CONFORMATION ABOUT THE GLYCOSIDIC BOND: RIBOSE–NICOTINAMIDE OF NAD$^+$ BOUND TO SOME DEHYDROGENASES

Jean-François BIELLMANN and Jean-Pierre SAMAMA
Institut de Chimie, Université Louis Pasteur, 1, rue Blaise Pascal, 67008 Strasbourg Cedex, France*

Received 12 October 1973

1. Introduction

The absence of a strong preference for the conformation about the glycosidic bond between nicotinamide and the C-1 of the ribose in NMN** which may be very likely extended to NAD$^+$ [1] (fig. 1), raises the question of the relative orientation of the two rings in the complexes with the dehydrogenases. The introduction of a methyl group at C-2 or C-6 of the nicotinamide favours strongly the conformation where the methyl group is *syn* to the hydrogen on C-1 of the ribose. The behaviour of the two analogues: 2-Me NAD$^+$ and 6-Me NAD$^+$ with the horse liver alcohol dehydrogenase and the dogfish lactate dehydrogenase, is in accord with the proposal that the NAD$^+$ is in the conformation where C-2 of the nicotinamide and the H of C-1 of the ribose are *syn* in the complexes with the two enzymes; in other words the conformation of this glycosidic bond is *anti* according to the general rule [2].

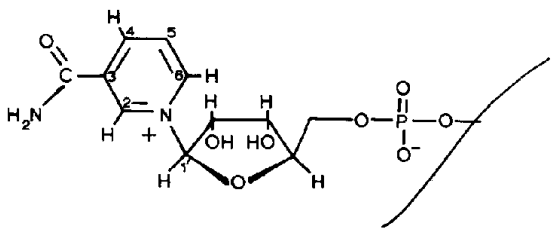

Fig. 1.

* Laboratory associated to the CNRS.

** *Abbreviations*: NAD$^+$: nicotinamide adenine dinucleotide; NMN: nicotinamide mononucleotide; 2-Me NAD$^+$: 2-methyl nicotinamide adenine dinucleotide; 6-Me NAD$^+$: 6-methyl nicotinamide adenine dinucleotide.

2. Materials and methods

Model compounds have been prepared in order to ascertain the structures of the NAD$^+$ derivatives and will not be described here [3]. The 2-methyl-3-cyanopyridine [4] and 6-methyl-3-cyanopyridine (product obtained from 6-methyl-3-cyano-2-chloropyridine [5] which is in turn synthesised from 6-methyl-3-cyano-2-pyridone [6]) are converted to the corresponding amide by hydrolysis with Dowex 1 X 8 [7]. The 2-Me and 6-Me NAD$^+$ are prepared by transglycosidation catalysed by the pig brain NAD glycohydrolase (Sigma) [8, 9] and purified by five chromatographies on Dowex 1 X 2 (200–400 mesh; formate form; gradient 0–0.4 N formic acid).

The efficiency of the separation was checked as follows: a mixture containing 18–20% of [4-^{3}H] NAD$^+$ and purified analogue are mixed and chromatographed; the purification process is performed until the ultraviolet control indicates only one product. The amount of NAD$^+$ in the 2-Me and 6-Me NAD$^+$ determined by radioactivity, is less than 0.2%. The ultraviolet data of both derivatives are very similar: in 0.1 M potassium pyrophosphate buffer pH 7.6 λ_{max} = 260 nm (ϵ = 20 000); in 1M KCN λ_{max} = 260 nm (ϵ = 15 000); λ_{max} = 332 nm (ϵ = 5000).

The isoenzymes III, IV and V [10] of the horse liver alcohol dehydrogenase were a generous gift from Professor J.P. von Wartburg (Bern) and the dogfish lactate dehydrogenase has been kindly provided by Dr. P.D.G. Dean (Liverpool). The oxidation of cinnamyl alcohol by the horse liver alcohol dehydrogenase has been studied in our laboratory: general conditions: cinnamyl alcohol 10^{-3} moles/litre, 0.1 M potassium pyrophosphate buffer pH 7.6 [11].

