Structural Basis for the Mechanistic Understanding of Human CD38-controlled Multiple Catalysis*

Received for publication, July 5, 2006, and in revised form, August 29, 2006. Published, JBC Papers in Press, September 2, 2006, DOI 10.1074/jbc.M606365200

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The enzymatic cleavage of the nicotinamide-glycosidic bond on nicotinamide adenine dinucleotide (NAD⁺) has been proposed to go through an oxocarbenium ion-like transition state. Because of the instability of the ionic intermediate, there has been no structural report on such a transient reactive species. Human CD38 is an ectoenzyme that can use NAD⁺ to synthesize two calcium-mobilizing molecules. By using NAD⁺ and a surrogate substrate, NGD⁺, we captured and determined crystal structures of the enzyme complexed with an intermediate, a substrate, and a product along the reaction pathway. Our results showed that the intermediate is stabilized by polar interactions with the catalytic residue Glu236 rather than by a covalent linkage. The polar interactions between Glu236 and the substrate 2',3'-OH groups are essential for initiating catalysis. Ser193 was demonstrated to have a regulative role during catalysis and is likely to be involved in intermediate stabilization. In addition, a product inhibition effect by ADP-ribose (through the reorientation of the product) or GDP-ribose (through the formation of a covalently linked GDP-ribose dimer) was observed. These structural data provide insights into the understanding of multiple catalysis and clues for drug design.

Human CD38 is a type II transmembrane glycoprotein first identified as a surface antigen of lymphocytes (1). It has since been shown to possess many cellular functions (2) and is directly involved in some human diseases such as B cell chronic lymphocytic leukemia (3), AIDS (4), and diabetes (5). In addition, the extramembrane domain of CD38 is found to be a multifunctional enzyme. As a member of the ADP-ribosyl cyclase family, CD38 not only can synthesize cyclic ADP-ribose (cADPR)⁴ from NAD⁺ but also can hydrolyze NAD⁺ and cADPR to produce ADPR (6–8). Furthermore, at acidic conditions and in the presence of nicotinic acid, CD38 can catalyze a nicotinamide exchange reaction to produce NAADP⁺ from NADP⁺ (9). Recent studies indicate that CD38 can also hydrolyze NAADP⁺ to ADPRP and that this hydrolysis reaction, likewise, occurs only at acidic pH (10). Biochemical and structural characterizations reveal that two acidic residues, Glu146 and Asp155, at the active site of CD38 are the critical determinants of this novel acidic dependence (10). The enzymatic products of CD38, cADPR, NAADP⁺, and ADPR, are all calcium messengers targeting different calcium channels and stores, indicating the central role of the enzyme in calcium signaling.

The enzymatic syntheses of these signal molecules first require the removal of the nicotinamide moiety from the substrate to form a reactive intermediate, which can be further catalyzed to produce divergent signals through divergent pathways (11). As the catalytic ability of an enzyme is dependent on the structure of the reaction intermediate, it is thus important to characterize the chemical nature of the intermediate. To describe the reaction mechanism, a covalent intermediate was proposed in which the remaining ribonucleotide is linked to the catalytic residue of the enzyme after the departure of the nicotinamide (12). However, mounting evidence points instead to the formation of an ionic intermediate, proposed to be an oxocarbenium ion that is stabilized by ion pair interactions with the catalytic residues (13–15). Similar intermediates have also been proposed to exist for other NAD⁺-utilizing enzymes such as Sir2 enzymes (16), ADP-ribosyltransferase (17), and ADP-ribosylating toxins (18). In solution, the lifetime of the transient existence of such a cation is ~10⁻¹² s, preventing it from structural detection. In the active site of an enzyme, the cation intermediate may be stabilized by the enzyme for a longer lifetime. For example, during NGD⁺ catalysis by human CD38, the intermediate can stay in the active site of the enzyme with a half-life of about 6 ms (12, 19). To our knowledge, there has been no structural report for the intermediate during nucleotide catalysis.

