. *Mol. Cell. Biol*. **2003**, *23*, 908-915.
19. Escalier, D.; Silvius, D.; Xu, X. *Mol. Reprod. Dev*. **2003**, *66*, 190-201.
20. Stalter, G.; Siemer, S.; Becht, E.; Ziegler, M.; Remberger, K.; Issinger, O. G. *Biochem. Biophys. Res. Commun*. **1994**, *202*, 141-147.
21. Daya-Makin, M.; Sanghera, J. S.; Mogentale, T. L.; Lipp, M.; Parchomchuk, J.; Hogg, J. C.; Pelech, S. L. *Cancer Res*. **1994**, *54*, 2262-2268.
22. Faust, R. A.; Tawfic, S.; Davis, A. T.; Bubash, L. A.; Ahmed, K.; *Head Neck* **2000**, *22*, 341-346.
23. Yenice, S.; Davis, A. T.; Goueli, S. A.; Akdas, A.; Limas, C.; Ahmed, K. *Prostate* **1994**, *24*, 11-16.
24. Landesman-Bollag, E.; Romieu-Mourez, R.; Song, D. H.; Sonenshein, G. E.; Cardiff, R. D.; Seldin, D. C. *Oncogene* **2001**, *20*, 3247-3257.
25. Seldin, D. C.; Leder, P. *Science* **1995**, *267*, 894-897.
26. Guerra, B.; Issinger, O. G. *Electrophoresis* **1999**, *20*, 391-408.
27. Homma, M. K.; Li, D.; Krebs, E. G.; Yuasa, Y.; Homma, Y. *Proc.*

Natl. Acad. Sci. USA **2002**, *99*, 5959-5964.
28. Kim, Y. K.; Lee, K. J.; Jeon, H.; Yu, Y. G. *J. Biol. Chem*. **2006**, *281*, 36752-36757.
29. Lee, W. K.; Lee, S. Y.; Kim, W. I.; Rho, Y. H.; Bae, Y. S.; Lee, C.; Kim, I. Y.; Yu, Y. G. *Biochem. Biophys. Res. Commun*. **2008**, *376*, 439-444.
30. Kim, Y. K.; Jin, Y.; Vukoti, K. M.; Park, J. K.; Kim, E. E.; Lee, K. J.; Yu, Y. G. *Protein Expr. Purif*. **2003**, *31*, 260-264.
31. Pelton, J. T.; McLean, L. R. *Anal. Biochem*. **2000**, *277*, 167-176.
32. Dosztanyi, Z.; Csizmok, V.; Tompa, P.; Simon, I. *Bioinformatics* **2005**, *21*, 3433-3434.
33. Chen, Y.; Briese, F. W.; Reeve, E. B. *Am. J. Physiol*. **1974**, *227*, 927-931.
34. Cole, C.; Barber, J. D.; Barton, G. J. *Nucleic Acids Res*. **2008**, *36*, W197-201.
35. Seker, U. O.; Wilson, B.; Dincer, S.; Kim, I. W.; Oren, E. E.; Evans, J. S.; Tamerler, C.; Sarikaya, M. *Langmuir* **2007**, *23*, 7895-7900.
36. Solyakov, L.; Cain, K.; Tracey, B. M.; Jukes, R.; Riley, A. M.; Potter, B. V.; Tobin, A. B. *J. Biol. Chem*. **2004**, *279*, 43403-43410.
37. Lee, H.; Mok, K. H.; Muhandiram, R.; Park, K. H.; Suk, J. E.; Kim, D. H.; Chang, J.; Sung, Y. C.; Choi, K. Y.; Han, K. H. *J. Biol. Chem*. **2000**, *275*, 29426-29432.
38. Fink, A. L. *Curr. Opin. Struct. Biol*. **2005**, *15*, 35-41.
39. Kim, D. H.; Lee, S. H.; Nam, K. H.; Chi, S. W.; Chang, I.; Han, K. H. *BMB Reports* **2009**, *42*, 411-417.
40. Chi, S. W.; Lee, S. H.; Kim, D. H.; Ahn, M. J.; Kim, J. S.; Woo, J. Y.; Torizawa, T.; Kainosho, M.; Han, K. H. *J. Biol. Chem*. **2005**, *280*, 38795-38802.
41. Lee, S. H.; Kim, D. H.; Han, J. J.; Cha, E. J.; Lim, J. E.; Cho, Y. J.; Lee, C.; Han, K. H. *Curr. Protein Pept. Lett*. **2012**, *13*, 34-54.
42. Follis, A. V.; Hammoudeh, D. I.; Wang, H.; Prochownik, E. V.; Metallo, S. *J. Chem. & Biol*. **2008**, *15* 1149-1155.
43. Yin, X.; Giap, C.; Lazo, J. S.; Prochownik, E. V. *Oncogene* **2003**, *22*, 6151-6159.
44. Stoll, R.; Renner, C.; Hansen, S.; Palme, S.; Klein, C.; Belling, A.; Zeslawski, W.; Kamionka, M.; Rehm, T.; Muhlhahn, P.; Schumacher, R.; Hesse, F.; Kaluza, B.; Voelter, W.; Engh, R. A.; Holak, T. A. *Biochemistry* **2001**, *40*, 336-344.