Table 1
K_M and V_{max} for the oxidation of cinnamyl alcohol with the isoenzymes of the horse liver alcohol dehydrogenase.

Isoenzymes	NAD^+ K_M(10^5 moles/litre)	2-methyl NAD^+ K_M(10^5 moles/litre)	V_{max}*
III	4	72	26%
IV	4	50	5%
V	10	8.5	50%

* Relative to the V_{max} of NAD^+ (100%).

The experimental conditions for the lactate dehydrogenase were: 0.1 M Tris–HCl buffer, pH 8.8, 10^{-3} M EDTA, 11.4 mM lithium lactate.

3. Results and discussion

With ethanol and 2-Me or 6-Me NAD^+ no activity was detected by spectroscopic methods with the alcohol dehydrogenase. The possibility that these analogues are not powerful enough oxidising agents due to the electron releasing power of the methyl group may explain these results. The use of cinnamyl alcohol which is more easely oxidised than ethanol and gives cinnamaldehyde strongly absorbing at 290 nm (ϵ = 25 000) is a very sensitive way of detection for activity of such analogues [11]. With cinnamyl alcohol 2-Me NAD^+ is active and the kinetic constants determined by Lineweaver–Burk plot [12] are presented in table 1 and fig. 2. The 6-Me NAD^+ does not show any activity with these isoenzymes by using ethanol or cinnamyl alcohol. We then undertook an inhibition study of oxidation of cinnamyl alcohol with NAD^+ by 6-Me NAD^+. We did not detect any inhibition with the three isoenzymes even at a concentration of 10^{-3} moles/litre of 6-Me NAD^+. If it is admitted that the inhibition is competitive, the inhibition constant is certainly larger than 10^{-3} moles/litre.

For dogfish lactate dehydrogenase, we could not find any activity with either analogue. This is most probably due to an unfavourable equilibrium and since there is no more convenient substrate available, we undertook an inhibition study. The 2-Me NAD^+ is a competitive inhibitor K_i 1.7×10^{-3} moles/litre and the 6-Me NAD^+ is a noncompetitive inhibitor K_i 1.7×10^{-3} moles/litre (see figs. 3 and 4) relative to NAD^+ (K_M for NAD^+ found here: 10^{-4} moles/litre; ref. [13]: 3×10^{-4} moles/litre).

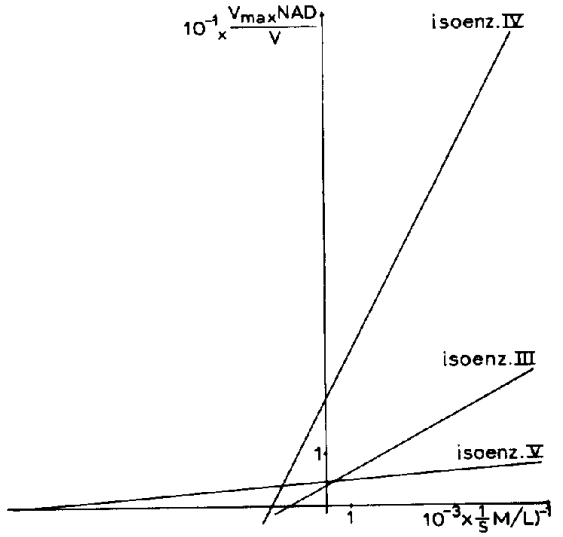

Fig. 2. Activity of 2-Me NAD^+ with the three major isoenzymes.

