Chemoenzymatic Synthesis of Analogues of the Second Messenger Candidate Cyclic Adenosine 5′-Diphosphate Ribose

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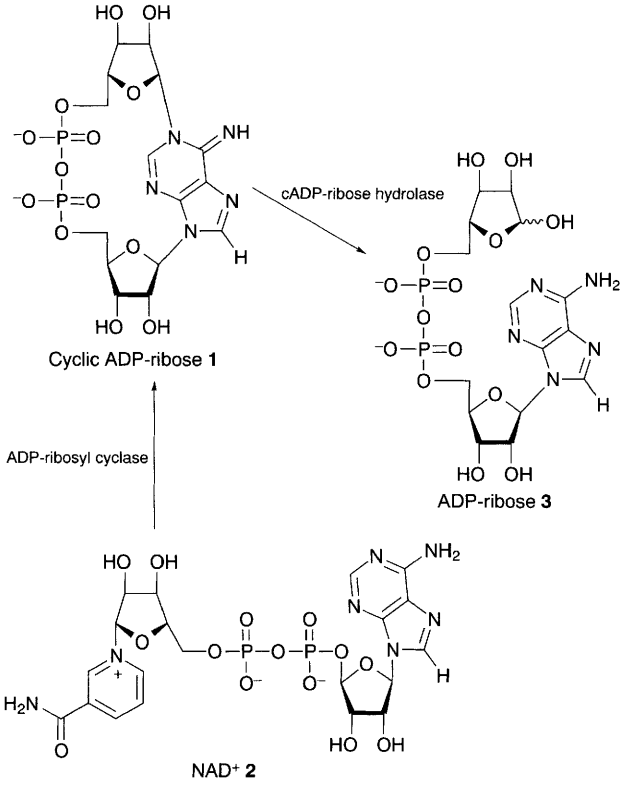
A broad substrate specificity for adenosine 5′-diphosphate ribosyl cyclase is demonstrated by cyclisation of ribose- and purine-modified nicotinamide adenine dinucleotide analogues to mimics of cyclic adenosine 5′-diphosphate ribose, generating a straightforward route for structural modification of this important Ca^{2+}-mobilising nucleotide.

Cyclic adenosine 5′-diphosphate ribose (cADPR **1**, Scheme 1) is a novel Ca^{2+}-releasing second messenger candidate, first discovered as a Ca^{2+}-mobilising metabolite of β-NAD^+ in 1987 from sea urchin egg microsomes.[1] A cyclic structure was proposed in 1989.[2] It releases intracellular Ca^{2+} as an endogenous regulator of ryanodine-sensitive Ca^{2+} channels and its action is independent of the other well known Ca^{2+}-mobilising second messenger *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3].[3] Its action, now established in a variety of cells,[4] is at a similar or lower concentration than Ins(1,4,5)P_3 and ryanodine receptor activation requires calmodulin in sea urchin eggs.[5] There is currently intense interest in the role of cADPR in cellular Ca^{2+} homeostasis as a potential second messenger. Indeed, cADPR has been proposed to have a role in insulin release as a possible second messenger of glucose.[6]

cADPR is synthesised from nicotinamide adenine dinucleotide (NAD^+ **2**, Scheme 1) by ADP-ribosyl cyclase,[7] which can be activated by cyclic 3′,5′-guanosine monophosphate *via* its kinase.[8] The net result is a linkage of the C^1 position on the nicotinamide-bearing ribose with the N^1 position of the adenine ring to form a cyclic structure. cADPR is degraded to ADP-ribose **3** at this N^1-glycosyl link by cADPR hydrolase. Ambiguity in the structure[9] of cADPR has now been removed by X-ray crystallography[10] and cADPR has been synthesised by the cyclisation of N^1-(5′-phosphoribosyl)ATP catalysed by NAD^+ pyrophosphorylase[11] and also by a biomimetic route by stereoselective cyclisation of β-NAD^+.[12]

Structural analogues of cADPR are currently urgently required as pharmacological tools for investigations of the properties of this topical molecule. Thus, analogues are needed to probe structure–activity relationships, provide non-hydrolysable mimics, radio-labelled photoaffinity and fluorescent analogues and receptor antagonists. While recent chemical advances are notable, yields are low and such routes are unlikely to be viable for the preparation of multiple structural analogues. We believe the most efficient approach lies in a chemoenzy-



