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CD38 as a Regulator of Cellular NAD: A Novel Potential Pharmacological Target for Metabolic Conditions

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Abstract

CD38 is a multifunctional enzyme that uses nicotinamide adenine dinucleotide (NAD) as a substrate to generate second messengers. Recently, CD38 was also identified as one of the main cellular NADases in mammalian tissues and appears to regulate cellular levels of NAD in multiple tissues and cells. Due to the emerging role of NAD as a key molecule in multiple signaling pathways, and metabolic conditions it is imperative to determine the cellular mechanisms that regulate the synthesis and degradation of this nucleotide. In fact, recently it has been shown that NAD participates in multiple physiological processes such as insulin secretion, control of energy metabolism, neuronal and cardiac cell survival, airway constriction, asthma, aging and longevity. The discovery of CD38 as the main cellular NADase in mammalian tissues, and the characterization of its role on the control of cellular NAD levels indicate that CD38 may serve as a pharmacological target for multiple conditions.

Keywords

CD38; NAD; SIRT1; aging; obesity; metabolic syndrome

1. INTRODUCTION

Nicotinamide adenine dinucleotide (NAD) is a key cellular metabolite that is involved in cellular energetics. In addition, NAD has recently emerged as a crucial regulator of signaling pathways implicated in multiple physiological conditions [1–12]. The two main signaling roles of NAD include its importance as a substrate for the generation of second messengers such as cyclic-ADP-ribose (cADPR) [1–9] and its role as a substrate and regulator of the NAD dependent deacetylases sirtuins [10–12]. Both these signaling pathways have been shown to be very important in many physiological conditions from egg fertilization all the way to the cellular mechanisms of aging, longevity, and death [1–12]. In these regards, a great new interest in NAD functions and metabolism has emerged. In fact, we have seen almost a second discovery of this molecule in recent years [13]. Due to the key role of NAD in cells, it is crucial to characterize the mechanisms that control NAD metabolism. In recent years, we have learned much about the cellular echanisms of NAD synthesis [12–14]. Intense research in this field culminated with the discovery of the role of the protein nicotinamide phosphoribosyltransferase (Nampt) (also known as visfatin or PBEF) as a key enzyme involved in *de novo* synthesis of NAD [15]. In fact, Nampt has been recently shown to modulate NAD levels and some of its cellular functions [15,16]. On the other hand, until recently, very little was known about the

*Address correspondence to this author a the Department of Anesthesiology, Mayo Clinic and Foundation, Rochester, MN 55905, USA; Tel: (507) 255- 0746; Fax: (507) 255-5300; chini.eduardo@mayo.edu its potential role as a pharmacological target for the control of conditions regulated by cellular NAD.

mechanisms that regulate NAD degradation in mammalian cells. Our recent studies clearly show that the multifunctional enzyme CD38 is a key enzyme involved in the degradation of NAD and appears to control cellular NAD levels [17–19]. In this review, I will focus on the role of CD38 as one of the main cellular NADases, and will discuss its potential role as a pharmacological target for the control of conditions regulated by ellular NAD.

