DONNÉES EXPERIMENTALES DISPONIBLES POUR LE N-DOMAIN

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The Distinct Functional Properties of the Nucleotide-binding Domain of ATP7B, the Human Copper-transporting ATPase*

ANALYSIS OF THE WILSON DISEASE MUTATIONS E1064A, H1069Q, R1151H, AND C1104F*

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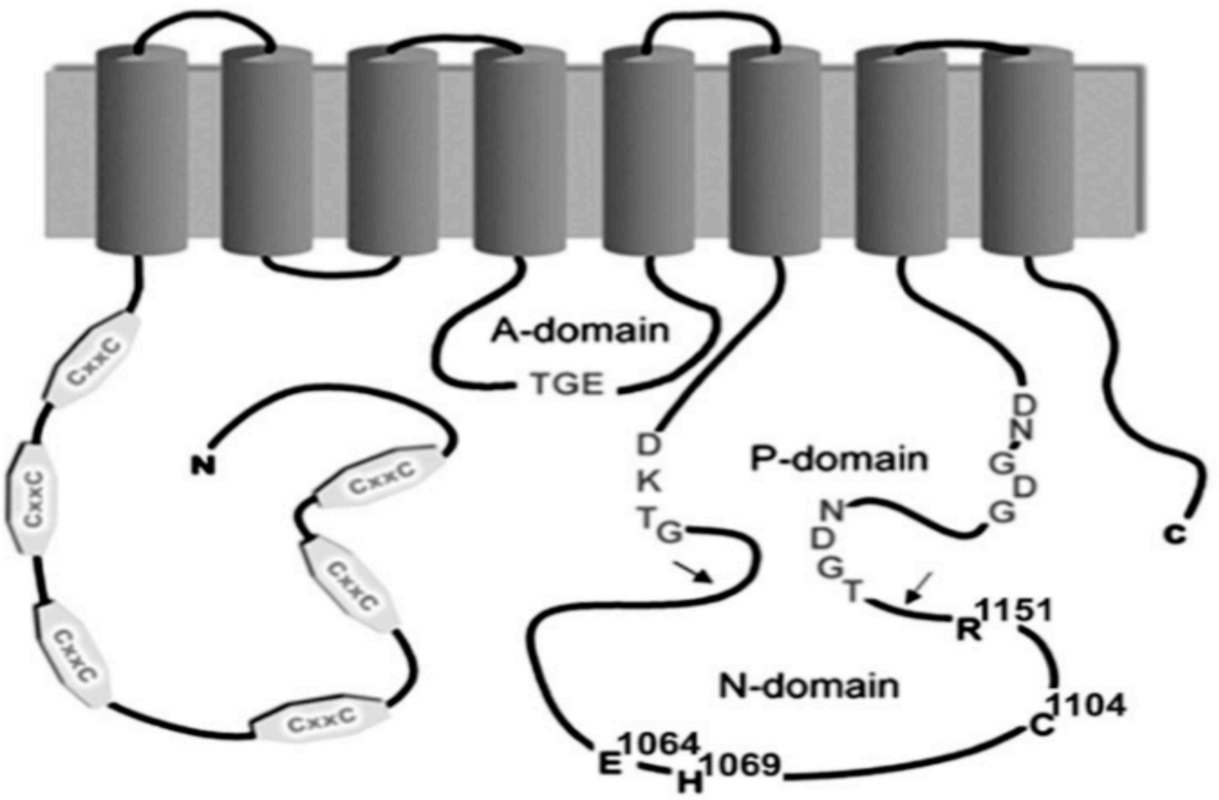
Clinton T. Morgan‡, Ruslan Tsivkovskii‡§, Yuri A. Kosinsky¶, Roman G. Efremov¶, and Svetlana Lutsenko‡¶‖

From the ‡Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, Oregon 97239-3098, and ¶Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia

A



B

AT7B_HUMAN	DKTGTITHGVPRVPR	(x_{20})	TA**E**ASSE**H**PLG	(x_{24})	VPGCGIG**C**KVSN	(x_{40})	GN**R**EW	(x_{34})	LCGMIAIADAVK
AT7A_HUMAN	DKTGTITHGTPVVPV	(x_{20})	TA**E**SNSE**H**PLG	(x_{24})	VPGCGIS**C**KVTN	(x_{57})	GN**R**EW	(x_{34})	LCGLIAIADTVK
ATZN_ECOLI	DKTGTLTVGKPRVPR	(x_{17})	AV**E**QGAT**H**PLA	(x_{22})	-VGSGIE**A**QVNG	(x_{6})	AA**G**K-	(x_{27})	VLGVIALQDTLR
ATU2_YEAST	DKTGTLTTGFMVVMV	(x_{21})	AT**E**SISD**H**PVS	(x_{27})	GKGIVSK**C**QVNG	(x_{6})	IG**N**EA	(x_{31})	VFGLFEINDEVK
CADA_STAAU	DKTGTLTKGVPVVPV	(x_{18})	AL**E**YRSQ**H**PLA	(x_{24})	ITGRGIK**G**IVNG	(x_{6})	SP**K**LF	(x_{34})	ILGVIAVADEVR
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QUESTIONS À SE POSER CONCERNANT LES RÉSULTATS

