Computational, biochemical, and NMR-driven structural studies on histone variant H2A.B

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Summary

Nature uses a special class of histone proteins, histone variants, to modulate the properties of chromatin at defined genomic locations. H2A.B is one of the most divergent H2A variants and is involved in important cellular functions, such as transcription and mRNA splicing. Incorporation of H2A.B in nucleosomes causes unwrapping of ~15 bp entry/ exit nucleosomal DNA from the histone octamer core. Yet, the molecular basis of such peculiar nucleosome conformation is unclear. The work described in this thesis aimed to determine the impact of H2A.B incorporation on the structural and dynamical properties of the nucleosome, primarily using nuclear magnetic resonance (NMR) spectroscopy.

Chapter 1 summarizes the current knowledge on the structure and function of histone variant H2A.B. I highlight the sequence differences between the variant H2A.B and canonical H2A, present an overview of the studies that uncovered the opened nucleosome structure for H2A.B, and conclude by discussing its role in active transcription and mRNA splicing. **Chapter 2** provides a compact review of isotope-labeling techniques used in solution NMR for studies of macro-molecular complexes, such as nucleosomes, with particular emphasis on the range of options available in terms of labeling strategy.

In the first step of our studies on H2A.B, the solution structure of the H2A.B-H2B heterodimer was determined using NMR spectroscopy and Rosetta, as described in **Chapter 3**. Through the combination of backbone chemical shifts and sparse intermolecular NOE distance restraints, a converged ensemble of structures could be obtained. The H2A.B-H2B structure shows that the dimer core is folded in the canonical histone handshake motif from which highly flexible N- and C-terminal regions protrude. The variant dimer structure is highly similar to the structure of canonical H2A-H2B dimer. I further found that the H2A.B-H2B dimer has a higher thermostability than the canonical H2A-H2B dimer. On the basis of an analysis of several mutant dimer proteins, I conclude that the lower the net charge of the H2A.B core region, resulting in reduced electrostatic repulsion with the H2B core, is responsible for the increased stability.

I next investigated the structural and dynamical properties of the H2A.B nucleosomes in **Chapter 4**. First, using an isotope-labeling strategy optimized for the highly flexible parts of the nucleosome, I studied in detail the H2A.B and H3 N-terminal histone tails within the variant nucleosome. Our data show the tails have similar length as the canonical nucleosome structure, indicating that the αN helix in H2A.B and H3 are well folded in the H2A.B nucleosome, despite its more open structure. Furthermore, our data suggest decreased DNA binding and increased flexibilty for the H3 N-tail in the variant nucleosome. Second, using methyl-specific labeling, I probed the conformation and dynamics of the H2A.B core within the nucleosome. Supported by both structural and dynamical data, I found that the H2A.B docking domain, which forms the interface to H3-H4, is folded into the canonical conformation upon incorporation into the nucleosome. On the basis of the updated structural model for the H2A.B nucleosome, I identified a register shift of a DNA-binding arginine in H2A.B that would cause a clash with the nucleosomal DNA and could thus cause opening of the DNA ends. Nucleosomes formed from an H2A mutant with the arginine shifted in position as in H2A.B showed significantly increased digestion of the nucleosomal DNA by micrococcal nuclease, highlighting that the arginine register shift is a contributing factor to the opened nucleosome conformation for H2A.B.

In **chapter 5**, I studied the acidity of the acidic patch, a key protein binding site on the surface of the nucleosome, by experimentally determining the pKa values of residues in and around the acidic patch by NMR. Prediction of pKa values based on the solution structure of the canonical H2A-H2B dimer, determined following a similar approach as in Chapter 3, indicated that several acidic residues may have elevated pKa's due to the clustering of many acidic residues in this patch. However, NMR pH titration experiments showed clearly that all glutamate side chains have pKa values below 4.5, in contrast to the predicted values. I further identified H2B H106 as the group that titrates most closely to the physiological pH range. Its proximity to the binding interface of several nucleosome-binding proteins suggests that protonation of this histidine upon lowering pH to sub-physiological values may be decrease the binding of acidic patch binding proteins.

Chapter 6 offers a general discussion of the results obtained in this thesis in perspective of NMR-driven integrative structural biology, the

structure-function relationship of H2A.B, and the role of protein electrostatics in general. Suggestions are made for future experiments that may add to our knowledge on the structure and function of H2A.B nucleosomes.