2. CD38 IS A SECOND MESSENGER ENZYME: SYNTHESIS AND DEGRADATION OF CADPR

2.1. Biochemistry and Metabolism of cADPR

Cyclic-ADP-ribose is a second messenger that induces calcium release from intracellular stores [2–9]. Cyclic-ADPribose is synthesized from β-NAD+ by an enzymatic activity named ADP-ribosyl cyclase and converted to ADPR in a reaction call cADPR hydrolase (Fig. **1**). It is interesting that cADPR metabolism resembles the cyclic AMP system; where a cyclic nucleotide compound with active biological activity (cADPR and cAMP) is hydrolyzed into an inactive non-cyclic compound (ADPR and 5'-AMP). It has to be noted, however, that in many cell types, studied so far, the precise nature of the enzyme(s) responsible for physiological ADP-ribosyl cyclase and cADPR hydrolase activities has not been well established. Nevertheless, ADP-ribosyl cyclase activity has been found across different species spanning from unicellular organisms, invertebrates (sea urchin eggs, *Aplysia*) to mammalian cells, plants, and parasites suggesting that cADPR metabolism has been preserved in evolution as an ubiquitous second messenger [20–39]. ADP-ribosyl cyclases have been found both in soluble and membrane-bound forms. The first characterization of ADP-ribosyl cyclase was performed in *Aplysia californic*a ovotestis [5,38,39]; this soluble 30-KDa enzyme was purified and found to have pure cyclase but no hydrolase activity [39]. It can use β-NAD+ as a substrate, but no α-NAD+ or NADH. It can also metabolize analogs of NAD+, such as nicotinamide guanine dinucleotide (NGD+) and nicotinamide hypoxanthine dinucleotide (NHD+), generating cyclic compounds (cGDPR and cIDPR, respectively) with fluorescent properties, but lacking calcium-releasing activity [40]. These fluorescent compounds are very useful as biochemical tools for studies of ADP-ribosyl cyclase activity [40]. The amino acid sequence of ADP-ribosyl cyclase from Aplysia has considerable homology with the human lymphocyte surface antigen CD38, which led to the discovery that CD38 has also ADP-ribosyl cyclase activity [41]. However, in contrast to Aplysia cyclase, CD38 is a transmembrane protein and has both ADP-ribosyl cyclase and cADPR hydrolase activities. Surprisingly, CD38 bound to the plasma membrane has its catalytic site located on the extracellular domain of the cell, which poses theoretical difficulties for understanding how CD38 generates cADPR in the cytoplasm, where it should be available to interact with calcium channels. Several mechanisms for generation of intracellular cADPR have been proposed, including the translocation of cADPR promoted by CD38 and internalization of CD38 molecules [42], but a clear molecular model remains to be established by further experimental evidence. In cells from vertebrates, the majority of data gathered about cADPR metabolism comes from the studies of cyclase and hydrolase activities of CD38. However, other enzymes though not as extensively characterized as CD38. Another lymphocyte surface antigen named BST-1 (CD157) also has ADP-ribosyl cyclase activity [43]. BST-1 appears to be the product of a gene duplication of CD38 [43].

Although it appears that CD38 clearly generates and degrades the second messenger cADPR and plays key roles in the regulation of intracellular calcium transients, CD38 may have other functions. For example, CD38 has been implicated as the enzyme responsible for the generation of other second messengers including NAADP *via* the base-exchange reaction [44–50]. However, the role of CD38 in NAADP generation is still controversial [50,51]. In fact, we and others have recently described that CD38 is not necessary for the intracellular generation of NAADP in some mammalian cells [50–52]. These indicate that yet another cellular enzyme

maybe responsible for some of the functions previously attributed to CD38. It is quite interesting that CD38 appears to be a very inefficient second messenger enzyme, as it will hydrolyze almost a hundred molecules of NAD to generate one molecule of the second messenger cADPR [53,54]. In this regard, we have recently focused on the possible role of CD38 not only as a second messenger enzyme, but also as a NADase that can control cellular levels of NAD and its physiological functions [17–19].

3. CD38 IS A CELLULAR NADase: ROLE ON THE DEGRADATION OF NAD AND CONTROL OF NAD LEVELS

CD38 is a multifunctional enzyme, ubiquitously distributed in mammalian tissues [26–36]. As discussed above its major enzymatic activity is the hydrolysis of NAD [21,24,26,35,53,54]. In fact, as discussed above, CD38 will generate one molecule of cADPR for almost every 100 molecules of NAD hydrolyzed [21]. Until recently, the role of CD38 as a modulator of NAD levels had not been explored. Recently, we postulated that CD38 is the major NADase in mammalian cells and that it regulates intracellular NAD levels (Fig. **2**). In fact, we examined the NADase activities and NAD levels in a variety of tissues from both wild-type and CD38 deficient mice [17]. In accordance with our hypothesis, we found that tissue levels of NAD in CD38 deficient mice were 10 to 20 fold higher than in wild-type animals [17], a result confirmed by others [55]. In addition, NADase activity was essentially absent in most of the tissues, from CD38 deficient mice [17–19]. These data support the novel concept that CD38 is a major regulator of cellular NAD levels. Since CD38 is distributed in nearly every mammalian tissue and cellular compartment, I have postulated that CD38 regulates cellular NAD levels. In particular, CD38 may have a role not only in the regulation of intracellular but also extracellular NAD, and may modulate the availability on extracellular applied NAD in some cellular systems. In addition, the presence of CD38 in different intracellular compartments may have a crucial role on the regulation of NAD functions in specific organelles. The role of nuclear CD38 has been recently explored. CD38 is located at the nuclear membrane and regulates the generation of the second messenger cADPR and nuclear stores calcium release [56]. In addition, we have also found that nuclear CD38 regulates the activity of the nuclear enzymes sirtuins [18]. In particular, we observed that CD38 degrades NAD and decreases the accessibility of NAD to the NAD-dependent acetylase SIRT1 [18,19]. It is also possible that generation of nicotinamide by CD38 may regulate SIRT1 activity (Fig. **2**) [18]; this is possible by the fact that nicotinamide is an endogenous inhibitor of the SIRT1 enzyme. The potential role of CD38 as a regulator of NAD levels and SIRT1 activity opens the possibility that CD38 may be a regulator of many of the SIRT1 functions including energy homeostasis, obesity, aging, and longevity. Next, I will briefly describe some key aspects of the SIRT1 pathway.