To structurally probe this transient intermediate and to understand the structural basis for the multiple catalysis, we combined molecular biology, low temperature soaking technique, and cryocrystallography to obtain CD38 complex structures along the reaction pathway. To obtain the Michaelis com-

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⁴ The abbreviations used are: cADPR, cyclic ADP-ribose; ADPR, ADP-ribose; NAADP⁺, nicotinic acid adenine dinucleotide phosphate; NGD⁺, nicotinamide guanine dinucleotide; GDP, GDP-ribose; cGDP, cyclic GDP-ribose; GDPRI, GDP-ribose intermediate; MES, 4-morpholinooethanesulfonic acid.

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OCTOBER 27, 2006 • VOLUME 281 • NUMBER 43 JOURNAL OF BIOLOGICAL CHEMISTRY 32861
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**TABLE 1**  
Crystallographic data and refinement statistics  
Values in parentheses are from the highest resolution shell. WT, wild-type CD38; r.m.s., root mean square.

|                | WT-ADPR     | E226Q-NAD | WT-GDPRI   |
|----------------|-------------|-----------|------------|
| Data collection|             |           |            |
| a, b, c (Å)    | 41.893      | 41.451    | 41.71      |
| α, β, γ (°)    | 51.152      | 50.715    | 52.812     |
| Space group    | P1          | P1        | P1         |
| Resolution (Å) | 50-1.7 (1.76-1.7) | 30-1.9 (1.97-1.9) | 30-1.7 (1.76-1.7) |
| Unique reflections | 52,170       | 37,732    | 56,343     |
| Multiplicity   | 3.6         | 3.3       | 3.9        |
| I/σ(I)         | 19.56 (3.34)| 17.72 (2.54)| 21.37 (2.95)|
| Rmerge (%)     | 6.2 (30.6)  | 6.2 (39.8)| 5.1 (46)   |
| Completeness (%)| 94.9 (71.8)| 95.8 (83.7)| 97.2 (95.9)|

| Refinement |            |           |            |
| R factor (%) | 17.92     | 17.63     | 18.25      |
| Rfree factor (%) | 23.13   | 23.39     | 23.57      |
| Protein atoms | 3,991      | 4,016     | 4,016      |
| Water molecules | 543        | 381       | 695        |
| Ligands (atoms) | 36 + 36 + 36 | 44 + 44 | 36 + 37 + 73 |
| r.m.s. deviations | 0.014     | 0.017     | 0.015      |
| Bond lengths (Å) | 1.531 | 1.767     | 1.702      |

* Rmerge = \( \frac{\sum I_i - \bar{I}}{\sum I_i} \), where \( I_i \) is the integrated intensity of a given reflection.

The soaked crystal was then directly mounted for data collection under the protection of a cold nitrogen stream. It should be noted that a soaking experiment in some cases might freeze out authentic conformational changes associated with substrate binding.

For the wild type-GDPR dimer and wild type-GDP-ribose intermediate (GDPR) complex, one wild-type CD38 crystal was incubated with 20 mM NGD\(^+\) soaking solution at 0 °C for 20 min. Soaking at 0 °C can greatly slow down the reaction rate of NGD\(^+\) and reduce the damage to the crystal.

**Data Collection, Reduction, and Structure Refinement**—All x-ray diffraction data were collected at the Cornell High Energy Synchrotron Source (CHESS) A1 station under cryoprotection at 100 K with a fixed wavelength of 0.976 Å. A total of 360 images with an oscillation angle of 1° each were collected for each crystal using a Quantum Q-210 CCD detector. The complete data sets were processed using the program package DENZO/SCALEPACK (24). The crystallographic statistics are listed in Table 1. The apostructure of soluble human CD38 was used as the initial model for structure solution by the molecular replacement method. Subsequent crystallographic refinements were done with the program REFMAC (25). All substrates and products were built using the program O (26).

