Peptide libraries as tools for studying histone interactions

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Abstract

Libraries of synthetic peptides can be used to investigate the post-translational modifications of the amino acids found in histones. These libraries can be printed as micro arrays, which enable large numbers of modifications to be screened simultaneously.

Histone proteins

Histones are the family of proteins that are the major constituent of chromatin, which acts as the regulator for DNA coiling, supercoiling and gene expression in eukaryotes, cells with nuclei. The alkaline histone molecules form complexes with the acidic DNA, locking it into place and enabling it to be packed very efficiently. The sequences of histone proteins are highly conserved between species and they also exhibit extensive post-translational modifications to the amino acids lysine, arginine, threonine and serine. These modifications, which alter the overall charge of the amino acid, act as 'molecular switches', altering the structure and function of the histones and have been discovered to play such a significant part in gene expression that the term 'Epigenome' is used to describe them. The action of these 'switches' is highly sophisticated and extensive research is currently underway in order to unravel their significance[1]. The locations, identity and frequency of the modified amino acids cause the genes of the organism to be expressed differently without any underlying change in the DNA of the genes themselves.

Peptide synthesis

As the modified amino acids found in histones are all now commercially available in forms suitable for solid phase peptide synthesis, it is now relatively straightforward to make peptides containing these modifications at any desired amino acid residue in the sequence.

Table 1 shows the side chain modifications, methylation of arginine and lysine, acetylation of lysine and phosphorylation of serine and threonine. During peptide synthesis, although some of the amino acids require extended coupling times, no major changes are required to the normal synthesis protocols and few significant side reactions are observed. The other two major histone modifications, sumoylation and ubiquitinylation, cannot be achieved by simple chemical synthesis so will not be discussed here.

Using standard chemistry, conventional synthesis instruments are used to make individual peptides or parallel synthesis machines can be used to make libraries of hundreds of histone peptides where every permutation and combination of side chain modification can be incorporated.

Table 1. *Amino acid modifications found in histones.*

Amino Acid	Side Chain Modifications
Arginine	Monomethyl, Dimethyl
Lysine	Acetyl, Monomethyl, Dimethyl, Trimethyl
Serine	Phospho
Threonine	Phospho

The instruments designed by Alta Bioscience can produce up to 6 x 96 well trays of peptides in one operation, in a format readily compatible with laboratory automation.

Micro arrays

Printing of histone peptide micro array slides[2] may be done with any commercial spotter, or in the case of Alta Bioscience, a custom made, contact spotter is used, capable of imprinting the exact layout of a micro titre plate, in duplicate, on up to 72 slides at a time. The spot diameter of 500micron, although large by modern micro array standards, has the advantage of easy visualisation and enables quantification of data.

Peptide design

A common strategy for studying antibody binding is to have the antigen, in this case a library of histone peptides, bound to a substrate such as a micro titre plate or glass micro array slide, as shown in Figure 1 below. Immobilisation is usually achieved by labelling the peptide antigen with biotin at either the amino or carboxy termini and binding the biotinylated peptide to a streptavidin coated surface. A spacer, usually aminohexanoic acid or a short PEG molecule, is required between the biotin and the rest of the peptide to ensure the peptide is sufficiently far enough away from the binding surface, preventing steric hindrance. Labelling with biotin enables precise orientation of the peptide chain on the substrate, allowing the chain to be fully exposed for any protein interaction.

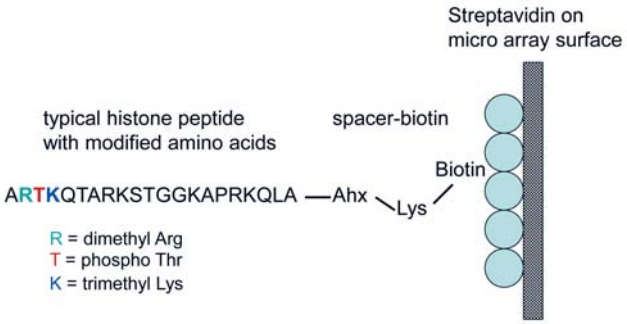

Figure 1. *Typical method of attaching a peptide to a surface.*

The choice of terminus for the biotin depends on the location of the peptide in the intact sequence. If the peptide is from an internal sequence, then it is usually easiest and cheapest to have the biotin-spacer at the N-terminus.

However, peptides derived from the N-terminal regions require their amino terminus to be free and available for interactions, so in these instances, the biotin-spacer tag must be at the carboxyl end of the peptide as shown in Figure 1.

Currently three peptide libraries are available[3], each having 94 different sequences, covering the following histones:-

1. Histone H3
2. Histone H2a, H2b and H4
3. Histone H3 and H4, N-terminal regions

Libraries 1 and 2 have the biotin anchor at their amino terminus while library 3 has the biotin at the C-terminus, leaving the amino terminal completely exposed and available for protein binding. The peptides in libraries 1 and 2 all have a C-terminal amide group which removes the positive charge which would otherwise be present, making them more like the natural material. Each library includes all the common permutations of modifications of methylation, acetylation and phosphorylation.