- Informations obtenues concernant la liaison

à l'ATP

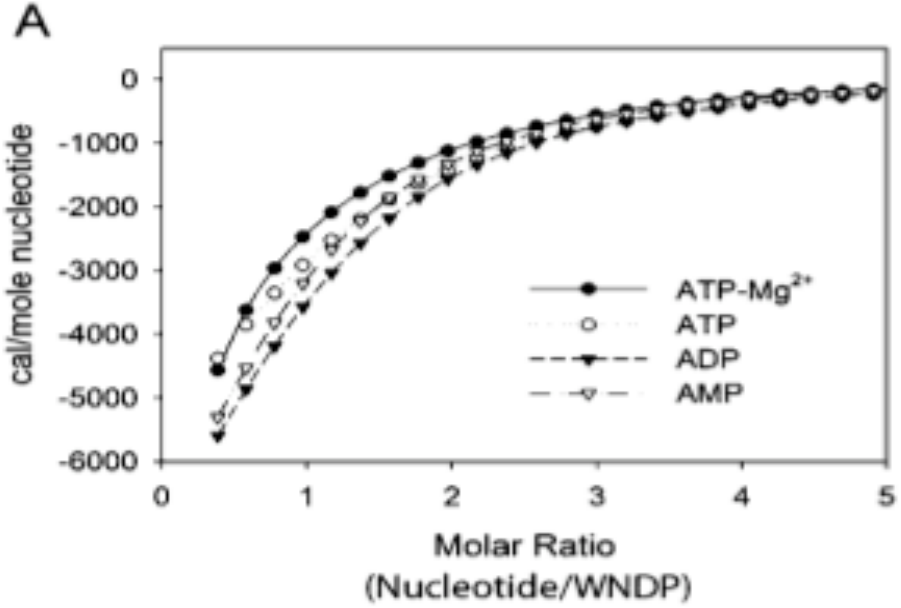
- Rôle du magnésium dans les expériences présentées
- Proposition d'un binding site par modélisation (méthode et template structural)

TABLE II
The K_d values for the experimental titrations of wt N-domains and the WNDP N-domain mutants with various nucleotides

	wt WNDP	E1064A	H1069Q	C1104A	R1151H	MNKP
				μM		
ATP	75.30 ± 3.62	No binding	~1200	59.97 ± 0.39	94.97 ± 0.39	83.00 ± 7.10
ADP	61.51 ± 6.05	No binding	~1000	48.06 ± 0.36	84.57 ± 1.29	44.5 ± 0.70
AMP	60.59 ± 1.31	ND[a]	ND	46.25 ± 3.68	68.60 ± 4.72	ND
ATP, Mg^{2+}	57.00 ± 3.00	ND	ND	ND	ND	ND

[a] ND, not determined.

An unexpected but reproducible finding was that the K_d values for ATP were slightly higher than those for ADP and AMP. Although the differences were not large, they were beyond error and observed repeatedly in both MNKP and WNDP N-domains. This result is consistent with the notion that in the isolated N-domain, the γ-phosphate of ATP experiences weak repulsive interactions. In support of this conclusion, the addition of Mg^{+2} increases the binding affinity for ATP, bringing the K_d value near those for ADP. It has been proposed that ATP binding facilitates movement of the N-domain toward the P-domain in the P-type ATPases (14). It is tempting to speculate that the observed repulsive interactions between the N-domain and ATP's γ-phosphate may contribute to such a movement. The structural analysis of the N-domain in a complex with the nucleotide would directly test this hypothesis; these experiments are currently underway in our laboratory.



Magnesium plays a critical role in the catalytic cycle of the P-type ATPase. The presence of Mg^{2+} is required for the hydrolysis of ATP by the full-length WNDP (our data), suggesting that ATP-Mg^{2+} is a substrate for this reaction. Although the N-domain lacks the catalytic aspartate required for the hydrolysis of ATP, it is unknown whether Mg^{2+} plays a role in the docking of ATP to the N-domain of WNDP. To address this issue, we examined the effect of Mg^{2+} on interaction between wt WNDP N-domain and ATP. The results shown in Fig. 4*A* indicate that Mg^{2+} increases the affinity of the N-domain for ATP, but only slightly (Table II). In addition, no interactions were observed when the N-domain was titrated with magnesium in the absence of ATP. We conclude that the nucleotide binding by the N-domain is a magnesium-independent event.

