PREPARATION AND PROPERTIES OF NICOTINAMIDE GUANINE DINUCLEOTIDE

A.M. STEIN*

Department of Biochemistry, University of Florida, College of Medicine, Gainesville, Florida 32601, USA

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1. Introduction

A number of pyridine nucleotide analogues have been prepared by modification or substitution of the adenine moiety of nicotinamide adenine dinucleotide. The hypoxanthine analogue has been prepared by nitrous acid [1] or enzymatic [2] deamination of NAD; the 1- and 6-hydroxyethyl adenine analogues by treatment of NAD with ethylene oxide [3]; the uracil [4, 5], thymine [4, 5], cytosine [5], guanine [5] and 6-mercaptopurine [6] analogues by the carbodiimide method. In addition, the guanine analogue has also been prepared enzymatically from GTP and NMN [7], the cytosine analogue by condensation of the mononucleotides in trifluoroacetic anhydride by the method of Shuster et al. [9] and the 3-iso-AMP analogue by exchange of the nucleotide with the methyl trioctyl ammonium salt of NMN [10].

Following the method of Shuster et al. [9], a rapid, large scale preparation of the guanine analogue (NGD) has been developed, utilizing NAD and GMP as starting materials. NGD is separated from the complex reaction mixture of adenine, guanine and nicotinamide mono- and dinucleotides by chromatography on long columns of Dowex-1-formate. This procedure avoids the costly initial preparation of NMN and has been scaled up to reaction mixtures starting with 2.5 g of NAD.

* Present address: Department of Biology, Florida International University, Miami, Florida 33144, and the Department of Biochemistry, University of Miami School of Medicine, Miami, Florida 33152, USA.

2. Methods

2.1. *Preparation of NGD*

Trifluoroacetic anhydride (about 5 g) was cautiously added to 1 g of a 3:1 mixture by weight of GMP and NAD in a pressure vial. The mixture was stirred for 20 hr at room temp. Most of the acetic anhydride was removed under reduced pressure; the crude nucleotides precipitated with excess ether, washed with ether and dried under reduced pressure. The product was dissolved in water, adjusted to pH 7 with KOH, filtered if necessary and applied to Dowex-1-formate columns. Chromatography is carried out as previously described for the large scale preparation of the thionicotinamide analogues of NAD and NADP [11]. Fig. 1 shows a typical chromatogram. Three pyridinium compounds are detected by their reaction in cyanide. The first component to emerge is free of adenine and guanine and is devoid of enzymatic activity. The second cyanide reactive component is identified as NAD by spectral properties and reactivity in the yeast alcohol dehydrogenase (YADH) system. The third component, identified as NGD, is weakly active in the YADH system. The compound is precipitated and dried as described previously [11]. The yield is 18–20% based on the starting NAD.

The spectra of NGD^+, NGDH and NGD.CN are shown in fig. 2a, the near UV bands of the cyanide and the enzymatically reduced compounds have maxima of 325 nm and 338 nm, respectively, indistinguishable in this regard and in extinction from the values for the corresponding NAD derivatives [12]. The spectra in the UV are similar to those of

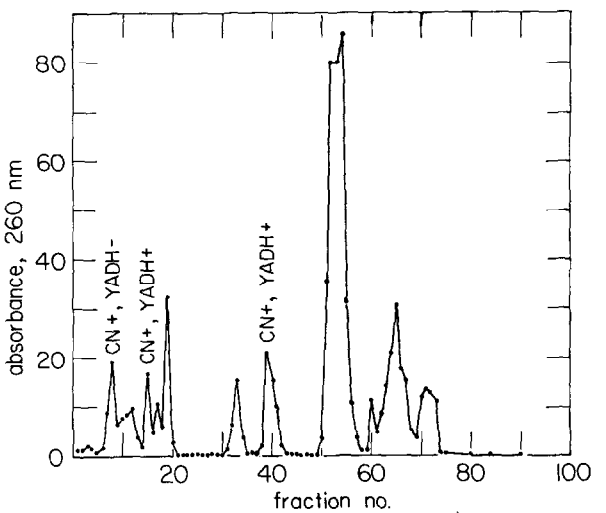

Fig. 1. Chromatography of the NGD reaction mixture on Dowex-1-formate. The nucleotide mixture derived from the reaction of 200 mg of NAD and 600 mg of GMP is added to a 1.5 × 55 cm column of Dowex-1-formate- X2 and eluted with a 0–3.5 M HCOOH gradient. Fraction volume is 4 ml. The peak tube of each component is tested for reaction in 1 M KCN; the YADH reaction mixture is 5% v/v in ethanol and 0.1 M in Tris base.

