Crystal Structure of a Complex of DNA with One AT-Hook of HMGA1

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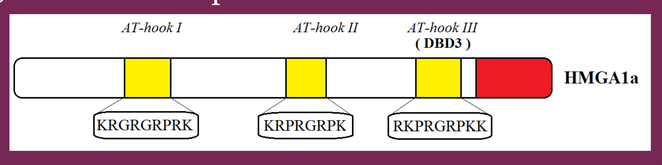
Introduction

High Mobility Group A proteins (HMGA) are intrinsically disordered non-histone chromosomal proteins characterized by containing three DNA binding domains called AT-hooks. These AT-hooks, which preferentially bind to the minor groove of short stretches of AT-rich DNA, are formed by a conserved core sequence, Pro-Arg-Gly-Arg-Pro [1]. By binding to AT-rich DNA regions and/or direct interaction with several transcription factors, HMGAs regulate the expression of numerous genes. In this way they influence many normal biological processes including growth, proliferation, differentiation and death [2-4]. Alterations or abnormal expression of HMGA proteins have also been related to several pathological processes and metabolic disorders, including obesity, type 2 diabetes mellitus and cancer [5-7].

Here we present the first crystal structure of an AT-hook domain. We show the structure of the complex of a DNA oligonucleotide d(CGAATTAATTCG)$_2$ with the third AT-hook (DBD3) of the HMGA1 protein.



Crystallization conditions	
Oligonucleotide	0.2 mM d(CGAATTAATTCG)$_2$
Peptide	0.4 mM Ac-RKPRGRPKK-NH$_2$
Buffer	5 mM $MgCl_2$, 25 mM NH_4Ac, 0.25 mM $NiCl_2$, 25 mM Tris-HCl pH=7.5 (MPD) 4ºC

Table 1. Crystallization conditions

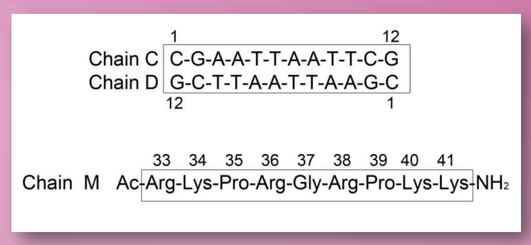


Figure 1. Numbering of the oligonucleotide and peptide residues used in this work.

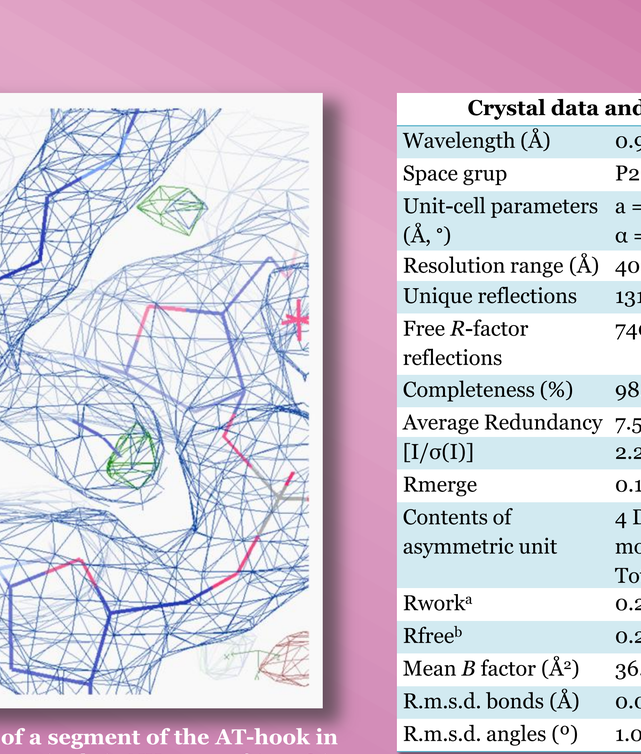


Figure 2. Diffraction pattern of the crystal (1º oscillation) and picture of the crystal diffracted.

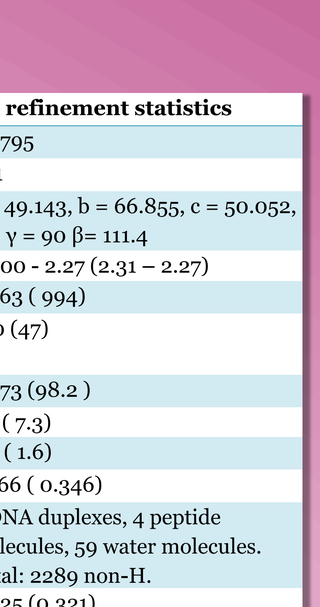


Figure 3. Previous binding assays. EMSA with the oligonucleotide CGAATTAATTCG and the HMGA1a(1-90) protein (full protein without the acidic tail). All the DNA is bound to the protein when the molar ratio (protein:DNA) is 3:1.

