Synthesis of Nicotinamide Adenine Dinucleotide (NAD) from Adenosine Monophosphate (AMP)

Sir:

The acceptance of oxoreductases as catalysts in organic synthesis has been slowed by the expense of the nicotinamide co-factors required by many of these enzymes. Effective procedures for nicotinamide co-factor recycling have decreased the effective cost of these substances by allowing them to be regenerated in situ.

The nicotinamide co-factors are, however, intrinsically unstable in solution, and the economic advantage to be gained by any recycling scheme is limited. It is thus also necessary to develop methods for producing them less expensively and for stabilizing them during use. Here we report a combined cell-free enzymatic and chemical synthesis of NAD starting from readily available AMP (Scheme I). This synthesis is a step toward the development of a practical nonfermentation route to NAD and NADP.

It also illustrates the utility of enzymatic methods for the synthesis of useful quantities of complex substances and provides a flexible route to derivatives of NAD.

The key intermediate in this synthesis, nicotinamide mononucleotide (NMN), was prepared from AMP in three steps. Ribose 5-phosphate (r-5-P) was obtained by acid-catalyzed hydrolysis of AMP. Treatment of r-5-P with anhydrous ammonia in dry ethylene glycol provided a solution of ribosylamine 5-phosphate (rA-5-P). This substance was isolated, but was condensed with Nt-(2,4-dinitrophenyl)-3-carbamoylpyridine chloride (NDC) to afford NMN in 25% yield based on r-5-P. The NMN (also not isolated) was coupled with ATP in a step catalyzed by NAD pyrophosphorylase (EC 2.7.7.1) immobilized in PAN gel.

This enzymatic coupling is an equilibrium reaction and was driven to completion by hydrolyzing the pyrophosphate formed by using pyrophosphatase (EC 3.6.1.1) in PAN. The yield of NAD was 90–97% based on NMN.

A typical reaction sequence follows: Dissodium ribose 5-

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(5) NAD is now isolated from yeast. One pound of yeast (~$1) yields 780 mL dry yeast (3A molecular sieves).


(9) Next of synthesis of NAD, see: Kornberg, A. J. Biol. Chem. 1950, 182, 779-793.


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(21) NADPP catalyzes the hydrolysis of ATP to ADP. It is, therefore, necessary to regenerate ATP continuously during the reaction which forms NAD.
to a volume of 2 L with distilled water, and the pH was adjusted
to 7.2. Magnesium chloride (50 mmol) and 1,3-dimercapto-2-
propanol (20 mmol, protein antioxidant)\(^\text{17}\) were added, and
the reaction was blanketed with argon. Diammonium acetyl phosphate
solution\(^\text{18}\) (AcP, 1 M, pH 7.0, stored at 0 °C) was added with
stirring by peristaltic pump to maintain an ATP concentration
above \(K_r\) for NADPP (0.5 mM). Additional NMN (20 mmol)
and AMP (25 mmol) were added over 10 days. At the conclusion
of the reaction, 100 mmol of AcP had been added and 39 mmol
of NAD produced (97% based on NMN). The enzyme-containing
gel was allowed to settle, and the reaction mixture was decanted.
A repetition of the reaction on the same scale and using the same
enzymes consumed 110 mmol of AcP and generated 37 mmol of
NAD (91% based on NMN).

The solutions containing NAD could be used directly, without
further purification, to provide NAD (or NADH) for cofactor-
requiring enzymatic synthesis.\(^\text{19}\) Treatment of this crude
NAD-containing solution with NAD kinase (EC 2.7.1.23) and
ATP (using the ATP regeneration system) also generated NADP
uneventfully. Thus, whatever the impurities present in the un-
purified NAD may be, they do not appear to inhibit or inactivate
other enzymes. If desired, however, solid NAD can be obtained
in >50% purity by acidifying the solution with Dowex 50 (H\(^+\)
form), precipitating impurities with Ba(OH)\(_2\), and precipitating
NAD\(^+\) with ethanol.

\(^{17}\) Szajewski, R. P.; Whitesides, G. M. J. Am. Chem. Soc. 1980, 102,
2011-2026.
44, 864-865.
\(^{19}\) For example, a turnover number of 1000 was obtained for NAD(H)
in the preparation of \(\alpha\)-lactate from pyruvate. The reaction mixture (20 mL)
contained 0.05 mM NAD (0.34 mL of the solution prepared as described),
glucose 6-phosphate (50 mM), pyruvate (50 mM), glucose-6-phosphate de-
hydrogenase (50 U) and \(\alpha\)-lactate dehydrogenase (50 U). Reaction was
complete in 24 h and generated \(\alpha\)-lactate quantitatively. Indistinguishable
results were obtained by using pure NAD (Sigma). Similar results have been
obtained with lipoamide dehydrogenase and horse liver alcohol dehydrogenase.
Impurities also do not seem to inhibit the enzymes used to make and assay
NAD and NADP.

This work has several interesting features. First, this synthesis
of NAD from readily available starting materials involves only
one isolation (of \(r\)-5-P; this isolation is required only to dry the
\(r\)-5-P and is straightforward). For all other steps, unpurified
reaction mixtures are used directly, and enzymatic selectivity is
used to direct reactants efficiently to products. Isolations and
separations of nucleotides are laborious: a synthesis which requires
only one simple separation has an advantage in convenience.
Second, the NAD produced appears to be suitable for use in
cofactor recycling procedures without further purification. Thus,
although the NAD produced here is only ~15-20% pure (without
purification), its simple synthesis and its demonstrated utility in
cofactor recycling should make it useful in enzyme-catalyzed
organic synthesis. Third, all of the enzymes required for the
synthesis are easily immobilized and very stable: the manipulation
of the enzymatic catalysts is thus straightforward. Finally, we
note that the facile synthesis of \(r\)-5-P should find application
in other areas of nucleotide chemistry, that the use of \(r\)-5-P as
starting material avoids many of the problems encountered in more
extensively developed synthetic routes to nucleotides, by avoiding
the protecting groups often required to generate a product having
the furanose configuration, and that preliminary studies suggest
that NADPP has sufficiently broad specificity to catalyze the
coupling of NMN and ATP moieties bearing at least some
structural modifications.

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