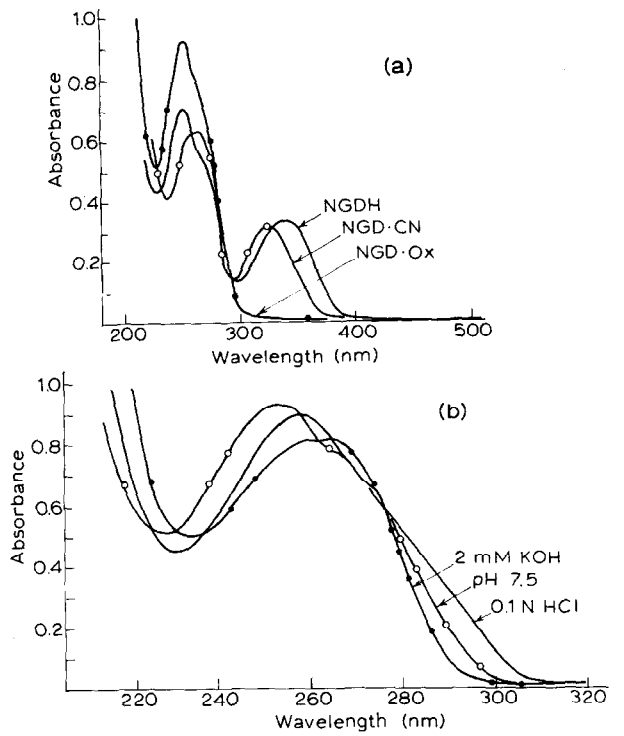

Fig. 2. Absorption spectra of NGD and derivatives. (a) NGD^+ (NGD.OX) in 0.1 M potassium phosphate, pH 7.5, NGD.CN in 1.0 M KCN and NGDH in the YADH reaction mixture (see fig. 1). (b) UV spectra of NGD in 0.1 N HCl, 0.002 KOH and in 0.1 M potassium phosphate, pH 7.5. All spectra recorded at 40 μg/ml on the Cary-14 recording spectrophotometer.

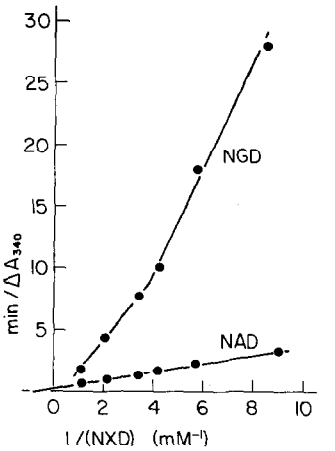

Fig. 3. Effect of NGD concentration on rate in the YADH system. The reaction is started by adding enzyme to the assay mixture, 2 M in ethanol and 0.1 M in Tris base. Ten times as much enzyme is used in the NGD reaction as in the reaction with NAD.

guanine, with 3 spectral types obtained in acid, alkali and at neutrality [12].

3. Results and discussion

3.1. *Coenzymatic activity of NGD*

At 0.1 mM, the concentration of pyridine nucleotide usually employed in assays in the YADH system, NGD is reduced at about 1/100 the rate of NAD (fig. 3, see also [5]). This difference is diminished with increasing NGD concentration; the effect of nucleotide concentration on rate appears to be cooperative, in contrast to the Michaelian

effect of NAD. Honjo et al. [5] have reported a synergistic effect of NAD and NGD in the YADH system. It is possible that the rate dependence on concentration observed in fig. 3 may explain this effect.

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