**Mutagenesis and Biochemical Assay**—The mutants of CD38 at the Ser\(^{193}\) position were prepared by site-directed mutagenesis as described (20, 23) and were verified and confirmed by DNA sequencing. Following a 24-h growth phase in YPD medium (1% yeast extract, 2% peptone, 2% glucose), protein expression was induced by addition of 0.5% methanol. The supernatants were harvested 72 h after methanol induction. The mutant proteins were purified by phenyl-Sepharose chromatography as described previously for proteins expressed with this system (27). The protein content was determined by the Bradford assay. The elution of protein corresponded to the elution of activity measured with NGD\(^+\) as substrate. The GDP-riboyl cyclase activity was measured based on fluorescence changes as described previously (28). NADase...
activity was determined for all Ser<sup>193</sup> mutants and compared with the NADase activities of wild-type CD38 and the E226G mutant. The Ser<sup>193</sup> and E226Q mutants were assayed at a protein concentration of 10 µg/ml and the wild-type CD38 at 1 µg/ml. The times of the assays were selected so that 10–20% of the substrate was consumed. The reactions were conducted in 25 mM Tris-HCl, pH 7, with 0.5 mM NAD<sup>+</sup> in volumes of 100 µl for times ranging from 1 to 30 min and were stopped by rapid mixing with 100 mM HCl.

Preliminary experiments showed that this was an effective method to disorient the adenine terminus of NAD<sup>+</sup> in the active site, consisting with its ability to refold toward the nicotinamide end of the molecule so that the two ends can be brought together to form cADPR.

The electron densities are clear for most parts of NAD<sup>+</sup>, except for the adenine ring and the ribose moiety attached to it (Fig. 1B). The adenine terminus of NAD<sup>+</sup> is out of the active site and thus not expected to be stabilized by the enzyme. The disordering of the adenine terminus reflects the flexibility of this portion of NAD<sup>+</sup> in the active site, consistent with its ability to refold toward the nicotinamide end of the molecule so that the two ends can be brought together to form cADPR.

The nicotinamide end of NAD<sup>+</sup> penetrates deep into the active site (Fig. 1A), which is also consistent with cleavage of the nicotinamide-glycosidic bond being the first step of catalysis. The nicotinamide group of the bound NAD<sup>+</sup> interacts with Glu<sup>146</sup> and Asp<sup>155</sup> through two hydrogen bonds to stabilize in the active site by extensive polar interactions involving residues Asp<sup>155</sup>, Glu<sup>146</sup>, Gln<sup>226</sup>, Trp<sup>125</sup>, Ser<sup>126</sup>, Arg<sup>127</sup>, and Thr<sup>221</sup> and a structural water molecule (Fig. 1B). The electron densities are clear for most parts of NAD<sup>+</sup>, except for the adenine ring and the ribose moiety attached to it (Fig. 1B). The adenine terminus of NAD<sup>+</sup> is out of the active site and thus not expected to be stabilized by the enzyme. The disordering of the adenine terminus reflects the flexibility of this portion of NAD<sup>+</sup> in the active site, consistent with its ability to refold toward the nicotinamide end of the molecule so that the two ends can be brought together to form cADPR.

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substrate nicotinamide moiety may be important for its cleavage, as has been observed in the structure of SIR2-NAD$^+$ complex (16).

Two other parts of NAD$^+$ that have strong interactions with the active site are the nicotinamide ribose and the diphosphate. The ribose is fixed by polar interactions with Gln$^{226}$, main-chain Trp$^{125}$ nitrogen, and a structural water molecule (Fig. 1B). The diphosphate forms four hydrogen bonds with residues, Ser$^{126}$, Arg$^{127}$, and Thr$^{221}$. Overall, the effect of interactions between substrate and enzyme is an activated Michaelis complex. In this activated complex, the labile nicotinamideribosyl bond is stretched from the ideal value bond length of 1.50 Å (29) to 1.65 Å, promoting the dissociation of the nicotinamide moiety from the substrate.

Reaction Intermediate Captured during NGD$^+$ Catalysis—Nicotinamide guanine dinucleotide (NGD$^+$) is a surrogate substrate of NAD$^+$ and differs from NAD$^+$ by replacing the NAD$^+$ adenine group with a guanine group. NGD$^+$ was used to obtain an intermediate because the reaction rate of NGD$^+$ is much slower than that of NAD$^+$ (by a factor of four (23)), suggesting a longer lived intermediate during the NGD$^+$ catalysis.

