

POTENTIAL PROTEIN POST-TRANSLATIONAL MODIFICATION IN ADIPONECTIN

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Key words: post-translational modifications, adiponectin, diabetes mellitus

SUMMARY

Post-translational modifications of proteins control many biological processes. This is also an important process in the pathogenesis of diabetes mellitus. Adiponectin is an insulin-sensitizing adipokine with antidiabetic, anti-atherogenic, anti-inflammatory and cardioprotective properties. The role of post-translational modifications in regulating the biosynthesis of high molecular weight adiponectin has presently come into the very focus of interest. In this study, the potential protein post-translational modifications in adiponectin were determined by a standard bioinformatics technique. Two vulnerable sites within the adiponectin molecule for post-translational modification were successfully identified and are reported herewith. This new knowledge can be useful for better understanding of the pathogenesis of diabetes relative to adiponectin and for developing new antidiabetic drugs.

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INTRODUCTION

Post-translational modifications of proteins control many biological processes, and examining their diversity is critical for understanding the mechanisms of cell regulation (1). This is also an important process in the pathogenesis of diabetes mellitus. Adiponectin is an insulin-sensitizing adipokine with antidiabetic, anti-atherogenic, anti-inflammatory and cardioprotective properties (2). Post-translational modifications of this protein have been proposed in association with diabetes mellitus type 2. The role of post-translational modifications in regulating the biosynthesis of high molecular weight adiponectin has presently come into the very focus of interest (2). Basically, post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex (3).

However, there is no in-depth study on post-translational modifications of this protein. In this work, the potential protein post-translational modifications in adiponectin protein were determined by a standard bioinformatics technique.

MATERIALS AND METHODS

Obtaining the sequence for adiponectin

The PubMed database (www.pubmed.com) was used to search for the amino acid sequence for adiponectin protein. The derived sequence was used for further predictive study.

Finding potential protein post-translational modifications

The derived amino acid sequence of adiponectin was further manipulated by the FindMod bioinformatics tool. Basically, it is a tool that can predict the potential protein post-translational modifications and find potential single amino acid substitutions in peptides. It examines peptide mass fingerprinting results of known proteins for the presence of 22 types of post-translational modifications of discrete mass: acetylation, amidation, biotin, C-mannosylation, deamidation, N-acyl diglyceride cysteine (tripalmitate), FAD, farnesylation, formylation, geranyl-geranyl, gamma-carboxyglutamic acid, O-GlcNAc, hydroxylation, lipoyl, methylation, myristoylation, palmitoylation, phosphorylation, pyridoxal phosphate, pyrrolidone carboxylic acid, and sulfatation. This is done by looking at mass differences between experimentally determined peptide masses and theoretical peptide masses calculated from a specified protein sequence (4). This tool is confirmed for reliability and accuracy comparing to mass spectrometry (4). The operative parameters in this study were tolerance equal to ± 0.5 Dalton and enzymes were set as (a) trypsin, allowing for up to 3 missed cleavages, (b) cysteine in reduced form, with acrylamide adducts, (c) methionine in oxidized form, and (d) tryptophan in oxidized form.

RESULTS

According to the search, adiponectin could be derived (ACCESSION ABZ10942) (Fig. 1). According to this work, 2 post-translational modifications could be identified (Table 1).

Table 1. **Potential post-translational modifications**

Potential modification	Peptide	Position
METH-SULF	K	101
METH-SULF	K	178

Figure 1. **Derived peptide sequences.**

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1 mlllgavll lalpghdqet ttqggpvllp lpgactgwm agipghpghn gapgrdgrdg
61 tpgekgekdg pgligpkgdi getgvpgaeg prgfpgiqgr kgepgegayv yrsafsvgle
121 tyvtipnmpi rftkifynqq nhydgstgkf hcniplgyyf ayhitvymkd vkvslfkkdk
181 amlftydqyq ennvdaqsgs vllhlevgdq vwlqvyegege rnglyadndn dstftgflly
241 hdtm

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DISCUSSION

Adiponectin is a recently described adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation (5). It is classified as a key adipokine in the metabolic syndrome (5). An increased amount of adipose tissue or its disproportionate distribution between central and peripheral body regions is related to the development of insulin resistance, type 2 diabetes mellitus, dyslipidemia, atherosclerosis, and coronary artery disease (6). In addition, expression and plasma levels of adiponectin, an insulin-sensitizing effector, are down-regulated in obesity (7). In conclusion, adiponectin plays a crucial role in the development of diabetes mellitus and high adiponectin levels should protect against impairment of glucose metabolism (8).

Post-translational modifications of adiponectin can be detected. Basically, the administration of recombinant adiponectin can increase glucose uptake and fat oxidation in muscle, reduce fatty acid uptake and hepatic glucose production in the liver, and improve whole body insulin resistance. However, the exact receptor and signaling systems are unknown but it is believed to be due to adiponectin activating 5' AMP-activated protein kinase (AMPK), a putative master metabolic regulator (9). AMPK is activated by rising AMP and falling ATP, either by inhibiting ATP production or by accelerating ATP consumption, by a complex mechanism and the system also regulates food intake and energy expenditure at the whole body level, in particular by mediating the effects of adipokines such as leptin and adiponectin (10). These processes are fundamental for many diabetic drugs. Vaspin is the best known novel drug the action of which is related to adiponectin. It is indicated that vaspin may be the compensatory molecule in the pathogenesis of metabolic syndrome (11). Hydroxylation and glycosylation of several conserved lysine

residues in the collagenous domain of adiponectin are necessary for the intracellular assembly and stabilization of its high-order oligomeric structures (2). This process is also related to the response to the diabetic drug. In the present study, the author successfully identified two vulnerable sites within the

adiponectin molecule for post-translational modification. This new knowledge can be useful for better understanding the pathogenesis of diabetes as related to adiponectin and for developing new antidiabetic drugs.

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