4. SIRT1 AND THE RENEWED INTEREST IN NAD

4.1. SIRT1, a New Key Regulator Energy Metabolism, Aging and Longevity

One of the main recent advances on the understanding of energy metabolism, and the subsequent development of metabolic syndrome, has been the discovery and characterization of the metabolic roles of the NAD dependent deacetylases sirtuins. In particular, activation of the sirtuin enzyme SIRT1 has been shown to regulate glucose and fat metabolism and protect animals from high fat diet (HFD)-induced metabolic syndrome, liver steatosis, and obesity. The protective effects of SIRT1 activation maybe mediated by both a systemic melioration of the metabolic syndrome and specific effect on tissue glucose and fat metabolism [57–60].

4.2. SIRT1 is a Mediator of Caloric Restriction

Several studies have clearly demonstrated that moderate caloric restriction (CR) slows aging, extending life span up to 30–50% [61], furthermore CR can protect animals from the development of metabolic syndrome [61]. Recently it has been shown that the effects of CR are mediated, at least in part, by SIRT1 [62]. SIRT1 uses NAD as a substrate to promote deacetylation of several target proteins. Increased activity of sirtuins leads to life extension in yeast, fruit flies, and *C. elegans* [62–64]. Sirtuins also have an important role in the regulation of body weight, and recently it has been clearly shown that activation of SIRT1 can lead to protection against the development of obesity and liver steatosis [10–12].

4.3. SIRT1, a Regulator of Systemic and Hepatic Glucose and Fat Metabolism

SIRT1 regulates energy metabolism, glucose and fat homeostasis both at the systemic and cellular level. In addition to its systemic effects, SIRT1 has liver specific affects on both glucose and fat metabolism [57–60]. SIRT1 induced mitochondrial biogenesis, and gene expression in several cell types [10,11]. Furthermore, hepatic SIRT1 activation leads to a protection against high fat diet (HFD)-induced steatosis, decreases SREBP1 expression, and the development of inflammation [10,11,59]. In addition, SIRT1 regulates the expression of the antioxidant genes SOD2 (manganese superoxide dismutase), NRF1, and UCP3. In cultured hepatocytes, SIRT1 inhibits glucose induced cellular fat accumulation *via* an AMPK dependent mechanism [65, 66]. It appears that all these effects may play a role on the protective effect of SIRT1 activation upon the development of metabolic syndrome, liver steatosis and obesity. Thus, activation of SIRT1 with small molecules, such as resveratrol, may represent a promising strategy for the treatment of metabolic syndrome, and obesity [10,11,19].

4.4. NAD, Sirtuins (SIRT1) and Obesity

NAD and nicotinamide play key roles in many cellular functions [1–11,19]. In addition to its well known importance in energy metabolism, NAD and nicotinamide also play a role in signal transduction. New evidence suggests that NAD and nicotinamide are regulators of the NAD dependent deacetylases (sirtuins also known as SIRT enzymes), which modulates, obesity, energy metabolism, aging, and longevity [10–12,57–66]. In fact, the drug resveratrol, an activator of the sirtuin enzymes, has been recently shown to protect animals against high fat (caloric) diet (HFD)-induced obesity, *via* an increase in energy expenditure [10–11,19]. In addition, activation of SIRT1 enzymes in mice feed HFD ameliorate pathological effects of obesity (including glucose tolerance), and increase longevity [10]. These data indicates that SIRT1 enzyme not only prevent obesity, but also promote salutary health benefits in HFD feed animals [10,11,19]. The effects of SIRT1 on obesity and energy metabolism are, at least in parts, mediated by deacetylation and activation of peroxisome proliferato ractivated receptor α coactivator, PGC1α [10,11,19] (Fig. **3**). Very strong evidence supports the notion that PGC1α is a key regulator of energy metabolism and mitochondrial biogenesis [67]. However, to date, the intracellular mechanisms that regulate SIRT1 mediated activation of PGC1α, inmammalian cells, have not been elucidate.

