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Review

Function and metabolism of sirtuin metabolite *O*-acetyl-ADP-ribose

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ABSTRACT

Sirtuins catalyze the NAD^+-dependent deacetylation of target proteins, which are regulated by this reversible lysine modification. During deacetylation, the glycosidic bond of the nicotinamide ribose is cleaved to yield nicotinamide and the ribose accepts the acetyl group from substrate to produce *O*-acetyl-ADP-ribose (*O*AADPr), which exists as an ~50:50 mixture of 2′ and 3′ isomers at neutral pH. Discovery of this metabolite has fueled the idea that *O*AADPr may play an important role in the biology associated with sirtuins, acting as a signaling molecule and/or an important substrate for downstream enzymatic processes. Evidence for *O*AADPr-metabolizing enzymes indicates that at least three distinct activities exist that could modulate the cellular levels of this NAD^+-derived metabolite. In *Saccharomyces cerevisiae*, NUDIX hydrolase Ysa1 cleaves *O*AADPr to AMP and 2- and 3-*O*-acetylribose-5-phosphate, lowering the cellular levels of *O*AADPr. A buildup of *O*AADPr and ADPr has been linked to a metabolic shift that lowers endogenous reactive oxygen species and diverts glucose towards preventing oxidative damage. *In vitro*, the mammalian enzyme ARH3 hydrolyzes *O*AADPr to acetate and ADPr. A third nuclear-localized activity appears to utilize *O*AADPr to transfer the acetyl-group to another small molecule, whose identity remains unknown. Recent studies suggest that *O*AADPr may regulate gene silencing by facilitating the assembly and loading of the Sir2–4 silencing complex onto nucleosomes. In mammalian cells, the Trpm2 cation channel is gated by both *O*AADPr and ADP-ribose. Binding is mediated by the NUDIX homology (NudT9H) domain found within the intracellular portion of the channel. *O*AADPr is capable of binding the Macro domain of splice variants from histone protein MacroH2A, which is highly enriched at heterochromatic regions. With recently developed tools, the pace of new discoveries of *O*AADPr-dependent processes should facilitate new molecular insight into the diverse biological processes modulated by sirtuins.

1. Introduction

Sirtuins are a conserved family of protein/histone deacetylases found in organisms ranging from bacteria to humans [1–3]. Members of this family share a similar enzymatic core domain of ~250 amino acids. The founding member Silencing Information Regulator 2 (Sir2) was originally discovered as a gene required for efficient mating in *Saccharomyces cerevisiae* [4]. Yeast Sir2p is involved in many processes that include gene silencing, ribosomal DNA recombination, DNA repair and longevity [5]. In higher organisms, Sir2 orthologs are also linked to lifespan regulation [6,7]. Expressing extra copies of Sir2 homologues leads to apparent lifespan extension in both *C. elegans* and *D. melanoganster* [2]. Although it is not yet clear if Sir2 homologs modulate lifespan in mammals, mammalian sirtuins are reported to induce positive effects on physiological processes, such as DNA repair, cell survival, stress resistance, metabolic control and insulin sensitivity [2]. Moreover, sirtuins may mediate the beneficial effects of caloric restriction (CR) [7–9], which is the only non-genetic method that extends lifespan in nearly all organisms where this regimen has been investigated.

1.1. Discovery of OAADPr

Once yeast Sir2 was identified as an essential factor in gene silencing [4,10,11], it was noted that Sir2 shared sequence homology to a bacterial enzyme, CobB that could partially complement a phosphoribosyltransferase defect in cobalamin biosynthesis [12–14]. Initial investigations of Sir2 enzymatic function reported a protein ADP-ribosylation activity, which required NAD^+ [15,16]. However, a number of subsequent reports began to reveal a more robust activity, NAD^+-dependent histone deacetylation [17–19]. In 2000, the surprising molecular role of NAD^+ in protein deacetylation was revealed [20,21]. The efficient histone/protein deacetylase reaction is tightly coupled to the formation of a novel acetyl-ADP ribose product *O*-acetyl-ADP ribose (*O*AADPr, also abbreviated as AAR and AADPR by some authors). One molecule of NAD^+ and acetyl-lysine are readily converted to one molecule of deacetylated lysine, nicotinamide, and *O*AADPr (Fig. 1). The details of the chemical reaction have been reported [22,23], and will not be reviewed here. However, the idea

