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Infrared spectroscopy of nucleoprotein complexes

A. Roque, I. Ponte and P. Suau*
Departamento de Bioquímica y Biología Molecular, Facultad de Biociencias, Universidad Autónoma de Barcelona, 08193 Bellaterra (Cerdanyola del Vallès), Barcelona, Spain. E-mail: pere.suau@uab.es

Introduction

H1 linker histones are thought to be primarily responsible for the condensation of the thick chromatin fibre. It is currently accepted that linker histones could have a regulatory role in transcription through the modulation of chromatin higher order structure. The carboxy-terminal domain of Histone H1 (CTD) is the primary determinant of histone H1 binding to DNA in chromatin. The CTD has ~100 amino acids, with ~40% lysine.

Protamines are small basic proteins that condense the DNA in mature spermatozoa. Typical protamines are of simple composition and very arginine-rich, usually in the range of 60–80%, and are characterised by a number of stretches of arginine residues separated by neutral amino acids. Salmine is a typical fish protamine with 32 amino acids, 67% of which are arginine. Squid protamine has 58 amino acids of which 79% are arginine.

The CTD of histone H1 and protamine have little structure in aqueous solution, but become folded upon interaction with DNA. They behave, therefore, as intrinsically disordered proteins with coupled binding and folding.[1,2] The inherent problems of the crystallographic approach have made it difficult for the study of the structure of the DNA-bound CTD and protamine. The complexes of DNA and the CTD have not been amenable to crystallisation so far and, in the case of nucleoprotamines, the protein, in contrast to DNA, is not sufficiently ordered to be visible in fibre-diffraction diagrams.[3]

We have applied Fourier transform infrared (FT-IR) spectroscopy to the study of a secondary structure of the DNA-bound CTD and also of protamine inside sperm nuclei. This technique is particularly useful for studying the complexes of DNA with basic peptides and proteins, because it is not affected by turbidity.

Experimental

The carboxy-terminal domain of histone H1 was measured at 5 mg mL^{-1} and the appropriate amounts of DNA in 10 mM HEPES buffering agent, pH 7, plus 10 mM or 140 mM NaCl. Salmon and squid sperm nuclei samples were measured at equivalent DNA concentrations of 5 mg mL^{-1} and 25 mg mL^{-1}, in 10 mM HEPES plus 140 mM NaCl, pH 7. Samples were exchanged with D_2O using successive steps of incubation with deuterated buffer.

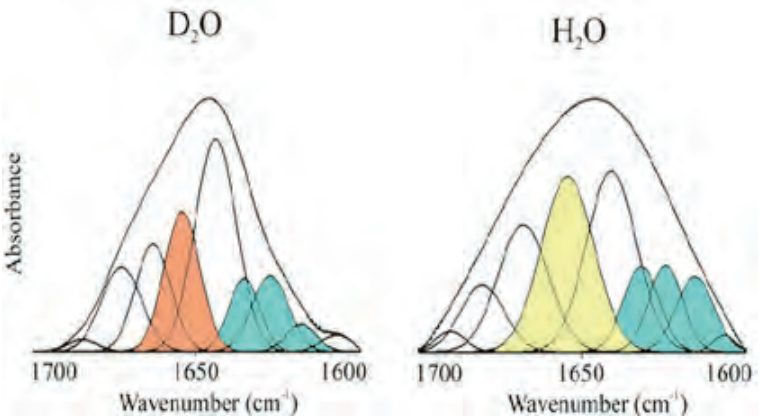
Spectra were obtained with a nominal resolution of 2 cm^{-1}. The number and position of the amide I band components were obtained by Fourier deconvolution and used for the curve fitting of the original envelope by an iterative process previously described.[4] The DNA contribution to the spectra was subtracted by using a DNA sample of the same concentration. To precisely adjust the subtraction, the DNA spectrum was weighted in order to cancel out the absorption at 1087 cm^{-1} in the difference spectra. The band at 1087 cm^{-1} corresponds to the symmetric stretching vibration of phosphate in the sugar–phosphate backbone. This band is the most intense in the DNA spectrum; it shows no overlap with other vibrations, it is not affected by the interaction of peptides or proteins and its intensity is proportional to the DNA concentration. The correctness of this procedure can be empirically checked by recording spectra of complexes of different protein/DNA ratio (r). The spectra were independent of r, which indicates that the amide I region is not significantly affected by DNA spectral changes that could occur following the interaction with protein.