4.5. SIRT1 has Multiple Cellular Targets

In addition to the deacetylation of PGC-1 [1–12], SIRT1deacetylates other diverse substrates including p53, forkheadtranscription factor (FOXO), NF-κβ, Ku70, MyoD, LXR ,and histones. Thus, it influences gene silencing, apoptosis, stress resistance, cellular senescence, fat and glucose metabolism. The combination of these cellular functions might contribute to the physiological effects of SIRT1. Despite the extensive studies of SIRT1 function, the regulation of SIRT1 is poorly understood.

5. CD38 AS A REGULATOR OF NAD AND SIRT1

5.1. CD38 and NAD Metabolism

As discussed above, CD38 is an enzyme that has been implicated on the generation of the second messenger cyclic- ADP-ribose (cADPR) [12–26]. However, its main enzymatic activity is the hydrolysis of NAD to nicotinamide and ADPR. We postulated that CD38 is the major NADase in mammalian cells and that it regulates intracellular NAD and nicotinamide levels. In fact, our recent publications indicate that in CD38 deficient mice, tissue levels of NAD are several folds higher than in wild type animals [17]. In addition, we observed that NADase activity is essentially absent in several tissues from CD38 deficient mice [17].

5.2. CD38, a Regulator of SIRT1 Activity?

We proposed that by modulating availability of NAD and nicotinamide to the SIRT1 enzyme, CD38 regulates SIRT1 activity. These findings have strong implications for the understanding of the basic mechanisms that modulate obesity, metabolic syndrome, energy homeostasis, longevity, and aging.

5.3. CD38 and Obesity

A correlation has been observed between chromosome 4 near marker D4S403, where the CD38 gene is located, and the development of metabolic syndrome that refers to the clustering of disease conditions such as obesity, insulin resistance, hyperinsulinemia, and dyslipidemia [68]. However, to date, except for our studies [17–19], no other studies have been published on the role of CD38 on diet-induced obesity. CD38 regulates SIRT1 *via* a Non-cADPR mediated mechanism. Although CD38 has been implicated as the enzyme responsible for the generation of the second messenger cADPR [5], CD38 also appears to have cADPR-independent functions. In the case of regulation of NAD, SIRT1 activity, and obesity, our data indicates that CD38 appears to do it *via* a cADPR independent way, but a SIRT1 dependent mechanism [19]. The regulation of NAD by CD38 and its implication for pharmacological approaches aim at increasing SIRT1 activity. We proposed that by augmenting NAD and decreasing nicotinamide levels, inhibition of CD38 will not only increase SIRT1 activity but also will increase the sensitivity of SIRT1 to its pharmacological agonists such as resveratrol. In fact, we have previously observed that the activation of recombinant SIRT1 by resveratrol is inhibited by the addition of active recombinant CD38 to the reaction media [17].

6. CD38 INHIBITORS

To date a few CD38 inhibitors have been reported including NAD analogs (arabiono-NAD), nicotinamide derivatives (nicotinamide and nicotinic acid), reducing agents (such as dithiothreitol), and other unrelated compounds (Fig. **4**; and reference [27,71]). The compound 2,2'-dihydroxyazobenzene (DAB) has been recently shown to protect against cardiac dysfunction-induced by angiotensin II [69]. At the present time, the search for specific and potent CD38 inhibitors remains elusive. However, a recent report on a new CD38 assays indicates that an intensive search for CD38 inhibitors is going at this time [70], and it maybe a question of time before potent and specific CD38 inhibitors are available atleast for research. In any case, some important aspects of the search for CD38 inhibitors deserve further discussion. First, it is important to say that it is possible that molecules that inhibit CD38 may also inhibit SIRT1 [71]. In fact, SIRT1 and CD38 have several similarities in their enzymatic and catalytical properties [71], and CD38 has been proposed as a model enzyme for the study of the mechanism of SIRT1 catalysis [71]. Both SIRT1 and CD38 degrades NAD to nicotinamide and an ADPR derivative. Furthermore, both enzymes are capable of base-exchange reaction (Fig. **1B**). In this regard, inhibitors of CD38 may also have effects upon SIRT1 activity, a potential undesirable “side-effect”. Secondly, as discussed above CD38 have

