A CIRCULAR DICHROIC ABSORPTION STUDY OF THE REACTION OF OXIDIZED PYRIDINE NUCLEOTIDES WITH CYANIDE IONS

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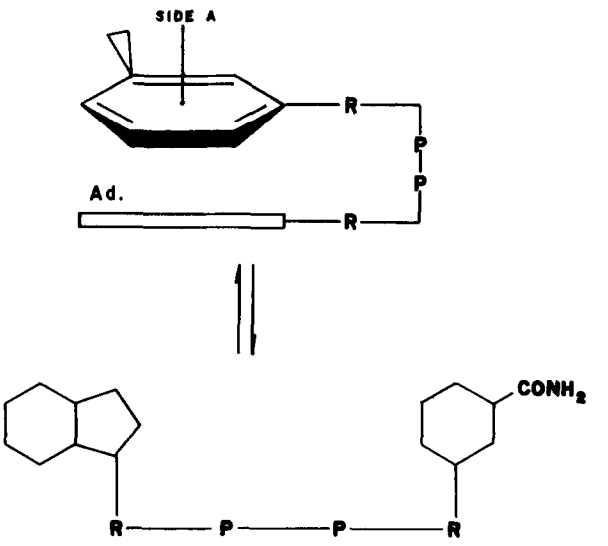
SUMMARY: A Circular Dichroic absorption study of the reaction of oxidized pyridine nucleotides with cyanide ions fully confirms the occurence of a very weak Cotton effect around 435 nm in the Circular Dichroic spectrum of the reduced coenzymes and therefore the very faint transition (λ_{max} = 435 nm; ε_{max} ~ 1 M^{-1} cm^{-1}) from which the Cotton effect originates.

INTRODUCTION

A NMR study by Oppenheimer et al. (1) of the addition of CN^- ions to NAD^+ and analogs indicates that NAD^+, like NADH, exists predominantly in one folded conformation which is in rapid equilibrium with an open form:



Abbreviation: CD - circular dichroic

The folded conformation should lead to a preferential formation of the enantiomer which has the CN group on the A-side of the ring. Hence it was of interest to study the system by circular dichroism. It was reasoned that the presence of a new chiral center might enhance, and thus confirm, the weak Cotton effect we recently observed (2) arising from the very faint transition ($\varepsilon_{max} \sim 1 M^{-1} cm^{-1}$) of the 1,4-dihydronicotinamide ring at 435 nm (3). Some information was also expected on the vexing question (4-8) of CN^- addition at other positions of the pyridinium ion in view of a possible enhancement of rotational strength because of a more hindered rotation.

EXPERIMENTAL SECTION

Circular dichroism measurements were made on a Cary 60 Spectropolarimeter equipped with the Model 6001 circular dichroism attachment. Circular cells of 1 cm optical path were used. Absorption curves were determined on a Zeiss DMR 21 Recording Spectrophotometer using 1 cm path cells.

β-NAD^+, α-NAD^+, and $NADP^+$ were obtained from Sigma Chemical Co. Their purity was 99%, 95%, and 98% respectively. ATP was obtained from BDH Chemical LTD. ; pig heart lactate dehydrogenase (EC 1.1.1.27) was obtained from Boehinger Mannhein GMbH. Rabbit muscle lactate dehydrogenase (EC 1.1.1.27), alchool dehydrogenase (EC 1.1.1.1.), glyceraldehydephosphate dehydrogenase (EC 1.2.1.12) and bovine serum albumin were obtained from Sigma Chemical Co.; KCN was a J.T.Baker Chemical product. 1,6-NADH was prepared according to the method of Chaykin et al. (9), from NAD^+ and $NaBH_4$.

To calculate the molar ellipticity, $[\theta]$, the following equation was employed:

$$[\theta]_\lambda = \frac{3300\Delta D}{cl} \quad \text{deg. cm}^2\text{. decimole}^{-1}$$

where D is the circular dichroic optical density, c is the molar concentration, and l is the path lenght in cm.

Control experiments ommitting cyanide showed that OH^- addition is not important under experimental conditions.

RESULTS AND DISCUSSION

The CD spectra of 1,4-α- and 1,4-β-NADH are known (10); however, that of 1,6-β-NADH has not yet been reported. We find that, contrary to the order of the molar absorptivities, the 340 nm CD band of 1,6-β-NADH ($[\theta]$ = -102,143 deg cm^2 $decimole^{-1}$) is stronger than that of the 1,4 isomer ($[\theta]$ = -40,200), whereas the opposite behavior is observed for the 260 nm band ($[\theta]$ = 204,280 and $[\theta]$ = 254,600, respectively).