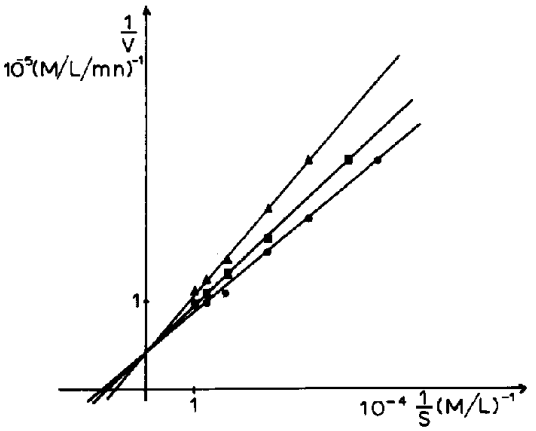

Fig. 3. Inhibition study with 2-Me NAD^+ of the lactate dehydrogenase: (●—●—●) NAD^+; (■—■—■) 3.8×10^{-4} moles/litre inhibitor; (▲—▲—▲) 7.6×10^{-4} moles/litre inhibitor.

The conformation about the glycosidic bond of 2-Me and 6-Me NAD^+ may be safely deduced from the results of studies on the conformation of cytidine [14] and uridine [14–16] and their C-6 methyl analogues [14, 17, 18]. Cytidine and uridine exist in the anti conformation in solution [14–16] whereas 6-methylcytidine and 6-methyluridine are *syn* under the same conditions [14, 17, 18]. In the crystalline state, cytidine is *anti* [19] and 6-methyluridine *syn* [20]

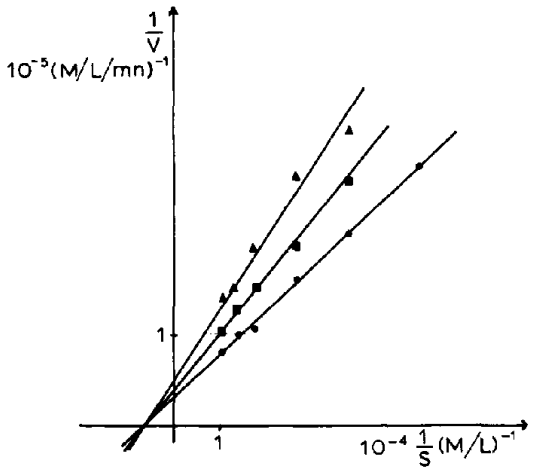

Fig. 4. Inhibition study with 6-Me NAD^+ of the lactate dehydrogenase: (●--●—●) NAD^+; (■—■—■) 6.5×10^{-4} moles/litre inhibitor; (▲—▲—▲) 9.75×10^{-4} moles/litre inhibitor.

(same results as in solution). The methyl group leads to a complete change in the conformation of the glycosidic bond, which probably results from the large steric effect of the methyl group compared to that of the carbonyl group at C-2 of the pyrimidines. The hydrogen being still smaller than the oxygen, the methyl group is expected to have a very large effect on the orientation of the nicotinamide ring, and so 2-Me NAD^+ is in the *anti* and 6-Me NAD^+ is the *syn* conformation.

The binding of 2-Me NAD^+ to the three major isoenzymes of horse liver alcohol dehydrogenase shown by its enzymatic activity, and the very loose binding, if any, of 6-Me NAD^+ to the same enzymes proved by the absence of activity, and by the large value of the lower limit of the inhibition constant, are in agreement with the proposal that NAD^+ is in the *anti* conformation in the complexes with the three major isoenzymes of horse liver alcohol dehydrogenase.

For the dogfish lactate dehydrogenase, 2-Me NAD^+ is a competitive inhibitor and 6-Me NAD^+ a noncompetitive inhibitor for the coenzyme: 2-Me NAD^+ is binding to the NAD^+ site whereas 6-Me NAD^+ is not binding to this site. From the discussion above, the conclusion is that the NAD^+ is also in the *anti* conformation when bound to the dogfish lactate dehydrogenase. This conformation has been suggested from data of the Rx structure determination for the bound