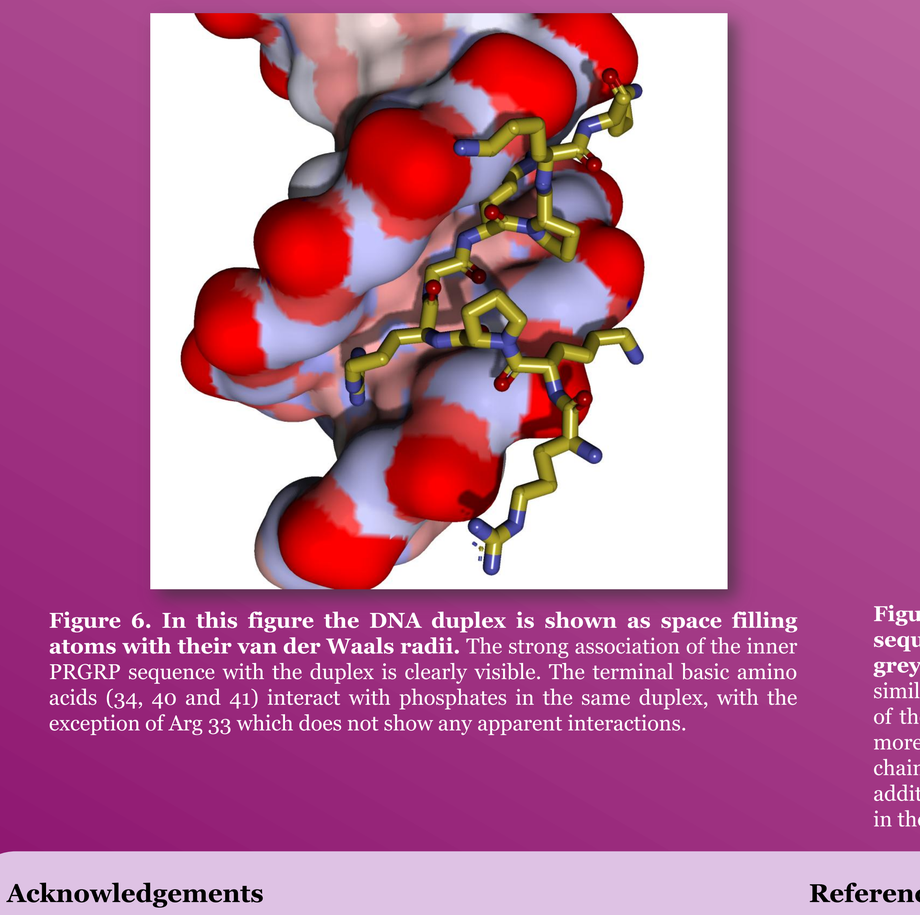


Figure 4. Stereoview of one AT-hook/DNA complex. We present one of the four slightly different complexes found in our crystal. The DNA duplex (shown as a partially transparent object) has a clear bend, localized in the region of base pairs 5 and 6. It determines an angle of 24º between the lower five bases and the upper seven bases of the duplex, represented by its helical axis in darker grey. The minor groove in one of the AATT region is occupied by the AT-hook, whereas the other region shows a clear spine of hydration, typical for the AATT sequence. Virtual bonds between phosphates are indicated by thin red lines: they demonstrate a much wider minor groove in the region where the AT-hook is inserted.

