Abstract

The insertion of posttranslational modifications (PTMs) is a well-known method exploited by organisms to expand the diversity of the proteome. Protein PTMs span from small groups, such as phosphorylation and acetylation, to actual proteins, such as ubiquitination and SUMOylation. Regardless of the moiety installed, PTMs feature dramatic effects on protein activity, localization, and interaction with other proteins. Among the known PTM categories, research in lysine acetylation has seen a renewed interest during the last twenty years, due to the simultaneous expansion of epigenetics. Additionally, the recent discovery of alternative acyl groups on lysine side chain has revealed the complexity of this regulatory system. As a result, the enzymes involved in the regulation of the acyl PTMs, such as histone deacetylases (HDACs), are currently investigated as pharmacological targets in several pathological conditions, such as cancers, inflammation, and neurodegenerative disorders. It is thus pivotal to characterize this family of enzymes in order to better understand their biological role. In order to do so, the development of methods for the incorporation of lysine acyl PTMs into proteins is instrumental, as this will enable the study of these modifications, and the enzymes involved, in more detail.

Substrate characterization of HDACs was addressed by designing, synthesizing, and testing a small library of fluorogenic chemotypes with potential as substrates. Preliminary screening experiments on class III HDACs (SIRTs) revealed the preference of SIRT2 for a myristoylated substrate (ETDKmyr), which was further kinetically characterized. Due to the favorable properties of the fatty acylated substrate, an inhibition assay for SIRT2 as deacylase was developed. In order to improve our assay and make it more easily accessible to other laboratories, the synthesis of ETDKmyr was optimized and resulted in a procedure that is scalable and features no purification steps. A substrate screening for class I,II and IV HDACs showed, interestingly, that the same myristoylated substrate was efficiently converted by HDAC11. This enzyme was originally described as a bona fide deacetylase, but a robust activity was never confirmed in vitro. A selection of long chain acylated substrates and known inhibitors were screened, which led to the identification of a novel type of substrate for the enzyme.

The development of methods for the installation of acyl PTMs on lysine residues was investigated for lysine crotonylation, a modification that was recently discovered. Currently, the crotonyl PTM has been incorporated into proteins only via amber codon suppression. For this reason, a chemical approach would be of great value. Mimicking of a crotonylated lysine via cysteine alkylation was explored and preliminary results are described. In parallel, a masked crotonylated lysine residue was designed, so that it could be used as building block for the synthesis and ligation of a peptide to a protein. Direct comparison of the masked analog with the crotonylated peptide showed that our approach can be beneficial, even though further investigation is required.