other functions that are mediated by the generation of calcium regulating second messengers such as smooth muscle contraction, cell death, and apoptosis, neural and hormonal signaling, egg fertilization and others [2–9,21,69,72,73]. In this regard, CD38 inhibitors may have beneficial effects upon conditions, where cellular calcium homeostasis is deregulated such as in hypertension, cardiac ischemia, asthma and dysfunctional labor [2–9,21,69,72,73]. On the other hand, CD38 has been implicated in the secretion and function of hormones such as oxytocin and ACTH [74,75], and may modulate maternal and social behavior [75]. These roles indicate that inhibition of CD38 may have potential deleterious effects. Potential immunologic dysfunction may be one of the worst possible "side-effects" of CD38 inhibitors. It has been shown that CD38 plays a key role in the mechanism by which the organism fights bacterial infection [76], and knockout of CD38 leads to increase susceptibility to lethal bacterial infection [76]. Despite these limitations, the search for CD38 inhibitors and the determination of their potential therapeutic roles will generate key new data that will provide new insights on multiple physiological and pathological conditions, and CD38 inhibitors may hold the key to new therapeutic strategies to multiple metabolic and inflammatory conditions.

7. CONCLUSION

Finally, it is important to discuss the fact that NADases are present in many other organisms such as bacteria and protozoans [77,78]. Group A streptococci produce several exoproteins that are thought to contribute to the pathogenesis of human infection. One of these proteins is a NAD+-glycohydrolase (NADase). When group A streptococci are bound to the surface of epithelial cells *in vitro*, pores in the cell membrane are form and bacterial NADase is delivered to the epithelial cell cytoplasm. *In vitro*, intoxication of keratinocytes with NADase is associated with cytotoxic effects and induction of apoptosis [77]. In this regard, bacterial NADase plays a key role on the virulence of some bacteria [77]. Furthermore, we have recently described that the parasite *Toxoplasma gondii* has a NADase/ADP-ribosyl cyclase [78,79]. Furthermore, in toxoplasma generation of cADPR, induced by abscisic acid, plays a key role in the mechanisms of cell invasion, differentiation and egress [78–80]. Inhibition of the NADase/ADP-ribosyl cyclase may be a novel target for pharmacological therapy against parasitic infection [78–80]. It is possible that CD38 inhibitors may cross many species barriers and may also be effective against microorganismal NADases, and maybe use for the treatment of microbiotic infection. In any case, search for specific bacterial and protozoa NADase inhibitors may also be of great importance. The recent development on the understanding of the catalytic properties of CD38 and the development of assays to study its NADase and base-exchange reaction maybe the initial step for the development of CD38 and species specific NADase inhibitors that may have multiple potential therapeutic roles in many human diseases. The future in this field is extremely exciting and provides promises of new and exciting pharmacological tools, let the hunt begin.

