

Enzyme Substrate Microarrays

ENZYME PROFILING

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. Acetyloyme 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 diagram 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 acetyloyme 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 acetyloyme 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 acetyloyme 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 acetyloyme 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 acetyloyme 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 acetyloyme 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 acetyloyme.

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