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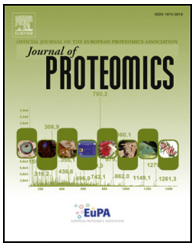

Characterisation of the influences of aspirin-acetylation and glycation on human plasma proteins

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ABSTRACT

The competition effect between aspirin-mediated acetylation and protein glycation has been a matter of concern for decades. However, the exact interactions between these two post-translational modifications are still not well understood. Several efforts have been made to explain how aspirin prevents glycation, but the influence of prior protein glycation on the action of aspirin has never been investigated. This study involved qualitative and quantitative analyses to: 1) identify acetylated and glycated proteins; 2) quantify rates of acetylation and glycation; and 3) elucidate the common modification sites. Human plasma was incubated with 30 mM glucose and then 500 μM aspirin. A label-free mass spectrometry approach indicated an increase in the acetylation level after this sequential glucose-then-aspirin incubation; these results were also confirmed by Western blot. Interestingly, for several proteins, decreases in glycation levels were evidenced after aspirin incubation. The common modification sites, where both acetylation and glycation took place, were also identified. The influence that glycation and acetylation processes have on each other could reflect conformational changes induced by glucose and aspirin. In future studies, in order to better understand the interactions between these two PTMs, we intend to apply this strategy to other blood compartments and to diabetic patients.

Biological significance
Non-enzymatic glycation represents an early stage in the development of the long-lasting complications that are associated with diabetes. Aspirin has been shown to prevent this process in a few reference proteins, but how the two post-translational modifications (PTMs) of aspirin-mediated acetylation and protein glycation interact with each other remains poorly investigated. This study used a label-free quantitative proteomic approach to characterise the extent of aspirin-induced acetylation and protein glycation in human plasma. The results clearly supported a mutual influence between these PTMs, which lead us to propose a potential model based on structural conformational changes.

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1. Introduction

Perturbations to glucose homeostasis, observed in type 1 and type 2 diabetes, develop in situations of hyperglycaemia. Specific perturbations not only determine the disease type, but they are also the cause of long-term complications, which include diabetic neuropathy [1], nephropathy [2], retinopathy [3] and cardiovascular diseases [4,5]. Circulating glucose reacts with proteins through a spontaneous, non-enzymatic post-translational modification (PTM), the rate of which is principally governed by blood glucose concentrations; this PTM is enhanced under hyperglycaemic conditions. The glycation process is the first step in a series of reactions that culminate in the development of the long-lasting deleterious effects typical of diabetes [6–9]. Most of these disorders are correlated to protein structure/function alterations. These include impairment of human albumin's drug-binding capacity [10,11] or its antioxidant activity [12,13]; the glycation of apolipoproteins and extracellular matrix proteins, which have been shown to be correlated to the pathogenesis of atherosclerosis [14–17] and an increased risk of cardiovascular diseases [18]; and the greatly enhanced toxicity of β-amyloid by glycation modification, that in turn increases the rate of protein aggregation into fibrils typical of neurodegenerative diseases [19,20].

Several efforts have been made to prevent excessive human protein glycation and most of these have concentrated on using compounds that act at different stages of the glycation process [21]. Among these, aspirin was probably the first agent shown to protect against protein glycation and to be associated with a decrease in cardiovascular events [22,23]. Aspirin acts through the acetylation of the reactive amino groups on proteins, i.e. the functional groups that are also the targets for glycation. Up until now, the aspirin's impact on protein glycation has been assessed using single reference proteins such as lens crystallins [24,25], collagen [26,27], haemoglobin [28] and fibrinogen [29], as

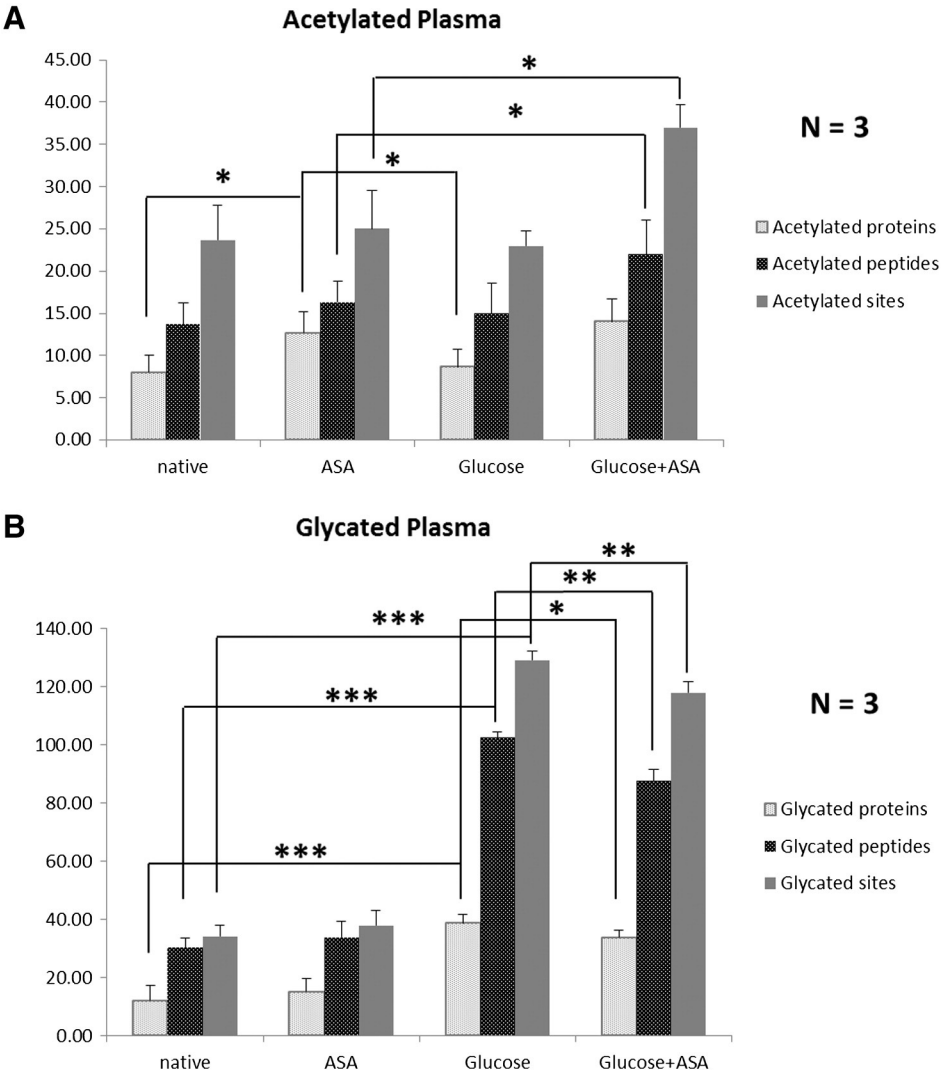

Fig. 1 – The mean number of unique proteins, peptides and sites detected on the acetylation (A) and glycation (B) data sets in native plasma, after 30 min 500 μM aspirin, after 24 h 30 mM glucose and after a sequential incubation of glucose followed by aspirin exposition at the same conditions (N = 3). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.005$.