ON THE STRUCTURE OF NADH-X

Klaus Zinner, Adelaide Faljoni and Giuseppe Cilento

Departamento de Bioquímica, Instituto de Química
Universidade de São Paulo, C. P. 20780, São Paulo, Brazil

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Summary: By following both the 285 nm Cotton effect and the increase in absorbance (ΔA_{285}) during the enzymic (glyceraldehyde-3-phosphate dehydrogenase) and the non-enzymic (pyrophosphate buffer) formation of NADH-X, it is inferred that (i) the configuration at C-1' is not changed, and (ii) the 285 nm Cotton effect in 6-hydroxy-1,4,5,6-tetrahydronicotinamide coenzymes arises not only from interaction with the ribose moiety but also from interaction with the C_6 chiral center.

NADH-X, the product resulting from the action of glyceraldehyde-3-phosphate dehydrogenase (GPD) upon NADH (1-3) or formed in the early stages of the action of polybasic anions upon NADH (4,5) is very similar to, but not identical with, the hydration product formed from NADH in dilute acids, i.e. the so-called primary acid product (p.a.p.). Since NADH-X can be transformed into p.a.p. (3) and since a $\beta \longrightarrow \alpha$ epimerization occurs in dilute acids (6), the most simple hypothesis concerning the structure of NADH-X is that it is the β-epimer of p.a.p. In other words, during the GPD or phosphate catalyzed addition of water, differing from the proton catalyzed addition, the configuration at the C-1' atom of the ribose moiety would not be

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affected; after hydration however, the product would change into the thermodynamically more stable α-epimer.

Since 5,6-saturated derivatives of NADH show a strong electronic transition near 285 nm, this hypothesis can be tested by following and comparing the absorbance and the Cotton effect at 285 nm as a function of time.

Material and Methods

β-NADH, α-NADH and GPD (yeast and muscle) were obtained from Sigma Chemical Company. The reaction was run in 0.02M pyrophosphate 0.012M maleate buffer at pHs 6.0 and 5.5, in the absence and in the presence of GPD (17 units/ml), the concentration of NADH being approximately 9×10^{-4}M. The absorbance at 285 nm was followed in 1 mm path cells, using a Zeiss DMR-21 Recording Spectrophotometer. For the C.D. spectra, either 1 mm path cells were employed or 0.2 ml aliquots of the reaction mixture were withdrawn, diluted to 3.1 ml with buffer or water and the spectrum taken in 1 cm cells at 27°C, a Cary 60 spectropolarimeter being employed.

Results and Discussion. In the presence of GPD from yeast, the 285 nm Cotton effect of β-NADH develops only in the later stages of the reaction; a representative example is shown in Fig. 1. In other experiments at pH 6.0, the C.D. spectrum (not illustrated) was recorded when 30%, 45% and 85% of the expected increase in A_{285} had already take place. However, within the limits of accuracy, no Cotton effect was detected. These results are consistent with the consecutive formation of two compounds with similar ε_{285} values but with 285 nm Cotton effects of very different intensities:

$$\beta\text{-NADH} \longrightarrow \text{NADH-X} \longrightarrow \text{p.a.p.}$$

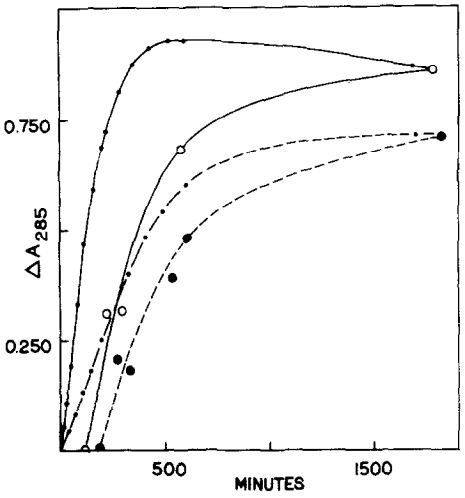

Figure 1. The increase in absorbance at 285 nm and the intensity of the 285 nm Cotton effect as a function of time when 9×10^{-4} M β-NADH is hydrated at pH 5.5 in the absence (broken curves) or presence of yeast GPD. In both cases the curve representing the intensity of the Cotton effect (large circles) was drawn at a height equal to that of ΔA_{285} at the end of the experiment. The molecular ellipticity $[\theta]$ at the end of the enzymic reaction, closely agrees with the data of Miles et al (6).

In the presence of GPD from muscle or in the absence of enzyme, the phenomenon was less striking. Undoubted this was because the hydration step, being slower (1), allowed a greater opportunity for the occurrence of a second step, most likely a $\beta \longrightarrow \alpha$ epimerization.

The 285 nm Cotton effect of p.a.p. is strong and positive (6); that of NADH-X appears to be very weak. Now, since water or $HgCl_2$ addition to α-NADH produces almost identical, strong, positive Cotton effects at 285 nm and since $HgCl_2$ addition to β-NADH or to β-NMNH produces a strong negative Cotton effect (6), one might also have anticipated a strong, negative, Cotton effect for NADH-X at 285 nm.

Useful information on this point was obtained by submitting α-NADH to the action of GPD. Surprisingly, it was observed that the absorbance at 285 nm increased even faster than with the β-anomer. The 285 nm Cotton effect (positive) only started developping after 60% of ΔA_{285} had already taken place. Therefore also with α-NADH an intermediate is first formed for which, within the limits of accuracy, the 285 nm Cotton effect is of zero intensity. Similar behavior, although less dramatic, was observed when α-NADH was hydrated in the presence of phosphate.

The fact that GPD and phosphate induce similar behavior with both α and β-NADH suggests that the "enzymic" action of GPD is not stereospecific and therefore that a mixture of C_6 epimers might be formed. This would explain why the Cotton effect is initially of zero intensity. It would also explain why the enzyme, which in the presence of ATP and Mg^{++} ions converts NADH-X back to NADH, acts very fast upon half of the substrate and slowly upon the other half (3). In this regard a recent analogy is the dehydration of pteridine 3,4 monohydrate in the presence of calf duodenal adenosine deaminase (7).

It would therefore appear that from the mixture of C_6 epimers with α-configuration, the thermodynamically more stable C_6-epimer will eventually predominate and produce the positive Cotton effect. The same epimer will also be formed from the mixture of C_6 epimers of β-configuration, after a $\beta \longrightarrow \alpha$ epimerization.

An alternative explanation for our results would require the hydration to be stereospecific, an initially attractive hypothesis in view of the fact that GPD holds β-NADH in a closed conformation (8). However the fact that α-NADH is also hydrated, and the observation that phosphate induces the same qualitative behavior as GPD, coupled with the difficulty to explain the absence of

Cotton effect in the initial stage makes this alternative less likely.

At any rate, the existence of three types of Cotton effects at 285 nm, positive, negative and one of nearly zero intensity, in the circular dichroism spectrum of 6-hydroxy-1,4,5,6-tetrahydro-nicotinamide coenzymes suggests that they arise not only from interaction with the ribose moiety close to the 285 nm chromophore but also from interaction with the C_6 chiral center.

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