Scheme 1 Formation and degradation of cyclic ADP ribose

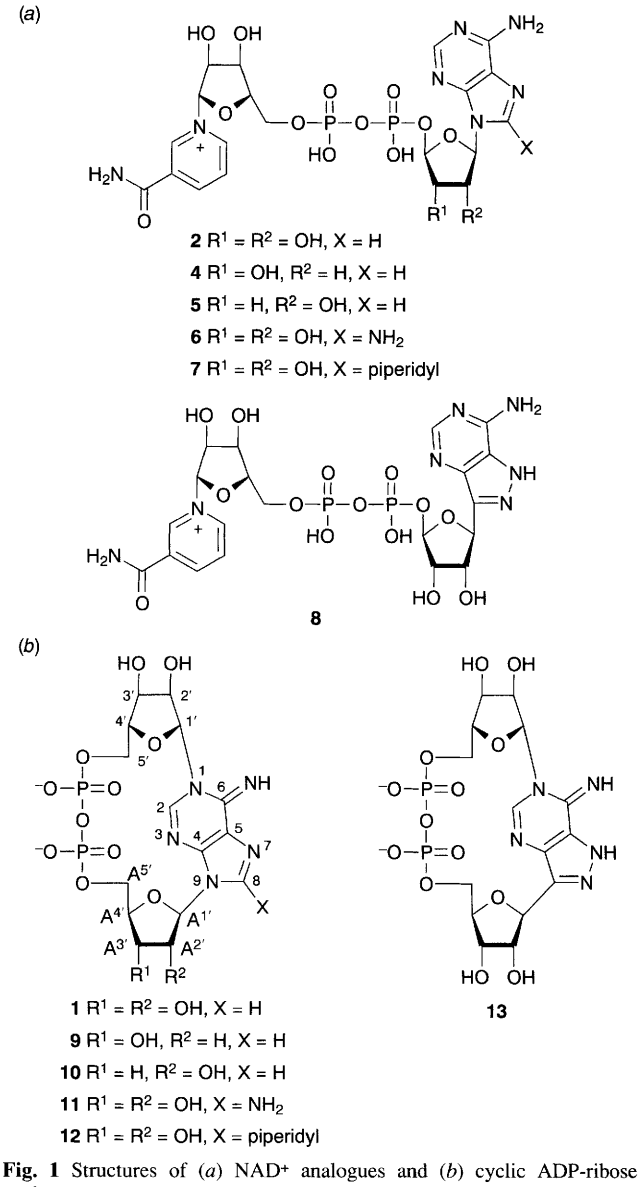


Fig. 1 Structures of (*a*) NAD^+ analogues and (*b*) cyclic ADP-ribose analogues

matic route involving the cyclisation of analogues of NAD^+ using ADP-ribosyl cyclase if the enzyme is sufficiently non-discriminatory. We demonstrate here the applicability of this approach and the loose substrate selectivity of this enzyme by the preparation of several cADPR analogues, structurally modified either in one of the ribose groups or in the purine ring, using ADP-ribosyl cyclase from *Aplysia californica*.

NAD^+ analogues [Fig. 1(*a*)] were synthesised by coupling suitably modified nucleotides with β-nicotinamide 5′-mononucleotide (β-NMN) using DCC.[13] Five NAD^+ analogues were prepared by coupling 2′-deoxy-5′-AMP, 3′-deoxy-5′-AMP, 8-amino-5′-AMP, 8-piperidino-5′-AMP and formycin A 5′-monophosphate with β-NMN to yield the corresponding NAD^+ analogues 2′-deoxy-β-NAD^+ **4**, 3′-deoxy-β-NAD^+ **5**, 8-amino-β-NAD^+ **6**, 8-piperidino-NAD^+ **7** and β-NFD^+ **8**, respectively. The resulting analogues were purified by ion-exchange chromatography.

NAD^+ **2** and NAD^+ analogues **4–8** were cyclised typically by incubating **2** or analogue (1.5 mmol dm^{-3}) in 25 mmol dm^{-3} HEPES {2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid} buffer pH 6.8 in (2.5 ml) with 10 μl of crude ADP-ribosyl cyclase from *Aplysia californica*[14] at room temp. for *ca.* 10 min to give cADPR **1** and cADPR analogues **9–13**, respectively [Fig. 1(*b*)]. The conversion was monitored by HPLC and, when complete, the product was purified by ion-exchange chromatography using a gradient of triethylammonium bicarbonate buffer. While relative conversion rates for analogue formation were not assessed with this crude enzyme preparation they were generally similar to that for formation of cADPR from NAD^+. Our results show clearly that a range of structural modifications both in the ribose and in the adenine ring are tolerated by ADP-ribosyl cyclase. A recent report on the preparation of cyclic guanosine diphosphate ribose (cGDPR) lends weight to this argument.[15] In contrast to cGDPR, however, the majority of our structural modifications were well tolerated in ryanodine receptor-mediated Ca^{2+} release and, when tested in Ca^{2+} release studies using sea urchin microsomes, all analogues, apart from **11** and **12**, were active. 8-Amino-cADPR **11** was an antagonist[16] and 8-piperidino-cADPR **12** was inactive. Full biological details will be published elsewhere. We have thus demonstrated an efficient chemoenzymatic route to cADPR analogues which has considerable advantages over total synthesis. Compounds produced by this method will be of use both to explore the recognition motifs associated with cADPR-mediated Ca^{2+} release and in the design of compounds to interfere with this pathway. These studies are in progress.

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