Results and discussion

IR spectroscopy of the carboxy-terminal domain of histone H1

As previously described,[1] in aqueous solution, the amide I of the CTD is dominated by the random coil and turns in rapid equilibrium with the unfolded state. Small amounts of β-structure (9%) are also present.

The spectra of the complexes of the CTD with DNA were recorded both in H_2O and D_2O to facilitate the assignment of the amide I components. The vibrations of loops/flexible regions and the α-helix are only slightly affected by isotopic substitution and are thus found at similar positions in H_2O and D_2O, the α-helix at ~1652 cm^{-1} and loops/flexible regions at ~1643 cm^{-1}; in contrast, deuteration has a great effect in the position of the random coil, so that in H_2O it overlaps with the α-helix, while in D_2O it overlaps with loops/flexible regions. Therefore, the α-helix can be estimated directly in D_2O, whereas loops/flexible regions can be estimated directly in H_2O. When both α-helix and loops/flexible regions are present in the protein structure, the percentage of random coil (RC) can be obtained either from the difference of the components around 1652 cm^{-1} in H_2O and D_2O or from the difference of the components around 1643 cm^{-1} in D_2O and H_2O, as indicated in the following equation:



Percentage (%) of secondary structure of the DNA-bound carboxy-terminal domain of histone $H1^0$ in 10 mM NaCl.

Assignment	D_2O		H_2O	
	Band (cm^{-1})	%	Band (cm^{-1})	%
Turns	1684	1	1688	2
Turns	1673	12	1679	8
Turns/helix 3_{10}	1663	14	1667	17
α-helix/random coil			1655	26
Flexible regions			1641	25
Random coil/flexible regions	1643	32		
Random coil[a]		7		
Random coil[b]				7
β-sheet	1634	8	1632	8
Low frequency β-sheet	1627	10	1625	7
Low frequency β-sheet	1617	4	1616	7

The values were taken from Reference 1.
[a]The value corresponds to the difference between the components at 1641–1646 cm^{-1} in D_2O and H_2O.
[b]The value corresponds to the difference between the components at 1650–1655 cm^{-1} H_2O in and D_2O.

Figure 1. Amide I decomposition of the spectra of the carboxy-terminal domain of histone $H1^0$ bound to DNA. The left spectrum was measured in D_2O and the right spectrum in H_2O. The salt concentration was 10 mM NaCl. The DNA contribution was subtracted as described in the Experimental section. *r* (protein/DNA ratio) = 0.7 (w/w). The protein concentration was 5 mg mL^{-1}. β-Structure components are highlighted in blue, the α-helix in red and the α-helix plus the random coil in yellow. The table shows the percentages of the secondary structure motifs.

$$cb^{H_2O}_{\alpha\text{-helix} + RC(\sim 1652\ cm^{-1})} - cb^{D_2O}_{\alpha\text{-helix}(\sim 1652\ cm^{-1})} = cb^{D_2O}_{loops + RC(\sim 1643\ cm^{-1})} - cb^{H_2O}_{loops(\sim 1643\ cm^{-1})} = cb_{RC}$$

where *cb* is the percentage of the assigned amide I component band in either H_2O or D_2O and cb_{RC} is the percentage of random coil. β-Structure components appear at the same frequency in D_2O and H_2O, whereas turn components are slightly shifted to lower frequencies in D_2O (Figure 1).

