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Commentary: Biochemistry

Non-Enzymatic Acetylation in Alkaline Environments

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Abstract. The mechanism behind the acetylation reactions in mitochondria has remained a mystery for a long time, until now. A recent study by Wagner and Payne (2013) revealed that mitochondrial proteins are acetylated non-enzymatically and such a mechanism has far reaching consequences in human diseases.

Protein acetylation is an important post-translational process that occurs on lysine residues and alters the function of various proteins, such as histones and mitochondrial proteins NADH dehydrogenase and malate dehydrogenase (Kim *et al.*, 2006). The modification of these and other enzymes in the mitochondria regulates various metabolic pathways. Acetylation of mitochondrial proteins is generally associated with the inhibition of their function. Hence, if this modification is not controlled, excessive mitochondrial acetylation could occur, leading to mitochondrial dysfunction. Indeed, increased mitochondrial protein acetylation has been linked to various diseases, such as metabolic diseases (Newman *et al.*, 2012) and cardiac hypertrophy (Hafner *et al.*, 2010).

The mechanism behind the acetylation of mitochondrial proteins has eluded researchers for long as there are no known mitochondrial acetyltransferases. A new study by Wagner and Payne (2013) demonstrated a possible enzyme-independent deacetylation mechanism for mitochondrial proteins. Wagner and Payne reported that the high pH and the millimolar concentrations of acetyl-CoA in the mitochondrial matrix are sufficient to induce non-enzymatic protein lysine acetylation. The researchers carried out their experiments under *in vitro* conditions using isolated mitochondria to ensure the absence of any enzymatic interference from non-mitochondrial enzymes. Firstly, they examined the effects of pH, incubation times, and acetyl-CoA concentrations on protein acetylation in isolated mitochondria. A pH-, dose-, and time-dependent increase in protein acetylation in mitochondria was observed. Furthermore, the researchers showed that a concentration of 1.5 mM acetyl-CoA at pH 8.0 alone was able to

induce protein acetylation of the non-mitochondrial protein bovine serum albumin (BSA). A similar phenomenon involving lysine succinylation was observed when BSA was subjected to equivalent concentrations of succinyl-CoA at pH 8.0.

The researchers also raised the possibility of enzyme-mediated mitochondrial protein acetylation and discussed the need to characterise the contribution of potential candidate enzymes such as GCN5L1 to protein acetylation in the mitochondrial matrix. It is however difficult to analyse matrix acetyltransferases as the very conditions that these enzymes would operate in would promote non-enzymatic acetylation. To resolve this complication, mitochondrial protein acetylation could be measured at pH 7.0 such that there is minimal non-enzymatic acetylation. A study that involves knocking down candidate acetyltransferases can then be carried out, followed by isolation of mitochondria and carrying out an *in vitro* acetylation assay of mitochondrial proteins at pH 7.0. Although it is expected that protein acetylation under non-optimal conditions will be lower, the experiments could reveal the involvement of enzymes to mitochondrial protein acetylation.

The research by Wagner and Payne (2013) is likely to have far reaching implications. As raised earlier, hyperacetylation of mitochondrial proteins is known to be involved in various diseases. Wagner and Payne (2013) demonstrated that the higher the pH of the medium, the greater the rate of non-enzymatic acetylation. A higher than normal pH in the mitochondrial matrix can be caused by hyperpolarisation of the inner mitochondrial membrane, leading to a steeper proton gradient and causing matrix alkalinisation. Protein acetylation may thus be induced by matrix alkalinisation under physiological conditions.

Matrix alkalinisation can arise as a result of various factors. For instance, matrix alkalinisation can occur as a result of reverse F0F1 ATP synthase activity. ATPase Inhibitory Factor 1(ATPIF1) is an enzyme that inhibits the ATPase activity of ATP synthase. Silencing of ATPIF1 has been reported to lead to an increase in matrix pH (Shah *et al.*, 2012). In pancreatic β cells, nutrient stimulation is able to stimulate matrix alkalinisation, which in turn promotes ATP synthesis (Wiederkehr *et al.*, 2009). These findings suggest that matrix alkalinisation may act as a form of signalling mechanism that regulates mitochondrial activity through changes in protein acetylation levels.

Could matrix alkalinisation be a cause of hyperacetylation in human diseases? There is a need to first establish the rates of non-enzymatic acetylation and the rates of enzymatic deacetylation by SIRT3 and SIRT5 beyond pH 8.0. If indeed the rates of acetylation exceed that of deacetylation, matrix alkalinisation can be a potential cause of hyperacetylation. It would be interesting to determine if conditions that induce matrix alkalinisation *in vivo* result in increased non-enzymatic acetylation of mitochondrial matrix proteins. On the other hand, conditions that decrease matrix alkalinisation *in vivo*, such as the expression of uncoupling protein-1(UCP1) in brown adipose tissue, might decrease mitochondrial protein acetylation. Such studies may reveal an important link between matrix alkalinisation and hyperacetylation under *in vivo* conditions.

Non-enzymatic mitochondrial protein acetylation has far reaching implications for human diseases as well as ageing. Future studies will likely reveal whether non-enzymatic acetylation

of matrix proteins is merely due to an undesired consequence of conditions that are present in the mitochondrial matrix or whether such a process can play an adaptive and protective role under specific conditions.

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