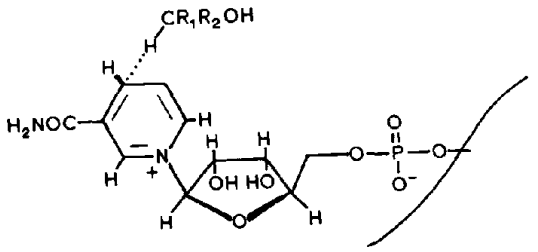

Fig. 5. Relative positions of coenzyme and substrate during hydride transfer.

coenzyme [21, 22]. Both dehydrogenases belong to class A: the transfer of the hydrogen from substrate to the coenzyme occurs to the *re* face of the nicotinamide ring. Considering the conformation about the glycosidic bond and the stereochemistry of the hydrogen transfer, the position of the substrate relative to the other portion of the coenzyme is determined as presented in fig. 5. Further study on these two analogues with other enzymes are in progress.

Acknowledgements

The financing of this work by the DGRST (contract no. 72.7.0139) and the Foundation pour la Recherche Medicale Francaise is greatfully acknowledged.

References

[1] Egan, W., Forsen, S. and Jacobus, J. (1973) Chem. Commun. 2, 42.
[2] Donohue, J. and Trueblood, K.N. (1960) J. Mol. Biol. 2, 363.
[3] Samama, J.P. and Wrixon, A.D., unpublished results.
[4] Patent: Badische Anilin und Soda Fabrik, A.G. (by Pasedach, H. and Seefelder, M.) Ger. 1 207 930; Chem. Abst. 64, 9694 d.
[5] Reider, M.J. and Elderfield R.C. (1942) J. Org. Chem. 7 286–296.
[6] Roblitt, J.M. and Scola, D.A. (1960) J. Org. Chem. 25, 560–564.
[7] Galat, A. (1948) J. Am. Chem. Soc. 70, 3945.
[8] Kaplan, N.O. and Ciotti, M.M. (1956) J. Biol. Chem. 221, 823–832.
[9] Anderson, B.M., Ciotti, C.J. and Kaplan, N.O. (1959) J. Biol. Chem. 234, 1219–1225.
[10] Lutstorf, U.M., Schurch, P.M. and Von Wartburg, J.P. (1970) Eur. J. Biochem. 17 497–508.

[11] Biellmann, J.F. and Samama, J.P., to be published. For reduction of cinnamaldehyde, see Winter, A.D. (1958) Acta Chim. Scand. 12, 1695-1696.
[12] Lineweaver, H. and Burk, D. (1934) J. Am. Chem. Soc. 56, 658–666
[13] Rosenheimer, N. (1973) These 3e Cycle, Strasbourg.
[14] Schweizer, M.P., Banta, E.B., Witkowski, J.T. and Robins, R.K. (1973) J. Am. Chem. Soc. 95 3770–3778.
[15] Hart, P.A. and Davis, J.P. (1972) J. Am. Chem. Soc. 94, 2572–2577.
[16] Hruska, F.E., Grey, A.A. and Smith, I.C.P. (1970) J. Am. Chem. Soc. 92, 4088–4094.
[17] Schweizer, M.P., Witowski, J.T. and Robbins, R.K. (1971) J. Am. Chem. Soc. 93 277–279.
[18] Miles, D.W., Robins, M.J., Robins, R.K., Winkley, W.M. and Eyring, H. (1969) J. Am. Chem. Soc. 91, 824–831.
[19] Furberg, S., Petersen, C.S. and Romming, C. (1965) Acta Cryst. 18, 313–320.
[20] Suck, D. and Sanger, W. (1972) J. Am. Chem. Soc. 94, 6520–6526.
[21] Adams, M.J., McPherson Jr., A., Rossmann, M.G., Schevitz, R.W. and Wonacott, A.J. (1970) J. Mol. Biol. 51 31–38.
[22] Adams, M.J , Buchner, M., Chaudrasekhar, K., Ford G.C Hackert, M.L., Liljas, A., Rossmann, M.G., Smiley, I.E., Allison. W.S., Everse, J., Kaplan N.O. and Taylor, S.S. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1968–1972.