STEREOSPECIFICITY OF THE ENZYMIC NAD SYNTHESIS

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There are two known isomers of NAD: α-NAD and β-NAD. The α isomer forms about 10-15 per cent of common NAD preparations and, contrary to the β isomer, is not active with alcohol dehydrogenase and is not hydrolyzed by NAD glycohydrolase (Kaplan et al.,1951). The difference lies in the nicotinamide-ribose bond, which has the β configuration in the enzymically active form (β-NAD) and the α configuration in the enzymically inactive form (α-NAD) (Kaplan et al.1955). It should be noted that almost all the naturally occurring nucleotides contain a β ribosidic link (Davoll et al.,1946).

As far as the origin of the two isomers of NAD is concerned, two hypothesis may be formulated. The two isomers could originate from a partially asymmetric synthesis; on the other hand, only one isomer could be synthesized and this isomer could subsequently undergo an isomerisation.

In this report evidence is presented that the β isomer is the only product of NAD synthesis in vitro and in vivo.

In vitro, NAD has been synthesized from nicotinic acid by human erythrocytes, under the classical conditions of Preiss and Handler (1957). NAD synthesis in vivo has been studied in rat liver after nicotinamide injection (Bonsi-

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gnore and Ricci,1949). NAD has been isolated from other pyridine nucleotides and from N-methylnicotinamide by chro= matography on a 1 x 12 cm column of Dowex 1 ($HCOO^-$), X8, 200-400 mesh. Elution was begun with 1N formic acid in the reservoir, following the method of Hurlbert et al.,(1954). The fractions in the NAD peak were pooled and lyophilized. Total NAD (α isomer + β isomer) was estimated by the reac= tion with KCN; a mM absorbancy coefficient of 5.9 at 327 mμ was used (Siegel et al.,1959). β-NAD was estimated by re= duction with alcohol dehydrogenase (Ciotti and Kaplan,1957); a mM absorbancy coefficient of 6.22 at 340 mμ was used (Horecker and Kornberg,1948). The amount of α-NAD was calculated by difference. This method has been checked with purified isomers purchased from the Sigma Chemical Co.; it is possible to detect amounts as low as 2% of one isomer in the presence of the other.

The results of the analysis on the NAD synthesized in vitro by erythrocytes are summarized in table I.

TABLE I
Stereospecific synthesis of NAD in human erythrocytes

Incubation time	total NAD (μmoles)	α isomer (μmoles)	(%)	β isomer (μmoles)	(%)
0^h	0.721	0.079	11	0.642	89
24^h	5.074	0.102	2	4.972	98

The reaction mixture contained: 140 μmoles nicotinic acid; 280 μmoles $MgCl_2$; 700 μmoles phosphate buffer pH 7.4; 314 mg glucose; 2800 μmoles NH_4Cl; defibrinated whole blood 42 ml. Incubation at 37°C. The reaction was stopped by adding 1 vol. of 10% TCA. NAD was precipitated overnight with 5 vol. of cold acetone. The precipitate was dissolved in wa= ter, neutralized and adsorbed on to the Dowex 1 column. The values of NAD are expressed as μmoles in the reaction mixture.

There is a 7 fold increase in the amount of NAD pres= ent in the reaction mixture after an incubation of 24 h.

At this time α-NAD is undetectable, while the enzymically reactive NAD (β-NAD) accounts for the total NAD present in the reaction mixture, as determined with KCN. This result strongly suggests that only β-NAD is synthesized from nicotinic acid by erythrocytes. Had the NAD synthesis been only partially asymmetrical, the ratio of α isomer to β isomer ought to remain the same at the beginning as at the end of the incubation.

The results of the analysis on the NAD synthesized in vivo by rat liver are summarized in table II.

TABLE II
Stereospecific synthesis of NAD in rat liver

Hours after injection	total NAD (μmoles/g)	α isomer (μmoles/g)	(%)	β isomer (μmoles/g)	(%)
0	0.602	0.079	13.2	0.523	86.8
2	1.690	0.034	2	1.656	98
5	2.263	0.045	2	2.218	98
8	2.858	0.057	2	2.801	98
12	5.004	0.115	2.3	4.889	97.7
16	2.879	0.135	4.7	2.744	95.3
24	0.554	0.043	7.7	0.511	92.3

The rats were injected intraperitoneally with 100 mg of nicotinamide per 100 g of body weight. Pyridine nucleotides have been extracted from the liver with perchloric acid and separated by column chromatography. Each value is the mean of two rats.

After the injection of nicotinamide there is an increase in the liver concentration of NAD which is the result of an enhancement in the rate of NAD synthesis. The maximum concentration of NAD is reached at the 12th h after the nicotinamide injection; then the synthesis of NAD returns to the normal rate. It should be noted that the percentage of α isomer decrease during the period of enhanced NAD synthesis. Only the β isomer accounts for the increased level of NAD; the amount of α isomer becomes undetectable. From the 12th to the 24th h the percentage of α isomer

tends to approach the normal value.

The concentration of α isomer shows variations which are not within the experimental error. An increase is noted, at the 12^{th} and 16^{th} h after the injection of nicotinamide, when the concentration of β-NAD begins to decrease. It is therefore probable that a conversion of β isomer to α isomer takes place. A decrease in the concentration of the α isomer is observed at earlier times after the injection of nicotinamide. The present work does not allow any conclusions about the exact significance of this decrease.

The data suggest that the β isomer is the only product of NAD synthesis both from nicotinic acid in human erythrocytes and from nicotinamide in rat liver. Further investigation is needed to determine whether the synthesis of NAD is stereospecific also when it occurs at the normal rate in the presence of physiological concentration of precursors.

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