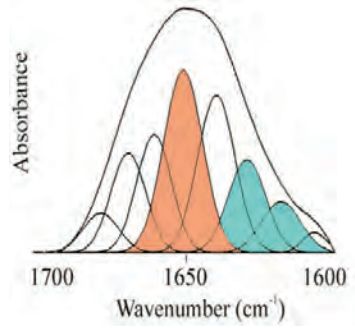
Upon interaction with DNA, the CTD of $H1^0$ acquired an extensively folded conformation. The structure of the bound domain was affected, to some extent, by salt concentration. In 10 mM NaCl, the DNA-bound CTD contained 19% α-helix, 22% β-structure, 27% turns, 25% loops/flexible regions and a residual 7% random coil. In physiological salt (140 mM NaCl), the CTD became fully structured, as indicated by the complete absence of random coil. Under these conditions, the CTD contained 24% α-helix, 25% β-structure, 18% loops flexible regions and 33% turns (Figure 2).

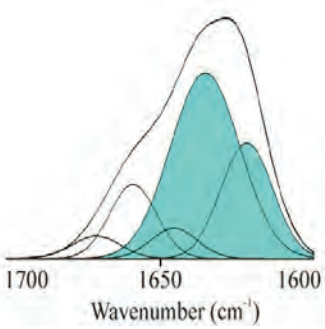
Phosphorylation by cyclin-dependent kinases at SPKK (Serine–Proline–Lysine–Lysine) motifs is the main post-translational modification of histone H1. We studied the effects of phosphorylation on the secondary structure of the DNA-bound CTD, which contains most of the phosphorylation sites of the molecule. The effects of phosphorylation on the secondary structure of the DNA-bound CTD were site-specific and depended on the number of phosphate groups. The greatest effects were observed in the fully phosphorylated (three phosphate groups) CTD that became an all-β protein with 72% β-structure and no α-helix (Figure 2). Partial phosphorylation increased the amount of undefined structure and decreased that of α-helix without a significant increase in β-structure.[5]

Secondary structure of protamine in sperm cell nuclei

The secondary structure of salmine and squid protamine bound to DNA inside sperm nuclei was examined by FT-IR spectroscopy.[2] Spectra were recorded in D_2O and 140 mM NaCl. Salmine had a component band representing 20% of the total amide I intensity at 1652 cm^{-1}, which is the canonical position of the α-helix. Two other component bands at 1674 cm^{-1} (16%) and 1663 cm^{-1} (22%) were assigned to β-turns. The main amide I component of salmine was at 1642 cm^{-1}, with 42% of the total intensity. Vibrations in this region are usually assigned to random coil/flexible regions, lacking stable patterns of intramolecular hydrogen bonding. Deconvolution of the amide I of squid protamine in sperm nuclei gave the same components as those observed in salmine. The β-turn components had similar proportions to those in salmine: 17% at 1669 cm^{-1} and 20% at 1662 cm^{-1}. In contrast, the α-helix was dominant with 40% of the total intensity, while the component of random coil/flexible regions decreased to 23%. β-Structure components were not observed in either salmine or in squid protamine (Figure 3).

Models of nucleoprotamine have the hexagonal packing of the DNA molecules in common, but differ in the conformation and location of the protamine in the complexes.[6–8] Some models assume that the protamine follows the path of either the narrow or the wide groove of DNA, with the guanidinium groups of consecutive arginines binding alternatively to the phosphate groups of either strand of the DNA double helix. In these models, the stretches of arginine lack intramolecular hydrogen bonding and, therefore, of a secondary structure of its own. Other models assume that the stretches of arginine adopt an α-helical structure when bound to DNA. In general, models of nucleoprotamine assume a uniform conformation for the arginine tracks and even for the entire protein. Our results show that the secondary structure of protamine in sperm nuclei