There are two CD38 molecules in the asymmetric unit of preformed wild-type crystals (21). By soaking these wild-type crystals with NGD$^+$, the active sites of both molecules were saturated with substrate NGD$^+$, and reaction proceeded in the crystal. By analyzing the data collected from these crystals, we found that the active sites of the two molecules contain different nucleotides. In fact, as can be seen in Fig. 2, molecule B contains two nucleotides, a GDP-ribose intermediate and a hydrolyzed product, GDPR, whereas molecule A contains only one nucleotide. It is reasonable to observe different occupants in the active sites of two molecules. We reported previously that two molecules in the crystallographic asymmetric unit have slightly different structures because of the crystal constraints (21). In molecule B, the electron densities for the active site indicate the presence of an intermediate and a guanine ring from a hydrolysis product, GDPR (Fig. 3A). Compared with the electron density for NAD$^+$ in Fig. 1B, the gap between the densities of GDPR and the guanine ring clearly prove that the nicotinamide has been cleaved in the GDPR complex. The electron density at the ribosyl C-1’ position clearly shows the absence of an OH group, or any linkage, attached to C-1’. This demonstrates that it is not a substrate or product but most likely an intermediate produced after the attached nicotinamide is released from the substrate, NGD$^+$, but before the nucleophilic attack by water that would lead to the formation of GDPR. The observed structure is thus consistent with the predicted oxocarbenium ion intermediate or its glycal analogue (30). The guanine ring of the GDPR is very close to C-1’, with its guanine oxygen only 2.16 Å away (Fig. 3A). The close proximity of the guanine ring to the intermediate presumably blocks the access of water and greatly delays its nucleophilic attack on C-1’ of the intermediate, allowing it to accumulate sufficiently for visualization. In molecule A, the electron density for the nucleotide clearly shows that the nucleotide is neither a GDPR (as there is no gap between densities of the ribose and guanine ring (Fig. 3B)) nor a rebound GDPR (as density attached to the far side of the ring extends out, whereas a rebound GDPR would end at guanine). Analysis of the electron density in molecule A points to a covalently linked GDPR dimer in the active site. That is, the newly formed oxocarbenium ion intermediate has been nucleophilically attacked by the guanine oxygen atom, resulting in a covalent C–O bond with a bond length of 1.67 Å. This bond length is similar to that observed for the nicotinamide-glycosidic bond in the Michaelis complex. The enzymatic formation of the GDPR dimer has not been reported previously, but an ADPR dimer has been detected during the enzymatic catalysis of NAD$^+$ (31).

Enzyme-ADPR Complex Reveals Products with Divergent Conformations—To further validate the intermediate and its hydrolysis, we soaked wild-type CD38 crystals with NAD$^+$ and determined their structure. In this way, we could observe the electron density for the final products, ADPRs, in the active site of both molecules (Fig. 4). In molecule B, we observed density similar to that in Fig. 3A but with additional density present around the ribosyl C-1’ carbon (Fig. 3C). This density corresponds to an OH group added to the C-1’ carbon after the nucleophilic attack of the intermediate by a nearby water molecule, indicating the presence of ADPR, the fully hydrolyzed product from the substrate NAD$^+$. It should be noted that the OH group is attached to the α-face of the ribosyl group (from the bottom), suggesting the existence of an inversion mechanism for NAD$^+$ hydrolysis. Such an inverted anomeric configuration is consistent with an ionic intermediate instead of a covalent intermediate linked to the C-1’ carbon from the α-face as reported previously (19).
In the active site of molecule B, we also observed a somewhat noisy electron density corresponding to the adenine ring from another ADPR. Comparing the densities of the guanine and adenine rings (Fig. 3, A and C, respectively), it is clear that the guanine ring does have higher affinity for the active site, as judged by its well ordered presence in the crystal structure. In molecule A, we cannot find an ADPR dimer, although it has been proposed to exist (31). Unexpectedly, the electron density in the active site clearly shows that the bound species is a hydrolyzed product, ADPR, which has reentered in a reversed orientation (Fig. 4A). The electron density for this ADPR is excellent except at the terminal ribose (Fig. 4A). This is consistent with the ribose having a freely rotational single bond to the adjacent oxygen atom. Two phosphates of this ADPR form four hydrogen bonds with active site residues Ser126, Arg127, and Phe222. Ser193 and one water molecule have direct polar interactions with the two hydroxyl groups of the ADPR adenine ribose. Differing from residue Glu226 in GDPRI or GDPR dimer complexes, Glu226 has only indirect interactions to ADPR via a water molecule (Fig. 4A). The reentered ADPR complex can also be obtained by cocrystallizing NAD+ with native CD38 (data not shown). During the cocrystallization, CD38 hydrolyzes NAD+ to ADPR, which can reenter the active site of the enzyme and form a complex.