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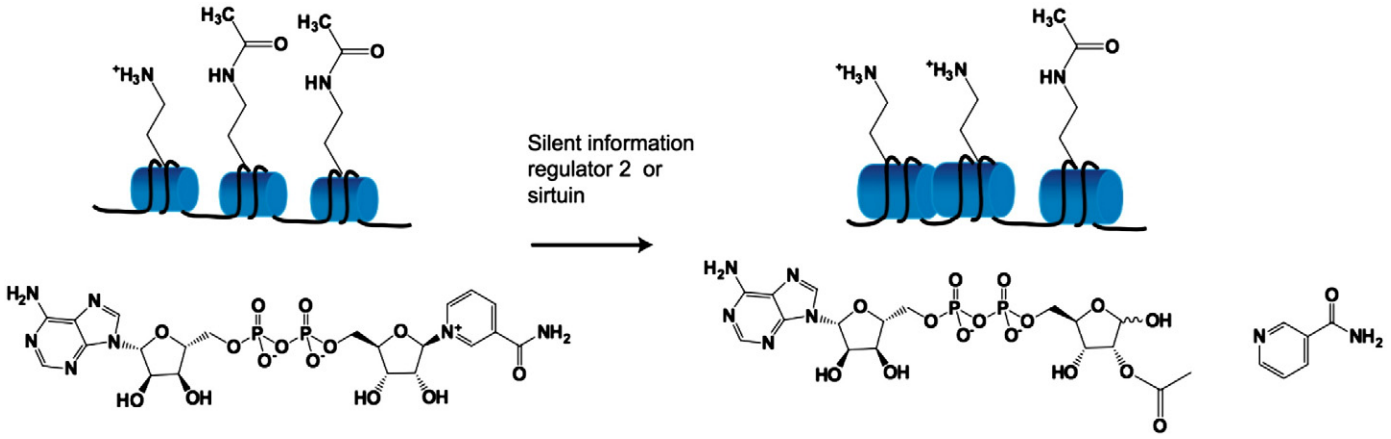

Fig. 1. *O*AADPr production by Sir2/sirtuins deacetylation reaction. Sir2 and sirtuins catalyze NAD^+ dependent deacetylation of histone tails, or other non-histone acetylated proteins. The reaction transfers the acetyl group from acetylated lysine residues to the ADP-ribose moiety of NAD^+, generating deacetylated histone tails, nicotinamide, and the novel metabolite *O*AADPr.

that some sirtuins mediate protein ADP-ribosylation, and not deacetylation, remains active. The potential ADP-ribosylating activity of sirtuins stems primarily from two main observations: Some sirtuins exhibit little or no detectable protein deacetylase activity on tested substrates, and low levels of ADPr transfer to protein are often detected under extremely long incubations with NAD^+. Several published studies have provided evidence that the reported ADP-ribosylation activity of sirtuins represents a slow side reaction that may not reflect an important physiological function of these enzymes [24–28].

From several mechanistic studies, the deacetylation product released by sirtuins appears to be the 2′-*O*-acetyl-ADP-ribose isomer of *O*AADPr [21,23,29]. At neutral pH, 2′-*O*-acetyl-ADP-ribose and 3′-*O*-acetyl-ADP-ribose were found to be the solution products, existing in ~1:1 ratio and generated through a non-enzymatic intramolecular transesterification [23,29] (Fig. 1). The ability of sirtuins to produce *O*AADPr is well conserved ranging from bacterial CobB to mammalian sirtuins ([3,19,27,30]). It is important to note that the energy released by hydrolysis of NAD^+ is 8.2 kcal/mol, in a comparable range for the hydrolysis of ATP to ADP [15,31]. It is striking that Sir2 enzymes (Class III protein deacetylases) retain this energetically expensive mechanism, compared to the Class I and II deacetylases, which directly produce acetate and do not require NAD^+. There is accumulating evidence that the elaborate mechanism of sirtuin catalysis renders *O*AADPr which can elicit downstream responses that might synergize or antagonize the biological functions of sirtuin genes. In the following sections, we will discuss several studies published over the last few years focusing on the metabolism and cellular functions of *O*AADPr.