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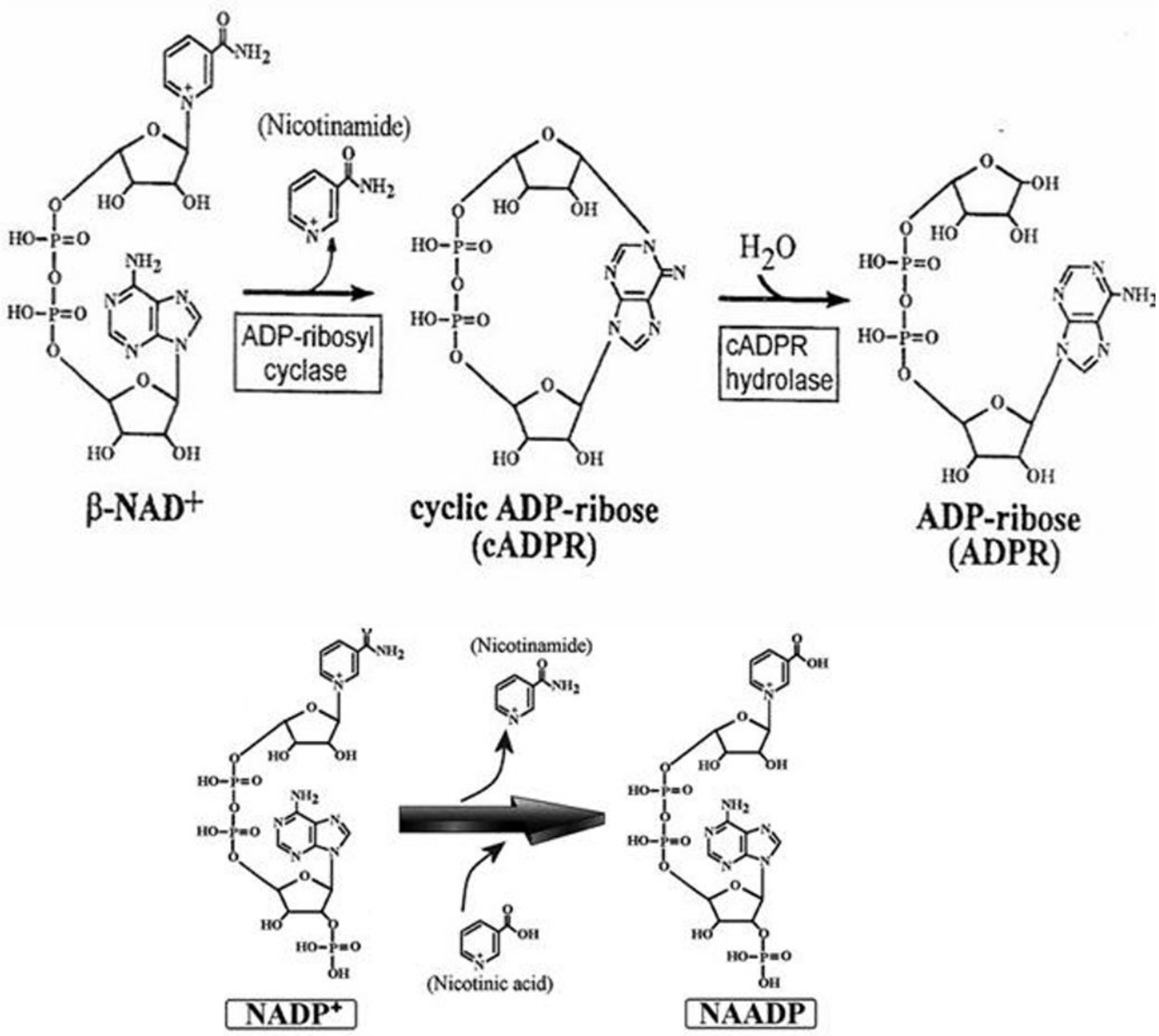

Fig. (1).

NADase activity: Synthesis and degradation of cyclic ADP-ribose is shown. (**A**). Synthesis of nicotinic acid adenine dinucleotide phosphate [NAADP+] from nicotinamide adenine dinucleotide phosphate [NADP+] by base-exchange reaction is shown.