Each of these libraries is available in three formats:-

1. Micro arrays on glass slides.
2. Coated onto streptavidin micro titre plates.
3. As 'free', soluble peptides.

Uses for the libraries

Two common uses for histone peptides in micro array format are the screening of histone antibodies and the study of protein – histone interactions. Large numbers of anti-histone antibodies are now commercially available and their specificity is often much broader than their data sheets suggest. By printing histone libraries as micro arrays, it is relatively straightforward to coat an antibody over a micro array and readily observe the extent of any cross reactivity to 94 different sequences in one operation. In a similar fashion, the affinity of other histone binding proteins such as methylases and transferases to specific peptide sequences can be investigated.

The following figures show an example of how proteins specific to methylated peptides can be examined. Figure 2 shows the array of different modified peptides attached to the surface of the micro array slide.

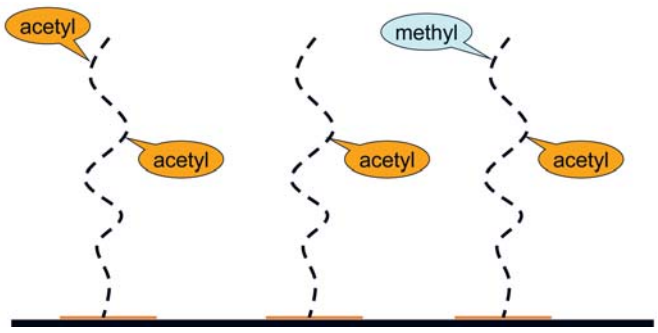

Figure 2. *The modified histone peptides are attached to the streptavidin coated surface with biotin.*

The micro array is then probed with the protein under investigation as shown in Figure 3. The binding affinity of the protein to the range of peptides is demonstrated by washing the array with a series of buffers of increasing salt content. High affinity binding being apparent where the protein remains bound at the maximum salt concentration.

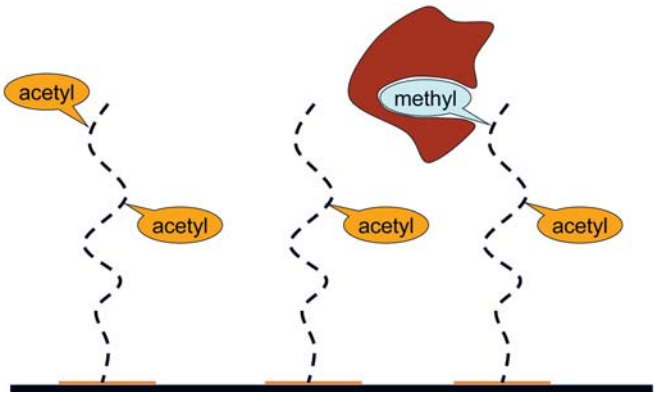

Figure 3. *The immobilised peptides are probed with the protein of interest.*

After excess probe protein has been washed away, it can be visualised by reacting with a labelled secondary antibody as shown in Figure 4.

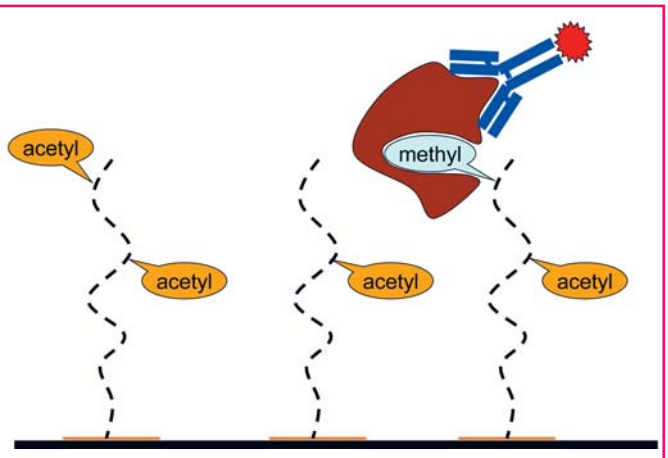

Figure 4. *Visualisation of any binding with labelled secondary antibodies.*

Detection methods

If the binding proteins are labelled with a suitable fluorescent dye, it is possible to directly observe them as they bind to the immobilised peptides. However, it is usual to use a labelled secondary antibody as a detection system. The dyes used can be of any convenient wavelength, depending on the scanner/detection system available. It has been found that IR dyes give very good signal to noise ratios.

References

1. Turner. B. M. "Defining an epigenetic code". Nature Cell Biology 9, 2 - 6 (2007)
2. USPTO Patent Application 20090149343, Histone peptide microarrays.
3. Peptide sequences. www.altabioscience.bham.ac.uk/services/peptide/histonearrays.shtml

Author profiles

Dr John Fox is the Director of Alta Bioscience. He has been involved with peptide synthesis for over 25 years and has designed and built an entire range of peptide synthesis instruments.
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