1.2. *O*AADPr metabolism

After the discovery of *O*AADPr, a quantitative microinjection assay of *O*AADPr into starfish oocytes caused a delay/block in oocyte maturation [32], supporting the hypothesis that *O*AADPr can evoke biological activity. It is worth noting that microinjection of purified sirtuin enzymes also led to the identical effect. Although the molecular basis behind these observations is unknown, it was reasonable to postulate that the metabolism of *O*AADPr *in vivo* might be tightly controlled. Several *O*AADPr-metabolizing enzymes have been reported (Fig. 2). The best characterized are the NUDIX hydrolases (hydrolysis of a nucleoside diphosphate linked to another moiety *x*), including Ysa1 from yeast and NudT5 from mouse [33]. Recombinant Ysa1 and mNudT5 cleave the pyrophosphate bond of *O*AADPr, generating 2- and 3-*O*-acetylribose-5-phosphate and AMP. ARH3 from the human ADP-ribosyl hydrolase (ARH) family hydrolyzes the acetyl group of *O*AADPr, generating ADPr, which might act as a negative feedback inhibitor of ARH3 [34]. Additionally, two uncharacterized enzyme activities detected in both yeast and human cells have been reported, namely, a cytoplasmic esterase that removes the acetyl group from *O*AADPr, and a nuclear acetyltransferase that transfers the acetyl group from *O*AADPr to an unknown molecule [33]. Future identification and detailed analysis of those activities will likely be important for clarifying the physiological roles of *O*AADPr.

1.2.1. *O*AADPr hydrolysis by NUDIX hydrolases

NUDIX hydrolases catalyze the hydrolysis of a nucleoside diphosphate linked to another moiety *x*, thus the abbreviation "NUDIX" [35,36]. They are found in more than 250 organisms and characterized by the highly conserved array of amino acids G*X*5E*X*7-REU*X*EE*X*GU (U represents a bulky, hydrophobic amino acid and *X* represents any amino acid). The nucleoside diphosphate linkage is common to the broad range of substrates utilized by the family, including NADH, dinucleoside polyphosphates, and nucleotide sugars such as ADPr. NUDIX hydrolases are further divided into sub-families based on their substrate specificity. Comparison among the members of the ADPr pyrophosphatase subfamily revealed a conserved proline, 16 amino acid residues downstream of the NUDIX box, which determines a catalytic preference for ADPr. Considering the structural similarity between *O*AADPr and ADPr, several members from the ADPr pyrophosphatase subfamily were analyzed for their activity against *O*AADPr [33]. Ysa1 from *S. cerevisiae* [37], NudT5 from mouse [38] and NudT9 from human [39] belong to this family and exhibit ADPrase activity. Ysa1 and mouse NUDT5 (mNUDT5) are able to catalyze hydrolysis of a variety of ADP sugar conjugates with a preference for ADPr. Human NUDT9 (hNUDT9) is highly specific for ADPr and non-physiological IDPR. The shared mechanism among ADPr pyrophosphatases is a nucleophilic attack by water on the pyrophosphate linkage of the substrate, producing AMP and ribose-5-phosphate. Thus, *O*AADPr hydrolysis yields the corresponding products AMP and 2- and 3-*O*-acetylribose-5-phosphate ([40–42]).

Steady-state kinetic analyses were performed with Ysa1, mNudT5 and NudT9 using *O*AADPr and ADPr as substrates (Table 1) [33]. Ysa1 hydrolyzes *O*AADPr with a ~2 fold lower k_{cat} value (37 s^{-1}) and a ~3.5 fold lower k_{cat}/K_m value (4.5×10^4 M^{-1} s^{-1}) compared to ADPr. mNudT5 catalyzes the hydrolysis of both ADPr and *O*AADPr at a similar maximal rate (0.8–0.9 s^{-1}) and k_{cat}/K_m value (1.96×10^4 vs. 1.73×10^4, respectively). This kinetic analysis demonstrates that Ysa1 and mNudT5 bind and hydrolyze *O*AADPr and ADPr with similar catalytic efficiency (Table 1). In contrast, NudT9 poorly catalyzes hydrolysis of *O*AADPr compared with ADPr. The K_m value for NudT9 catalysis of *O*AADPr is ~500-fold higher than that of ADPr, suggesting that the low