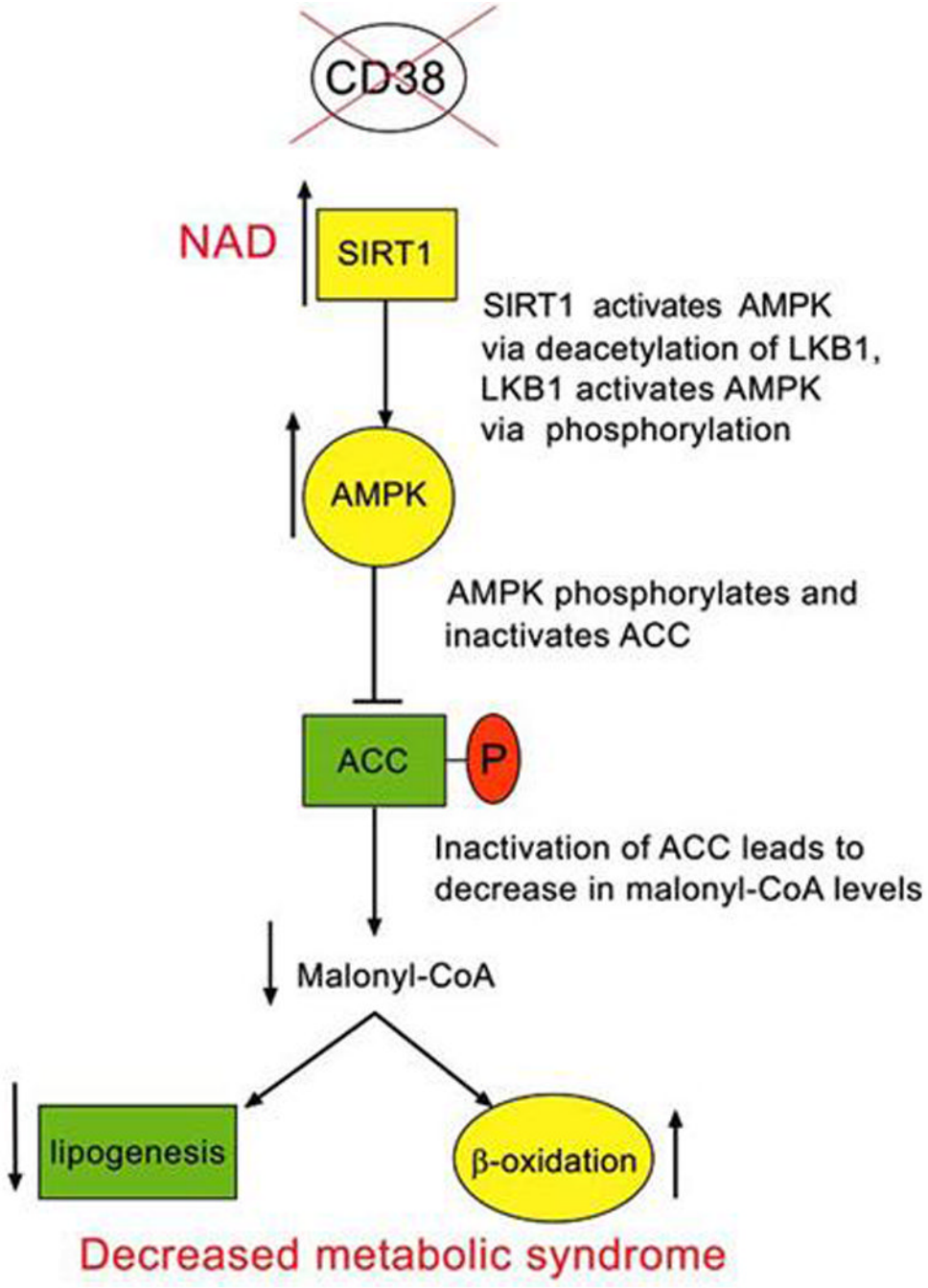

Fig. (2).
Possible mechanism of regulation of SIRT1 and AMPK pathway by CD38 inhibition.