**Product Inhibition and Identification of CD38 Inhibitors Useful for Drug Design**—Because the active site of CD38 has affinity for ADPR and GDPR, it has been suggested that ADPR and GDPR might exert product inhibition. Therefore, we measured the effect of ADPR and GDPR on the cyclization activity of CD38 using NGD/H11001 as a substrate, forming cyclic GDP-ribose (cGDPR). As shown in Fig. 4B, inhibition by ADPR and GDPR was indeed observed in the mM range. The biochemical data confirm that the rebinding of ADPR to the active site of the enzyme can compete with other substrates and thus inhibit the enzymatic activities of CD38. ATP has long been identified as a CD38 inhibitor and is implicated in insulin secretion and diabetes (32). As ATP also has an adenine ring and guanine ring has a higher affinity to the active site over the adenine ring, it might be the case that guanine-containing nucleotides also inhibit CD38 activity. We therefore measured the inhibitory effect of GTP, GDP, and cGMP on CD38 cyclization activities and found that indeed all of them produce obvious inhibi-
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FIGURE 4. Enzyme-ADPR complex and the identification of potent inhibitors. A, complex structure of wild-type CD38 with three ADPRs in the active sites of two CD38 molecules in the asymmetric unit. Two ADPRs are in molecule B with one (green) blocking the release of the other from the active site. The surfaces are shown with different colors for different molecules in the crystallographic asymmetric unit. Inset, close-up view of the ADPR in the active site of molecule A. Protein residues involved in polar interactions with this ADPR are drawn as pink sticks. Trp189, which has hydrophobic interactions with the ADPR adenine ring, is represented by blue sticks. Two water molecules between the ribose and Glu226 are drawn as small red spheres. B, inhibition of the CD38 NGD cyclization activity by various inhibitors containing either a guanine ring or an adenine ring.

FIGURE 5. Structural characterization of the reaction cycle. The color scheme is the same as described in the legend for Fig. 1A, except that the right-hand nucleotide has cyan carbons. Nucleotide groups outside of the active site were omitted for clarity. A, protein-nucleotide interactions for the intermediate. Polar interactions involved in stabilizing the intermediate are shown as dashed cyan lines. Interactions between the two phosphate groups and protein residues, identical to those in the enzyme-NAD complex (Fig. 1B), are omitted here for purposes of clarity. Ser193 is now 4.76 Å away from the proposed C-1 position and therefore is unlikely to stabilize the intermediate. B and C, structural comparisons of other complexes with the intermediate shown in A. B, E226Q-NAD complex. The aligned E226Q-NAD models are shown as green sticks, and the intermediate is kept the same as in A. C, unreleased ADPR plus the adenine ring from a second ADPR.

bition of the cyclization reaction of NGD (Fig. 4B). For example, 0.1 mM GTP can inhibit cGDPR formation by 50%, revealing that GTP is an inhibitor. The affinity of the CD38 active site for guanine and adenine rings suggests the inclusion of the guanine ring moiety in the design of effective human CD38 inhibitors. Based on complex structures we determined an analogue of the GDPR dimer might be one of the human CD38 inhibitors. As the catalytic activity of CD38 has been confirmed to relate directly to some diseases such as diabetes (32), asthma (33), and inflammation (34), the effective inhibitors of human CD38 activity might be good drug candidates for treating these diseases.

