Exploring the Human Acetylome Using High-Density Peptide Microarrays

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Modification of lysine residues in proteins represents a dynamic and reversible posttranslational modification, playing a major role in regulating transcription, protein stability and activity of metabolic enzymes. Mass spectrometric investigations identified several thousand acceptor sites in human proteins. We created a peptide microarray displaying all known human lysine acetylation sites. Such peptide microarrays enable both, analysis of binding specificities of acetylation-specific protein domains, and determination of subsite specificities of lysine-acetyltransferases and lysine-deacetylases.

Introduction

Modification of lysine residues in proteins, especially acetylation, represents a dynamic and reversible posttranslational modification playing a major role in regulating transcription and metabolic processes. Regulation of enzymatic activities by reversible acetylation is highly conserved and seems to be one of the most abundant modifications of proteins. Originally, this modification was discovered for histone proteins about 40 years ago. However, the extent of this modification in other cellular proteins, so-called non-histone targets, is largely underestimated. Protein acetylation levels are regulated by lysine-acetyltransferases and lysine-deacetylases. Recent mass spectrometric investigations identified more than 5,500 acetylation sites in human proteins. We created a high-density peptide microarray displaying more than 6,800 peptides derived from all known human lysine acetylation sites in both unmodified and acetylated form resulting in more than 13,600 different features per microarray pair (**Deacetylase & Acetyltransferase Microarrays**).

Each of these features is displayed in triplicates for quality control, resulting in more than 40,000 data points per experiment. Such Acetylome Microarrays were used to analyze the reactivities of commercially available, pan-specific anti-acetyl-lysine-antibodies. Treatment of acetylome microarrays with either lysine-deacetylases or lysine-acetyltransferases in the presence of acetyl-CoA followed by an optimized mixture of anti-acetyl-lysine-antibodies yielded unique substrate patterns enabling deduction of subsite specificity profiles for each individual enzyme. Additionally, we were able to analyze