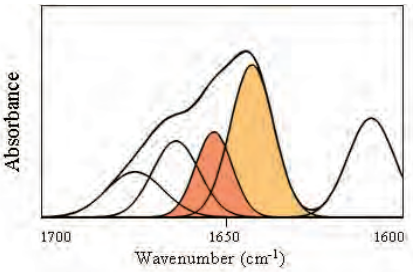


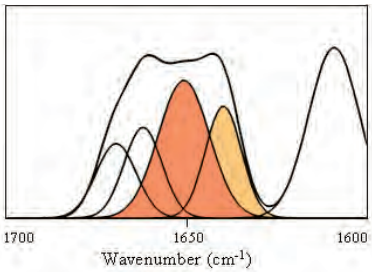
Percentages (%) of secondary structure of the DNA-bound unphosphorylated and triphosphorylated carboxy-terminal domain of histone H1° in 140 mM NaCl

Assignment	r=0.7			
	0p		3p	
	Band (cm^{-1})	%	Band (cm^{-1})	%
Turns	1677	6		
Turns	1668	13	1671	4
Turns	1659	15	1659	14
α-helix	1650	24		
Random coil/flexible regions	1639	18	1646	6
β–sheet	1630	16	1636	52
Low frequency β-sheet	1617	8	1623	24

The values corresponding to the unphosphorylated (0p) and the triphosphorylated domain were taken from Reference 2 and Reference 5, respectively

Figure 2. Amide I decomposition of the DNA-bound carboxy-terminal domain of histone $H1^0$. 0p, unphosphorylated domain. 3p, triphosphorylated domain. The protein/DNA ratio (*r*) was 0.7 w/w. The spectra were measured in D_2O. The buffer was 10 mM HEPES plus 140 mM NaCl, pH 7.0, at 20°C. Protein concentration was 5 mg mL^{-1}. β-structure components are highlighted in blue and the α-helix component is highlighted in red. The table shows the percentages of the secondary structure motifs.





Percentage of secondary structure of salmon and squid protamine in sperm nuclei in 140 mM Nacl

Assignment	Salmon protamine		Squid protamine	
	Band (cm^{-1})	%	Band (cm^{-1})	%
Turn	1674	16	1669	17
Turn	1663	22	1662	20
α-helix	1652	20	1650	40
Random coil/flexible regions	1642	42	1640	23
Side chain	1609	–	1609	–

The data were taken from Reference 2.

Figure 3. Amide I decomposition of salmine and squid protamine in purified sperm nuclei. Spectra were measured in D_2O. The buffer was 10 mM HEPES plus 140 mM NaCl, pH 7.0, at 20°C. The concentration of nuclei expressed as DNA concentration was 5 mg mL^{-1}. The peak at 1609 cm^{-1} corresponds to the arginine side-chains plus, in the case of squid protamine, a contribution from tyrosine side-chains. The α-helix component is highlighted in red and the random coil/flexible component is highlighted in orange. The table shows the percentages of secondary structure of salmon and squid protamines in sperm heads.

is heterogeneous and it contains α-helix, β-turns and non-hydrogen bonded conformations. The results showing large differences in secondary structure between salmine and squid protamine indicate that there is not a single conformation for protamine in sperm nuclei, in spite of the common hexagonal packing of DNA molecules.

Conclusions

The possibility of deconvoluting the amide I band in components arising from different secondary structure motifs after precise subtraction of the DNA contribution, together with the insensitivity of IR spectroscopy to light scattering artefacts, has allowed us to show that both the CTD of histone H1 and protamines behave as intrinsically disordered proteins with coupled binding and folding. The CTD becomes fully structured upon interaction with DNA. The DNA-bound structure contains α-helix, loops/flexible regions, turns and β-structure. Phosphorylation can have profound effects on the DNA-bound structure. In sperm nuclei, protamines contain large amounts of defined secondary structure (α-helix and turns) stabilised by intramolecular hydrogen bonding.

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