DISCUSSION

Structural Features of the Intermediate—After the cleavage and release of nicotinamide, the newly formed oxocarbenium ion intermediate is stabilized by the formation of two hydrogen bonds between its two ribosyl 2',3'-OH groups and the carboxyl group of the catalytic residue Glu226. Structurally conserved water may also contribute to stabilization by forming an additional hydrogen bond with ribosyl 2'-OH (Fig. 5A). The guanine ring from a product GDPR is tightly associated within the active
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Structural Basis for Nicotinamide Cleavage—To examine the structural changes that occur during the cleavage of nicotinamide from NAD⁺ and the formation of the intermediate, the Michaelis complex and the intermediate complex were aligned based on least-squares minimization of all main-chain atoms on active site residues Trp¹⁸⁹, Glu¹⁴⁶, Asp¹⁵⁵, Trp¹₂⁵, Ser¹⁹³, and Glu²²⁶ (Fig. 5B). In the aligned model, the nicotinamide ring is coplanar with the guanine ring, with its amide nitrogen fixed by interactions to Glu¹⁴⁶, Asp¹⁵⁵, and Trp¹⁸⁹ such that the anomeric C-1' of the nicotinamide ribose is 3.22 Å from the catalytic residue Glu²²⁶ and 3.19 Å from the Ser¹⁹³ OH group. In wild-type CD38, a glutamate is at position 226. Its carboxyl group has higher nucleophilicity than the carbonyl group in the E226Q mutant and favors the formation of an extra hydrogen bond with the 2'-OH of the nicotinamide ribose. This extra interaction appears to be critical for the enzymatic activity of CD38, as the E226Q mutant has greatly reduced activity. This is consistent with observations in the crystal structure of CD157 complexed with nicotinamide mononucleotide (NMN⁺). In that case, the hydroxyl groups of the NMN⁺-ribose were also observed to form hydrogen bonds with a glutamate (15). The aligned structures thus suggest that, after the release of the nicotinamide group, the ribose moiety is no longer restrained by steric hindrance from triplet Glu¹⁴⁶-Asp¹⁵⁵-Trp¹⁸⁹ and rotates toward Glu²²⁶, with which it forms an additional hydrogen bond (Fig. 5B). The additional H-bond is seen in the structure of the intermediate.

Intermediate Catalysis—The intermediate plays a central role in the multiple reactions catalyzed by human CD38. If NAD⁺ is used as the substrate, there are individual pathways from the intermediate to produce ADPR, cADPR, or an ADPR dimer. In the ADPR pathway, nucleophilic attack by a water molecule present in the active site forms ADPR (Fig. 5C). After hydrolysis of the intermediate, the newly formed ribosyl C-1'-OH group forms an additional hydrogen bond to Glu²²⁶, with a C-2' ring pucker similar to that found in the Michaelis complex (Fig. 5C). The 2'-OH group, previously found to interact with Glu²²⁶, interacts with Ser¹⁹³ OH through a hydrogen bond. In the cADPR pathway, the adenine end of the intermediate folds back into the active site, so that its N-1 atom can attack the C-1' carbon of the intermediate to form cADPR (35). If the adenine N-1 of another ADPR attacks the intermediate, the dimerization reaction is favored to form an ADPR dimer.
dimer. If NGD\(^+\) is used as the substrate, human CD38 can generate the corresponding products, GDPR, cGDPR (28), and GDPR dimer.