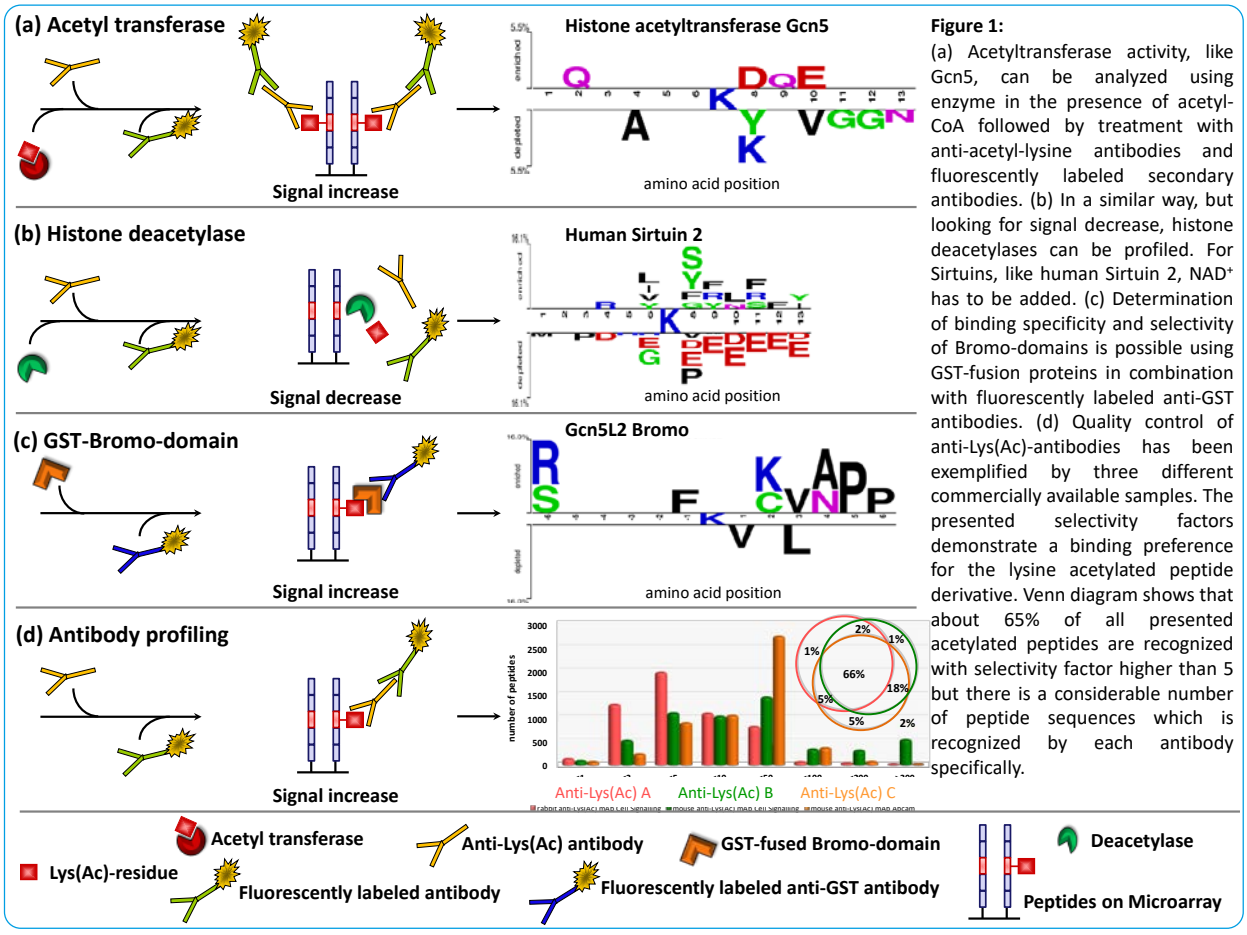

Figure 1:
(a) Acetyltransferase activity, like Gcn5, can be analyzed using enzyme in the presence of acetyl-CoA followed by treatment with anti-acetyl-lysine antibodies and fluorescently labeled secondary antibodies. (b) In a similar way, but looking for signal decrease, histone deacetylases can be profiled. For Sirtuins, like human Sirtuin 2, NAD^+ has to be added. (c) Determination of binding specificity and selectivity of Bromo-domains is possible using GST-fusion proteins in combination with fluorescently labeled anti-GST antibodies. (d) Quality control of anti-Lys(Ac)-antibodies has been exemplified by three different commercially available samples. The presented selectivity factors demonstrate a binding preference for the lysine acetylated peptide derivative. Venn diagram shows that about 65% of all presented acetylated peptides are recognized with selectivity factor higher than 5 but there is a considerable number of peptide sequences which is recognized by each antibody specifically.

the binding specificities of acetyl-lysine-specific protein domains using GST-fusion of these domains in combination with fluorescently labeled anti-GST-antibodies.

Results

We developed an antibody-based readout assay for the analysis of enzymatic activities of epigenetic targets like lysine-acetyltransferases and lysine-deacetylases. Acetylome Microarrays were used to profile the (cross) reactivities of different, commercially available anti-acetyl-lysine-antibodies (see **Figure 1d**).

Because pairs of peptides were displayed onto the microarrays differing in the acetyl residue on one lysine side chain only, we were able to extract selectivity factors for each pair. The selectivity factors show how much better the acetylated peptide is recognized compared to the non-acetylated form. Available anti-acetyl-lysine-antibodies differ regarding their selectivity factors and subsite specificities (see Venn diagramm in **Figure 1d**). The majority of the acetylated peptides is recognized by all or at least two antibodies. Nevertheless, each of the tested antibodies binds to several unique epitopes. Therefore, we decided to use an optimized mixture of the 3 different anti-acetyl-lysine-antibodies depicted in **Figure 1d** for the profiling of the epigenetic targets.

Treatment of the acetylome microarray with acetyltransferases, like p300, PCAF, Tip60 or Gcn5, in the presence of acetyl-CoA followed by treatment with the optimized anti-acetyl-lysine-antibody mixture yielded information about amino acid residues which are preferred or disfavoured in the different subsites (see **Figure 1a**). Moreover, identified peptide substrates are derived from naturally occurring proteins enabling deduction of potential in vivo targets of the enzymes. Treatment of the acetylome microarray with Sirtuins, NAD^+-dependent protein lysine-deacetylases involved in regulation of central physiological functions, such as energy metabolism, cell cycle progression, and aging processes, resulted in decrease of signals for several of the acetylated peptides in comparison to the control experiment in the absence of NAD^+. We were able to identify subsite specificities (see **Figure 1b**) and substrate sequences for all of the seven human Sirtuin isoforms. In addition to known substrates our results provide novel substrate candidates for the different Sirtuin isoforms which could be confirmed in subsequent solution phase experiments.

Principally, each enzyme transferring residues to the side chains of lysines could be analyzed with the acetylome microarray using either radioisotopic labelling (lysine-methyltransferases and labeled AdoMet, poly-ADP-ribosyltransferases and labeled NAD^+) or antibody-based readout (poly-ADP-ribosyltransferases and ethenoNAD$^+$ followed by anti-ethenoNAD$^+$-antibody, ubiquitin/SUMO transferring enzymes followed by anti-ubiquitin/SUMO-antibodies). Moreover, investigation of crosstalk for different posttranslational modifications is possible with the acetylome microarray. Treatment of the microarray with different kinases followed by fluorescently labeled phospho-specific stains showed that phosphorylation reactions are influenced by acetyl-lysine residues surrounding the phosphorylation site.

Enzymatic modification of proteins could be recognized by protein domains specific for the appropriate posttranslational modification. BROMO-domains are claimed to be specific for lysine-acetylated peptide motifs. Using GST-fusions of BROMO-domains in combination with the acetylome microarray allows reliable determination of binding specificities of the individual domains (see **Figure 1c**) in a similar way as described for GST-fusions of human SH3 domains on peptide microarrays displaying proline-rich peptides (1). Profiling of the human BROMO-domains together with the human lysine-acetyltransferases on the acetylome microarray will yield a comprehensive understanding of how the action of lysine-acetyltransferases forces BROMO-domain mediated protein complex formation in signal transduction.

Conclusion

Peptide microarrays represent a robust platform for the reliable identification of binding specificities of protein domains and subsite specificities of epigenetic targets involved in the human acetylome.

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Reference

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