BINDING SITE PROPOSÉ

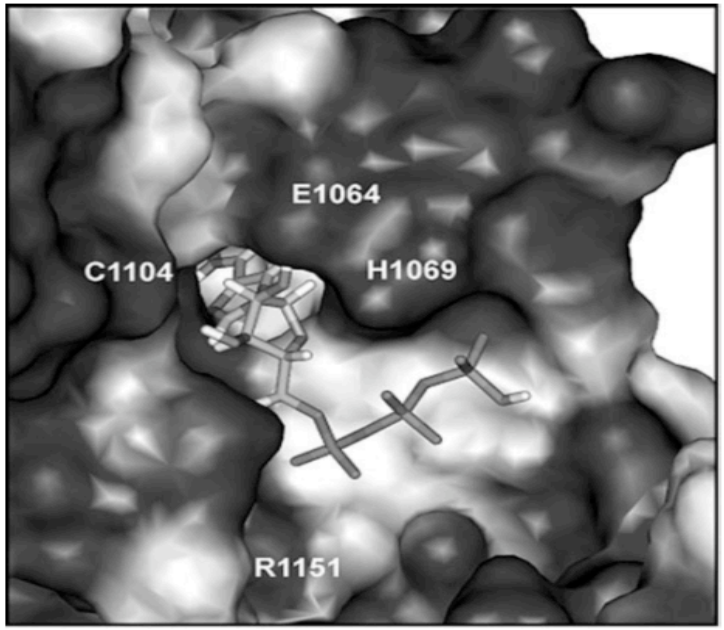


FIG. 7. **Structural model of the ATP binding site in the N-domain of WNDP obtained via molecular dynamics and docking simulations.** The calculated orientation of ATP in the binding site and

Molecular Modeling and Molecular Dynamics Simulations—A spatial model of the ATP-binding domain of WNDP (residues M996-R1322) was built *via* homology modeling based on the x-ray structure of the nucleotide-binding domain (residues Ala-320–Lys-758) of Ca^{2+}-ATPase in the E1 ("open") state (PDB entry 1EUL (13)). The details of the modeling experiments and validation criteria were described previously (24). In the current work, minor conformational changes were introduced into the loop 1062–1071 of the model using the following procedure. First, twenty models with different conformations of this loop were generated. Two models in which the side chains of Glu-1064 and His-1069 point toward the adenine binding cleft in the N-domain were selected for future studies. Both models were subjected to 5-ns molecular dynamics simulations in explicit water. The conformers extracted from the equilibrium parts of molecular dynamics trajectories were then employed in docking simulations with the ATP molecule as described previously (24). The molecular surfaces were mapped according to their hydrophobic properties using the molecular hydrophobicity potential approach (25).

CONCLUSION

Copper transport by the P_1-ATPase ATP7B, or Wilson disease protein (WNDP),[1] is essential for human metabolism. Perturbation of WNDP function causes intracellular copper accumulation and severe pathology, known as Wilson disease (WD). Several WD mutations are clustered within the WNDP nucleotide-binding domain (N-domain), where they are predicted to disrupt ATP binding. The mechanism by which the N-domain coordinates ATP is presently unknown, because residues important for nucleotide binding in the better characterized P_2-ATPases are not conserved within the P_1-ATPase subfamily. To gain insight into nucleotide binding under normal and disease conditions, we generated the recombinant WNDP N-domain and several WD mutants. Using isothermal titration calorimetry, we demonstrate that the N-domain binds ATP in a Mg^{2+}-independent manner with a relatively high affinity of 75 μM, compared with millimolar affinities observed for the P_2-ATPase N-domains. The WNDP N-domain shows minimal discrimination between ATP, ADP, and AMP, yet discriminates well between ATP and GTP. Similar results were obtained for the N-domain of ATP7A, another P_1-ATPase. Mutations of the invariant WNDP residues E1064A and H1069Q drastically reduce nucleotide affinities, pointing to the likely role of these residues in nucleotide coordination. In contrast, the R1151H mutant exhibits only a 1.3-fold reduction in affinity for ATP. The C1104F mutation significantly alters protein folding, whereas C1104A does not affect the structure or function of the N-domain. Together, the results directly demonstrate the phenotypic diversity of WD mutations within the N-domain and indicate that the nucleotide-binding properties of the P_1-ATPases are distinct from those of the P_2-ATPases.