**Regulative Role of Residue Ser\(^{193}\) during Catalysis**—As described above, we observed the intermediate GDPRI in the active site of molecule B but a product GDPR-dimer in the other. Why do the occupants of the sites differ? In Fig. 6A, the two molecules have been aligned based on main-chain atoms of the conserved active site residues. According to the aligned structures, two guanine rings, one from the GDPR dimer and the other from the product GDPR, take the same orientation and position with their guanine oxygens close to the C-1\(^{1}\) carbon. Except for Ser\(^{193}\), all other active site residues from two molecules overlap quite well. As a hydrogen donor, the Ser\(^{193}\) OH group forms a hydrogen bond with the Trp189 main-chain oxygen (Fig. 6C), bringing the lone pair electrons of the oxygen in the -OH group to a suitable distance for a nucleophilic attack at the scissile bond in the NAD\(^+\) complex (Fig. 5B). Indeed, the importance of Ser\(^{193}\) in catalysis was verified by site-directed mutagenesis of Ser\(^{193}\), which resulted in greatly reduced enzymatic activity (Table 2). Sequence alignments of CD38 homologues show that Ser\(^{193}\) is strongly conserved among various species from invertebrate to human, supporting its functional role in the active site (Fig. 6B). As the nucleophilicity of the Glu\(^{226}\) carboxyl group is higher than that of the Ser\(^{193}\) hydroxyl group, the functional role of Glu\(^{226}\) is likely to be dominant in effecting the cleavage of the nicotinamide group. This is consistent with mutagenesis results showing that mutations at Glu\(^{226}\) are more effective in reducing the enzymatic activities than changes at Ser\(^{193}\). Nevertheless, the fact that both residues are close to C-1\(^{1}\) suggests that they may work in concert for efficient catalysis.

The side chain of Ser\(^{193}\) can adopt two alternative conformations. In the NAD\(^+\) and GDPR dimer complexes, Ser\(^{193}\) is close to the C-1\(^{1}\) carbon at distances of 3.19 and 3.39 Å, respectively. In the GDPRI intermediate, it shifts to the other conformation, with a longer distance of 4.76 Å to the C-1\(^{1}\) carbon (Fig. 6C). For both conformations of Ser\(^{193}\), its OH group forms a hydrogen bond with the Trp189 main-chain oxygen (Fig. 6C). It is thus likely that the long distance between the Ser\(^{193}\) OH group and C-1\(^{1}\) carbon reduces the stability of the intermediate and is responsible for the relatively weak density observed for C-1\(^{1}\) carbon seen in Fig. 3A. The unstable intermediate in molecule B could be the reason that the guanine oxygen does not attack the GDPRI intermediate nucleophilically even at a distance of 2.16 Å. In molecule A, Ser\(^{193}\) is only 3.39 Å from the C-1\(^{1}\) carbon, and the partial negative charge on its lone pair electrons can act to stabilize the cation intermediate. Hence the guanine oxygen can approach and attack the C-1\(^{1}\) carbon, forming the C–O bond seen in Figs. 3B and 6A. To summarize, the role of Glu\(^{226}\) in the activation of substrate NAD\(^+\), the formation of an ionic reaction intermediate after nicotinamide cleavage, the stabilization of the intermediate by residues Glu\(^{226}\) and Ser\(^{193}\), and the competitive pathways toward cADPR or ADPR are illustrated in Fig. 7.

Acknowledgments—We thank Marliijn Hoogendoorn for expert help in producing and purifying the recombinant CD38, Tadhg Begley for useful discussion, and Marian Szepenyi for critical reading of the manuscript. The crystallographic data were collected at the Cornell High Energy Synchrotron Source (CHESS), which is supported by the National Science Foundation and National Institutes of Health NIGMS under Award DMR-0225180.
REFERENCES