The addition of CN^- ions to β-NAD^+ or β-$NADP^+$ resulted in CD spectra having features in common with that of the reduced form (10). The presence of glyceraldehyde phosphate dehydrogenase, alchool dehydrogenase, lactate dehydrogenase and bovine serum albumin did not significantly influence the NAD^+/CN^- system as shown by the fact that neither the velocity nor the final CD spectrum were appreciably affected. Our negative results with various samples of lactate dehydrogenase are at variance with the work of Gerlach et al (11) who found that pig heart lactate dehydrogenase catalyzes the addition of CN^- to NAD^+. Under the experimental conditions used, CN^- did not react with NAD^+, which normally occurs bound to glyceraldehydephosphate dehydrogenase. This may be related to the fact that NAD^+ occurs bound to glyceraldehydephosphate dehydrogenase in a folded conformation in which the other

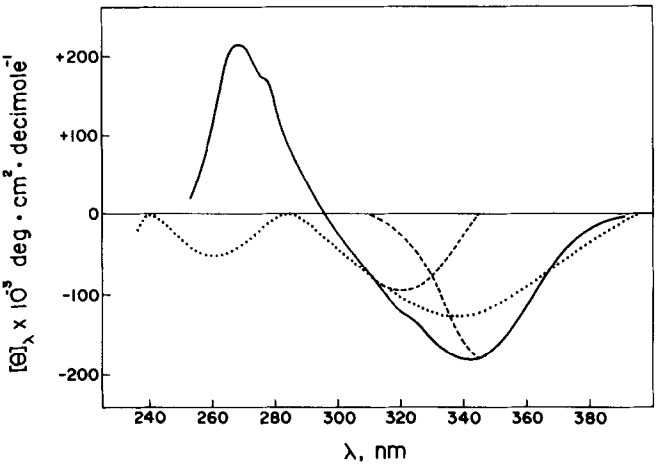


Fig. 1. Circular dichroic absorption spectrum of the α-NAD^+/CN^-adduct. The dotted lines show the resolution of the long wavelength CD band. Also shown is the CD spectrum of α-NADH (•••••).

side of the pyridinium ring is involved in charge-transfer complexation with an indole side chain of the enzyme (12).

Fig. 1 shows the CD spectrum of the α-NAD^+/CN^- system; for comparative purposes, the CD spectrum of 1,4-α-NADH is also presented. There is a clear indication of a hidden CD band at 320 nm. This feature proved to be reproducible over several trials. By analogy to the results of Reference (13), the longer wavelength band (λ_{max} = 341 nm) is tentatively assigned to the 1,6 adduct and that at 320 nm to the 1,4 adduct.

Interaction at high concentration of the partners. Using high β-NAD^+ and CN^- ion concentrations, it is possible to observe a very weak, broad, positive CD band above 420 nm (Figure 2). This band is not shown by β-NAD^+ alone or by the ATP/CN^- system. This band or part of it, should certainly correspond to the very weak CD band shown by β-NADH at 435 nm. The corresponding band observed with α-NAD^+ is stronger and is negative (Figure 2).

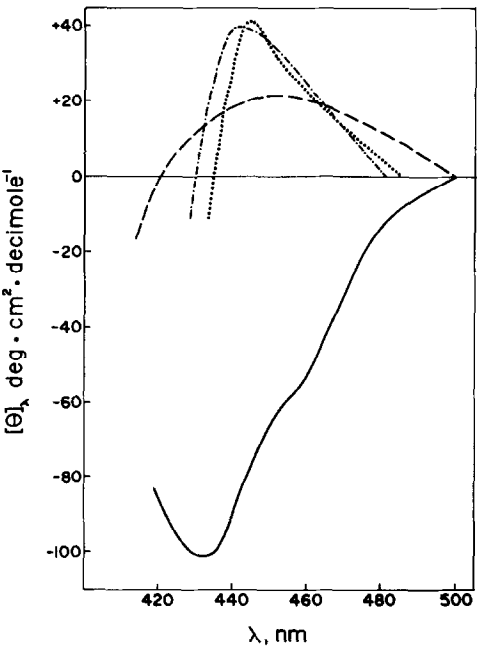


Fig. 2. Circular dichroic absorption spectrum of the α-NADH$^+$/CN$^-$ adduct (———) and of the β-NAD$^+$/CN$^-$ adduct (- - - -). Also shown are the spectra of α-NADH(••••••) and of β-NADH(-•-•-•).

The present results fully confirm the existence of a very weak Cotton effect in the visible spectrum of the 1,4-dihydronicotinamide coenzymes (2) and the very faint transition (λ_{max} = 435 nm; ε_{max} ~ $1M^{-1}$ cm^{-1}) from which the Cotton effect originates (3).

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