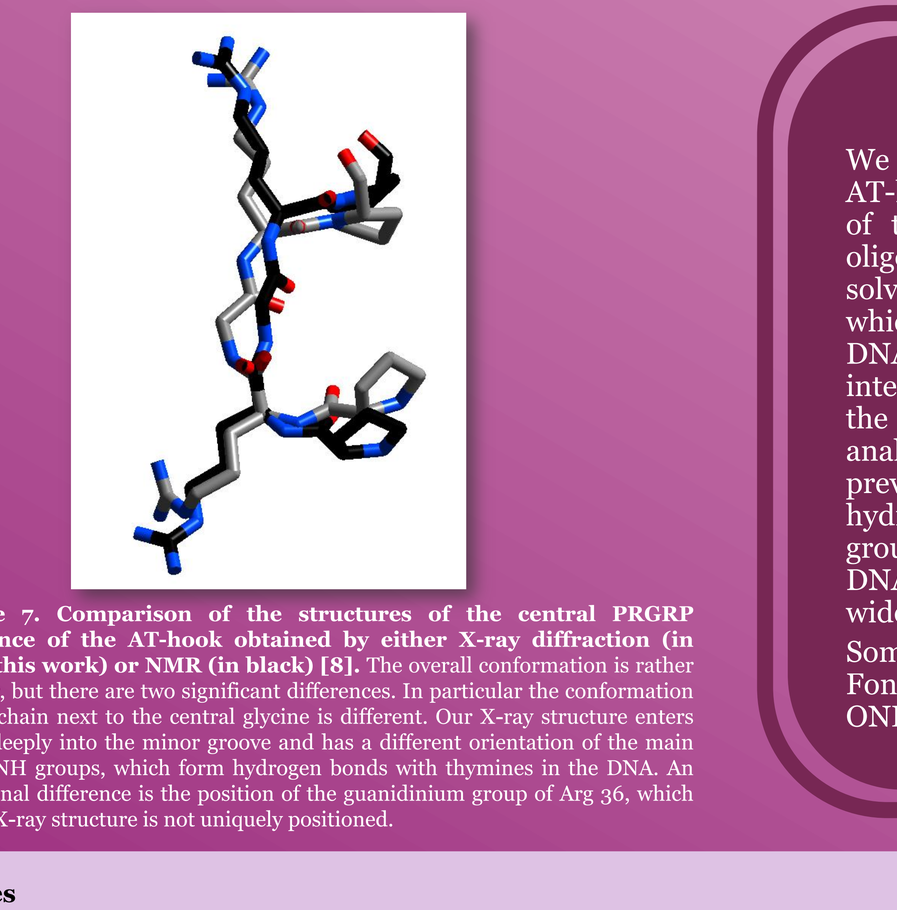


Figure 5. Electron density map ($2F_o$-F_c at 1σ level) of a segment of the AT-hook in the minor groove of DNA. A clear hydrogen bond of the main chain NH group of Arg 38 is formed with one thymine oxygen. An additional weaker bifurcated hydrogen bond is formed by the NH group of Gly and two thymine oxygen atoms from the opposite strands of the duplex. Both hydrogen bonds are indicated as red dashed lines.

Crystal data and refinement statistics	
Wavelength (Å)	0.9795
Space grup	P21
Unit-cell parameters (Å, °)	a = 49.143, b = 66.855, c = 50.052, α = γ = 90 β= 111.4
Resolution range (Å)	40.00 - 2.27 (2.31 – 2.27)
Unique reflections	13163 (994)
Free *R*-factor reflections	740 (47)
Completeness (%)	98.73 (98.2)
Average Redundancy	7.5 (7.3)
[I/σ(I)]	2.2 (1.6)
Rmerge	0.166 (0.346)
Contents of asymmetric unit	4 DNA duplexes, 4 peptide molecules, 59 water molecules. Total: 2289 non-H.
Rwork[a]	0.225 (0.321)
Rfree[b]	0.271 (0.387)
Mean *B* factor (Å^2)	36.36
R.m.s.d. bonds (Å)	0.006
R.m.s.d. angles (º)	1.062

Table 2. Crystal data and refinement statistics. Values in parentheses are for the last shell

[a] $R_{work} = \sum_{hkl}|F_0(hkl) - KF_c(hkl)| / \sum_{hkl} F_c(hkl)$

[b] R_{free} = *R* factor of reflections used for cross validation in the refinement

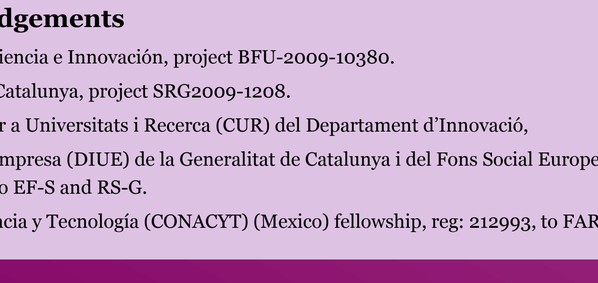


Figure 6. In this figure the DNA duplex is shown as space filling atoms with their van der Waals radii. The strong association of the inner PRGRP sequence with the duplex is clearly visible. The terminal basic amino acids (34, 40 and 41) interact with phosphates in the same duplex, with the exception of Arg 33 which does not show any apparent interactions.

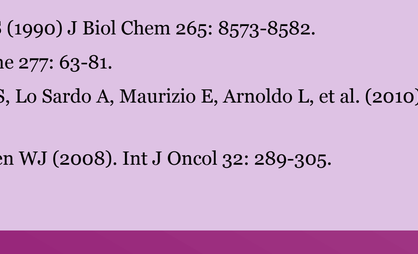


Figure 7. Comparison of the structures of the central PRGRP sequence of the AT-hook obtained by either X-ray diffraction (in grey, this work) or NMR (in black) [8]. The overall conformation is rather similar, but there are two significant differences. In particular the conformation of the chain next to the central glycine is different. Our X-ray structure enters more deeply into the minor groove and has a different orientation of the main chain NH groups, which form hydrogen bonds with thymines in the DNA. An additional difference is the position of the guanidinium group of Arg 36, which in the X-ray structure is not uniquely positioned.

Conclusion

We have crystallized for the first time an AT-hook domain (Ac-RKPRGRPKK-NH$_2$) of the nuclear HMGA protein with the oligonucleotide d(CGAATTAATTCG)$_2$. The solved structure reveals four complexes which are very similar in our crystal. The DNA duplex has two potential AATT interacting groups, one of them binds with the AT-hook. The structure presents analogies and significant differences with previous NMR studies; the AT-hook forms hydrogen bonds between main-chain NH groups and thymines in the minor groove, DNA is bent and the minor groove is widened.

Some of our results are published at Fonfría-Subirós E, et al. (2012). PLoS ONE 7 (5): e37120.

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