1. Jackson, D. G., and Bell, J. I. (1990) J. Immunol. 144, 2811–2815
2. Deaglio, S., Capobianco, A., Bergui, L., Durig, J., Morabito, F., Duhrsens, U., and Malavasi, F. (2003) Blood 102, 2146–2155
3. Thornton, P. D., Fernandez, C., Giustolisi, G. M., Morilla, R., Atkinson, S., A’Hern, R. P., Matutes, E., and Catovsky, D. (2004) Hemato. J. 5, 145–151
4. Roussanov, B. V., Taylor, J. M., and Giorgi, J. V. (2000) AIDS 14, 2715–2722
5. Antonelli, A., and Ferrannini, E. (2004) J. Endocrinol. Investig. 27, 695–707
6. Lee, H. C., Zocchi, E., Guida, L., Franco, L., Benatti, U., and De Flora, A. (1993) Biochem. Biophys. Res. Commun. 191, 639–645
7. Howard, M., Grimaldi, J. C., Bazan, J. F., Lund, F. E., Santos-Argumedo, L., Parkhouse, R. M., Walseth, T. F., and Lee, H. C. (1993) Science 262, 1056–1059
8. Takasawa, S., Tohgo, A., Noguchi, N., Koguma, T., Nata, K., Sugimoto, T., Yonekura, H., and Okamoto, H. (1993) J. Biol. Chem. 268, 26052–26054
9. Aarhus, R., Graeff, R. M., Dickey, D. M., Walseth, T. F., and Lee, H. C. (1995) J. Biol. Chem. 270, 30327–30333
10. Graeff, R., Liu, Q., Kriksunov, I. A., Hao, Q., and Lee, H. C. (2006) J. Biol. Chem. 281, 28951–28957
11. Schuber, F., and Lund, F. E. (2004) Curr. Mol. Med. 4, 249–261
12. Sauve, A. A., Deng, H. T., Angeletti, R. H., and Schramm, V. L. (2000) J. Am. Chem. Soc. 122, 7855–7859
13. Oppenheimer, N. (1994) Mol. Cell Biochem. 138, 245–251
14. Muller-Steffner, H. M., Augustin, A., and Schuber, F. (1996) J. Biol. Chem. 271, 23967–23972
15. Yamamoto-Katayama, S., Ariyoshi, M., Ishihara, K., Hirano, T., Jingami, H., and Morikawa, K. (2002) J. Mol. Biol. 316, 711–723
16. Avalos, J. L., Boeke, J. D., and Wolberger, C. (2004) Mol. Cell 13, 639–648
17. Seman, M., Adriouch, S., Haag, F., and Koch-Nolte, F. (2004) Curr. Med. Chem. 11, 857–872
18. Han, S., Craig, J. A., Putnam, C. D., Carozzi, N. B., and Tainer, J. A. (1999) Nat. Struct. Biol. 6, 932–936
19. Sauve, A. A., Munshi, C., Lee, H. C., and Schramm, V. L. (1998) Biochemistry 37, 13239–13249
20. Munshi, C., Aarhus, R., Graeff, R., Walseth, T. F., Levitt, D., and Lee, H. C. (2000) J. Biol. Chem. 275, 21566–21571
21. Liu, Q., Kriksunov, I. A., Graeff, R., Munshi, C., Lee, H. C., and Hao, Q. (2005) Structure (Camb.) 13, 1331–1339
22. Mushi, C., Fryxell, K. B., Lee, H. C., and Branton, W. D. (1997) Methods Enzymol. 280, 318–330
23. Graeff, R., Munshi, C., Aarhus, R., Johns, M., and Lee, H. C. (2001) J. Biol. Chem. 276, 12169–12173
24. Ortonowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
25. Collaborative Computational Project, Number 4 (CCP4) Suite: Programs for Protein Crystallography (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763
26. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
27. Munshi, C. B., and Lee, H. C. (2002) in Cyclic ADP-ribose and NAADP: Structures, Metabolism, and Functions (Lee, H. C., ed) pp. 81–99, Kluwer Academic Publishers, Dordrecht, the Netherlands
28. Graeff, R. M., Walseth, T. F., Fryxell, K., Branton, W. D., and Lee, H. C. (1994) J. Biol. Chem. 269, 30260–30267
29. Guilhot, B., Jelsch, C., and Lecomte, C. (2000) Acta Crystallogr. Sect. C 56, 726–728
30. Smar, M., Short, S. A., and Wol fendren, R. (1991) Biochemistry 30, 7908–7912
31. De Flora, A., Guida, L., Franco, L., Zocchi, E., Bruzzzone, S., Benatti, U., Damonte, G., and Lee, H. C. (1997) J. Biol. Chem. 272, 12945–12951
32. Okamoto, H. (2003) Ave. Exp. Med. Biol. 527, 243–252
33. Deshpande, D. A., White, T. A., Dogan, S., Walseth, T. F., Panettieri, R. A., and Kannan, M. S. (2005) Am. J. Physiol. 288, L773–L788
34. Partida-Sanchez, S., Goodrich, S., Kusser, K., Oppenheimer, N., Randall, T. D., and Lund, F. E. (2004) Immunity 20, 279–291
35. Lee, H. C., Aarhus, R., and Levitt, D. (1994) Nat. Struct. Biol. 1, 143–144