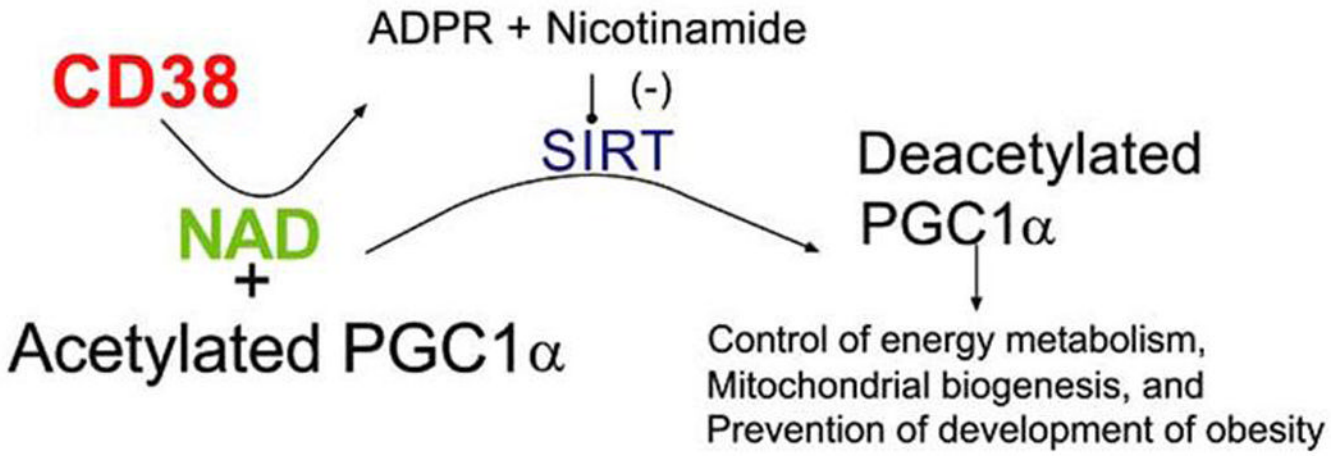

Fig. (3).
Possible mechanism of regulation of SIRT1, PGC1 pathway, and obesity by CD38 inhibition.

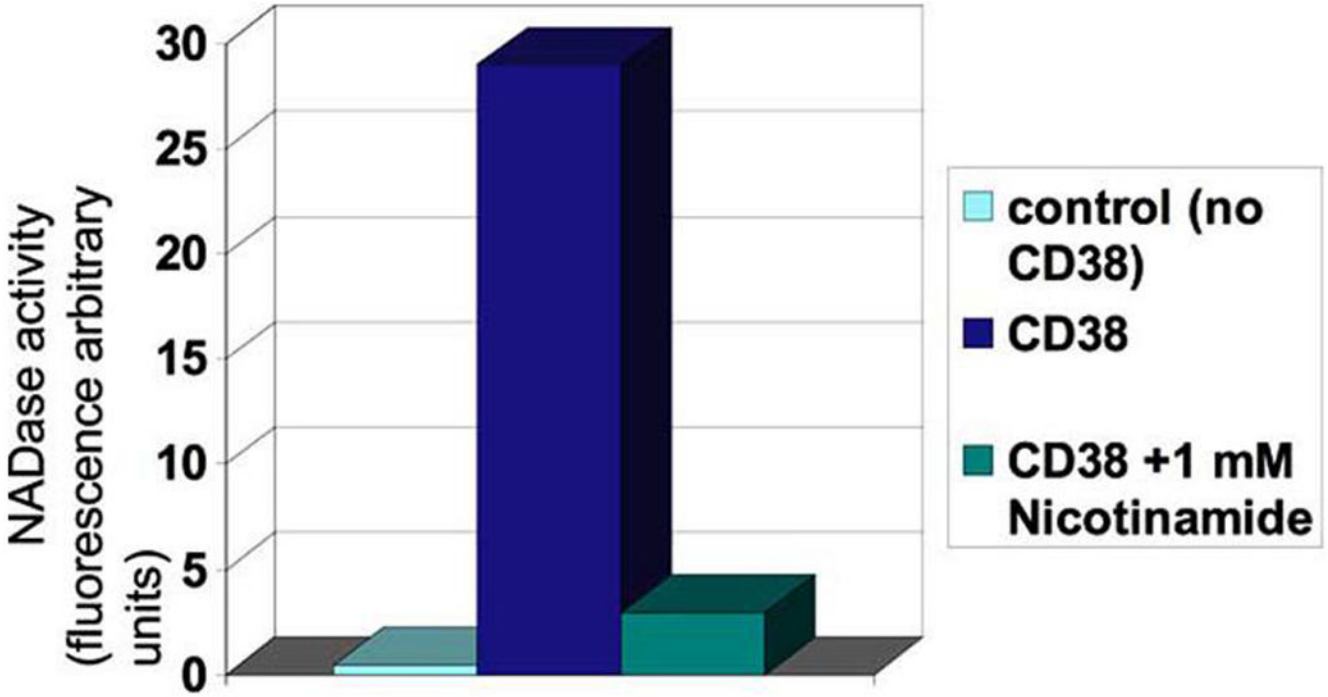

Fig. (4).
A typical CD38-NADase assay using etheno-NAD and recombinant CD38. Activity is inhibited by the reaction product nicotinamide. NADase acitivity is defined using etheno-NAD as a substrate and adding CD38 in the presence or absence of 1mM nicotinamide. Samples were incubated for 10 min.