Neutron Scatter Studies of Chromatin Structures
Related to Functions

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Technical Progress Report
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Summary

In the original application, we proposed to study the following experiments:

1. Structure of reconstituted fully acetylated chromatin.
2. Structure of TFI_{II}A/DNA complex.
3. Structure of human sperm chromatin.

Our primary source of neutron has been LANSCE, LANL. However during the funded period LANSCE has been plagued by many problems. The hydrogen moderator at LQD leaked in May 1990 and was replaced with a water moderator. This configuration has effectively changed the Q range for the experiment and made our proposed studies difficult. LANSCE has been shut down since August 1991 due to safety problems at LAMPF. The start-up date is still uncertain. Our alternate neutron source at Institut Laue Langevin, Grenoble France has also been down since April 1991. The earliest start up date is 1993. Despite of difficulties with neutron sources, we have managed to complete the study on the structure of trypsin trimmed histone octamers using small angle neutron and X-ray scattering studies (Appendix 1) and Nuclear Magnetic Resonance (Appendix 2). We have also completed studies on the structure of TFI_{II}A induced DNA bending by a circular permutation gel electrophoresis assay (Appendix 3). Individual acetylated species of core histones from butyrate treated HeLa cells were isolated and reconstituted into nucleosomes using a 5S rDNA nucleosome positioning DNA sequence from sea urchin. These nucleosomes were characterized by sulfhydryl group probing, nucleoprotein particle gel electrophoresis and DNase I footprinting (Appendix 4). Fully acetylated species of histones H3 and H4 were also reconstituted in closed circular minichromosomes and the effect of DNA topology changes caused by acetylation was studied (Appendix 5). Finally, protamines isolated from human sperm were characterized (Appendix 6) and a full set of core histones were isolated and characterized (Appendix 7).

Completed Projects:

1. Neutron and X-ray scatter studies of the histone octamer and amino and carboxyl domain trimmed octamers. (Appendix 1)

The Rg of the histone octamer inside the nucleosome as determined by neutron scattering contrast variation studies at 63% $^2\text{H}_2\text{O}$ is 3.3 nm. The reported value from x-ray crystallography studies at 0.7 and 0.8 nm resolutions is 3.0 nm. Isolated histone octamers in 2M NaCl gave a Rg of 3.3 nm by a combination of small X-ray and neutron scattering studies. Histone octamers which were trimmed of its amino and carboxyl "tails" by limited trypsin digestion gave a Rg of 3.0 nm, which is in full accord with that obtained by x-ray crystallography. We concluded that the amino and carboxyl domains of the histones in the histone octamers are probably disordered either statically or dynamically and therefore its electron density not fully accounted for in the crystal structure analysis.

2. An NMR study of trypsin trimmed histone octamers. (Appendix 2)

A study of the histone octamer and trypsin trimmed histone octamer by NMR has confirmed that the discernible signals seen by NMR on chromatin are contributed by the amino and carboxyl domains of the histones. These discrete signals disappeared when the octamers were treated with trypsin. This result is in good agreement with the above mentioned work using small angle scattering.

3. TFIIIA induced DNA bending: effect of low ionic strength electrophoresis buffer conditions. (Appendix 3)

Using a circular permutation gel shift assay, we have shown that TFIIIA when bound to the 5S rDNA gene induced bending of the DNA. This bending is extremely sensitive to the ionic strength of the buffer used during the analysis. By modifying the conditions of the electrophoresis, we were able to detect two distinct conformations for the TFIIIA/DNA complex. In very low ionic strength, the complex is estimated to be bending at 25-30 degrees. When analysed in higher ionic strength, the bending is increased to about 60-65 degrees. These results also demonstrate that the TFIIIA/DNA complex has a large degree of conformational flexibility. Both DNA bending and conformational flexibility are structural features which may provide a key insight into the function of TFIIIA as a positive transcription factor.

4. Isolation and characterization of acetylated histones H3 and H4 and their assembly into nucleosomes. (Appendix 4)

Using a combination of reverse phase and cation exchange HPLC, we were able to isolate large quantities of individual acetylated species of histone H3 and H4 for standard biochemical and structural analysis. Fully acetylated histones H3, H4 and control histones H2A, H2B were used to assemble nucleosome monomers with the Simpson nucleosome positioning sequence. These reconstituted nucleosomes were characterized with regard to their mobility on nucleoprotein particle gel electrophoresis, accessibility to labelling by iodoacetamide and DNase I footprinting. The results obtained indicate acetylation of histones probably exert its influence either in the DNA region beyond the 146 bp of the core particle, or on the internucleosome interactions that involve the

amino-terminal domains of the core histones in organization and stability of higher order chromatin structures.

5. Nucleosome linking number change controlled by acetylation of histones H3 and H4. (Appendix 5)

The first demonstration of a significant structural effect of histone acetylation in the nucleosome was our finding with high levels of acetylation of the histone octamer caused a reduction in the DNA linking number change from -1.04 to -0.81. Because H3 and H4 have been strictly correlated with transcriptionally active chromatin, we prepared chromatin using fully acetylated H3 and H4 isolated by the method mentioned in Appendix 4. Topological analysis using supercoiling gel electrophoresis and electron microscopy has shown that the reduction in linking number change by acetylation can be accounted for solely by acetylation of H3 and H4.

6. Zinc-induced secondary structure transitions in human sperm protamines. (Appendix 6)

Protamines are arginine-rich structural proteins associated with about 85% of the chromatin in matured sperm. Using circular dichroism we have shown that human group II protamines undergoes secondary structural transitions from random coil to a structure with increase in beta turns and anti-parallel beta sheet upon addition of 1 mM Zinc. Our data support a model in which protamine adopts a folded structure in the presence of zinc. The zinc induced secondary structure by zinc is probably of significant physiological relevance considering the high levels of zinc present in human sperm.

7. Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. (Appendix 7)

A full set of histone has been isolated and characterized from human sperm. These histones are highly modified in regard to acetylation, and the H2A consists predominantly of H2A.X, a minor variant found in somatic cells. Based on the amount of the histones obtained, we concluded that the histones are an integral part of the matured sperm chromatin. Given the unique nature of the histone variant present in sperm, we propose that this chromatin component has a specific function and may facilitate the programming of genes which will be active in early development.

Experiments in progress:

1. Chromatosome positioning on assembled long chromatin and its mobility.

Chromatosome is a subnucleosomal chromatin particle consists of a minimum of 168 bp DNA, a histone octamer and a molecule of the very lysine rich histone H1 or H5. When H1 or H5 is added to chromatin assembled on the tandem repeats of the Simpson nucleosome positioning sequences 207-18 or 172-45 derived from sea urchin 5S rDNA, its positioning boundaries remained the same as that in the absence of H1 or H5. They however are decisive in determining the chromatosome position and their distribution. We are studying the ability of the histone octamer to precisely position itself on this strongly positioning sequence. Most histone octamers predominantly position themselves on the precise sequence surrounded by minor positions ten base pairs on both sides of the sequence, therefore preserving the same rotational phase of the DNA coil. The work was done by restriction enzyme analysis, micrococcal nuclease digestion and DNase I footprinting.

2. Interactions of histone H4 amino terminal peptide 1-23 with DNA.

All of the in vivo reversible acetylation sites of histone H4 are located in the very highly mobile and flexible amino terminal domain 1-23 peptide. We have prepared peptide 1-23 by acetic acid hydrolysis and HPLC. The peptide can be acetylated to the same in vivo level of modification by titration with acetic anhydride. We are comparing the binding constants of the control and acetylated peptides to DNA using thermal denaturation and nuclear magnetic resonance. We also will study the effects of different cations on the DNA/protein complex interactions.

3. Neutron scattering studies of fully acetylated nucleosomes.

This experiment was originally planned to be completed in the current funding period, however a combination of equipment failure and safety problems at LANSCE and ILL has forced us to postpone the study until we have a reliable source of neutron beam. We have already submitted a proposal to the new Cold Neutron Reactor Facility at National Institute of Standards and Technology at Gaithersburg for neutron beam allocation. We anticipate to have completed at least two contrast matching experiments during this coming year on the fully acetylated nucleosomes and nucleosomes containing fully acetylated H4 and control H3, H2A and H2B.

4. Study of chromatosome by small angle X-ray scattering.

In collaboration with Dr. Jill Trehwella and Dr. Brian Imai of the Life Sciences Division at Los Alamos National Laboratory, we are studying the structure of the 168 bp nucleosomal particle containing the very lysine histone H1 and H5. Since there has been no reported attempts of crystallizing the chromatosome, we propose to study the location of the very lysine histone and the path of the DNA beyond the core particle in the nucleosome by small angle X-ray scattering.

5. Study of DNA/protein complex using metal cluster label for small angle X-ray scattering.

Tungstate clusters were synthesized and originally used as site specific probes for electron microscopy. These clusters are resistant to high electron beam, electron dense and the two derivatives so far available can be covalently attached to amines and sulfhydryl groups. The use of these probes can potentially be extended to label specific regions of DNA/protein complex for use in small X-ray scattering studies. We propose to label DNA ends with Tungstate clusters then reconstitute the labelled DNA with different chromatin variables. These tungstate clusters may serve as indicator of the outer limits of the DNA of the DNA/protein complex being studied in the x-ray beam.

6. Study of a non-palindromic, non-Watson-Crick base pairing DNA loop structure

In collaboration with the Life Sciences Division at Los Alamos National Laboratory, we have begun a study of a non-palindromic, non-Watson-Crick base pairing DNA loop structure found associated with the centromeric region of the human chromosomes. The DNA is a six fold tandem repeat of a pentanucleotide GGAAT. NMR and x-ray crystallographic data has indicated the existence of a G.A mismatch base pairing. We are in the process of analyzing the structure of the oligonucleotide and its significance and role in the centromeric region of human chromosomes by a combination of thermal analysis, nuclease sensitivity, molecular modeling and high resolution 2D NMR studies.

7. Role of histones and protamines in human sperm

Earlier study from our group reported the presence of a full set of histones in human sperm. These histones are moderately acetylated and the H2A is predominantly H2A.X, a minor variant found in somatic cells. We are in the process of confirming the presence of histones with different sperm isolation procedures to rule out contamination with other immature sperms and other cell types from matured sperm. In addition we are performing detailed immunological identification of these histones including the presence of the very lysine rich histone H1 in human sperm.

8. Effects of chromatin variables on the structure of nucleosomes by protein/DNA crosslinking.

Modifications of histones such as acetylation and phosphorylation may affect the structure of nucleosome in many possible ways: 1) it may change the path of the DNA entering and exiting the nucleosome, 2) it may change the shape of the histone octamer and 3) the interactions of the amino and carboxyl domains of the histones inside the histone octamer may be changed. We propose to study nucleosomes reconstituted with acetylated histones, histone H1, phosphorylated H1, HMG proteins and ubiquitinated histones (uH2A and uH2B).

Appendix

1. Neutron and X-ray Scatter Studies of the Histone Octamer and Amino and Carboxyl Domain Trimmed Octamers. Wood, M.J., Yau, P., Goldberg, M.W., Lambert, S.J., Fowler, A.G., Baldwin, J.P., Godfrey, J.E., Moudrianakis, E.N., Koch, M.H.J., Ibel, K., May, R.P. and Bradbury, E.M. *J. Biol. Chem.* (1991) **266**, 5696-5702.
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