

OPTIMIZATION OF THE SYNTHESIS OF N(1)-(2-AMINOETHYL)-NAD(P)

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Abstract - The alkylation of NAD(P) to N(1)-(2-aminoethyl)-NAD(P) with ethyleneimine in aqueous solution was optimized. First the pH-dependency of the alkylation of the N(1)-position of the adenine nucleus of NAD was studied in the pH range 2 - 5.5 at 20 °C, followed by adjusting the temperature and the concentration of ethyleneimine to achieve acceptable reaction times. The results were directly applicable with respect to the synthesis of N(1)-(2-aminoethyl)-NADP.

N(1)-(2-aminoethyl)-adenine derivatives of NAD(P) are important starting compounds for the efficient synthesis of column bound and water-soluble macromolecular derivatives of NAD(P)(H) e.g. Sepharose- and polyethylene-glycol (PEG)-N⁶-(2-aminoethyl)-NAD(P)(H)¹⁻⁴.

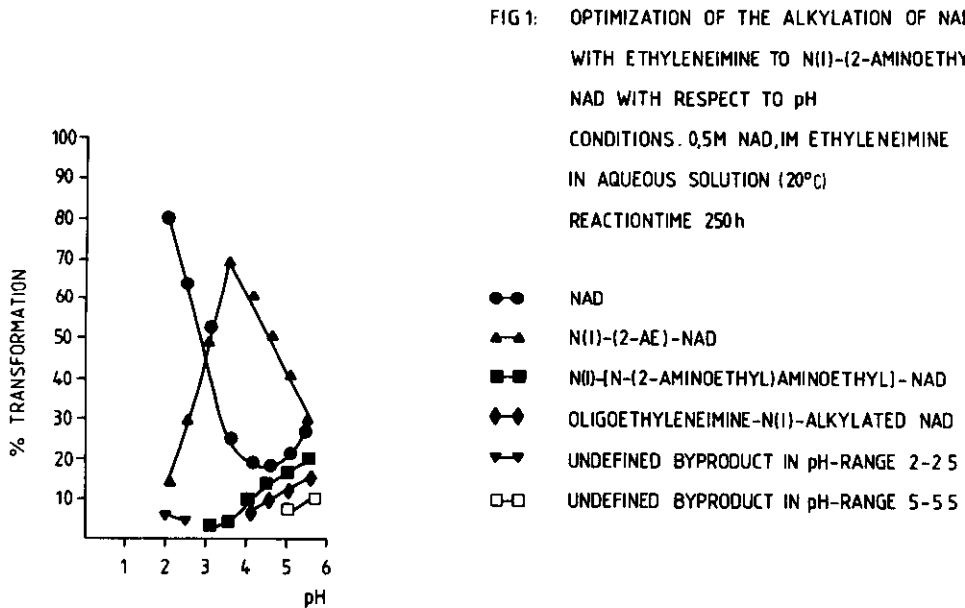
These derivatives of NAD(P) are synthesized by alkylation of the N(1)-position of the adenine with ethyleneimine in aqueous solution.

It became evident that ethyleneimine reacts further with the primary amino group of the aminoethyl group introduced giving N(1)-N-(2-aminoethyl)aminoethyl - and N(1)-(oligoethyleneimine)-NAD(P) as byproducts in considerable amounts². In consequence it became urgent to check if the formation of these byproducts could be minimized in favour of the formation of N(1)-(2-aminoethyl)-NAD(P).

NAD reacted with ethyleneimine at relatively high concentration in aqueous solution at 20 °C varying the pH in the range 2 - 5.5.

The course of the reaction, disappearance of NAD and formation of N(1)-(2-aminoethyl)-NAD, N(1)-N-(2-aminoethyl)aminoethyl -NAD (byproduct I) and

N(1)-(oligoethyleneimine)-NAD (byproduct II), was followed by quantitative UV-scanning after thin-layer chromatography (see experimental section). The composition (%) of the reaction mixture after 250 h was plotted in relation to the fixed pH values resulting in Fig. 1.



This plot proved that optimal conditions with respect to pH could be achieved restricting the formation of N(1)-NAD byproducts. For instance, at pH 3.5, 70 % N(1)-(2-aminoethyl)-NAD could be obtained with just 4 % N(1)-NAD byproducts leaving 26 % of the NAD unreacted. This composition was actually found after 200 h, which is still a long reaction time. Keeping the NAD concentration at 0.5 M and the pH at 3.25 and 3.5, the influence of raising the temperature to 30 °C and the ethyleneimine concentration up to 1.66 M on the composition of the reaction mixture and the reaction time has been investigated (Table 1).

Table 1. Comparison of the alkylation of NAD (0.5 M) with ethyleneimine (1.25 - 1.66 M) at 30 °C and at pH 3.25 and 3.5.

Nr. Experiment	Conc. Ethyleneimine (M)	pH (\pm 0.05)	NAD (%)	N(1)-(2-AE)-NAD (%)	N(1)-NAD by-products (%)	Reaction time (h)
I	1.25	3.5	20	70	10	55
II	1.25	3.25	24	70	6	96
III	1.41	3.25	20	72	8	96
IVa	1.66	3.25	26	69	5	52
IVb	1.66	3.25	11	79	10	96

2-AE: 2-Aminoethyl

The data of Table 1 point to a strong effect of a slight pH change on the reaction time to obtain a composition with 70 % N(1)-(2-aminoethyl)-NAD (I and II). By increasing at pH 3.25 the ethyleneimine concentration (IVa), a composition similar to II could be achieved decreasing the reaction time approximately one half to that of I. The reaction conditions of IVa should be chosen if a limited formation of the N(1)-NAD byproducts is required e.g. in the case of the synthesis of technical grade PEG (M_r 20 000)-N⁶-(2-aminoethyl)-NADH³.

If pure N(1)-(2-aminoethyl)-NAD is the final aim the reaction time should be longer (IV^b).

The optimal conditions found for N(1)-(2-aminoethyl)-NAD could directly be adapted for the synthesis of N(1)-(2-aminoethyl)-NADP approaching or exceeding the 70 % margin (Table 2). This has led to conditions where the formation of similar N(1)-NADP byproducts is suppressed, combined with a rather long reaction time (Table 2, I). By increasing both the NADP and the ethyleneimine concentration the reaction time can be shortened increasing the formation of the N(1)-(NADP) byproducts (Table 2, II).

Table 2. The alkylation of NADP with ethyleneimine at optimal conditions

No. Experiment	Conc. Ethyleneimine (M)	Conc. NADP (M)	pH (± 0.05)	NADP (%)	N(1)-(2-AE)-NADP (%)	N(1)-NAD byproducts (%)	Reaction time (h)
I	1.12	0.38	3.25	27.5	68	4.5	120
II	1.75	0.6	3.25	16	73	11	55

2-AE: 2-aminoethyl

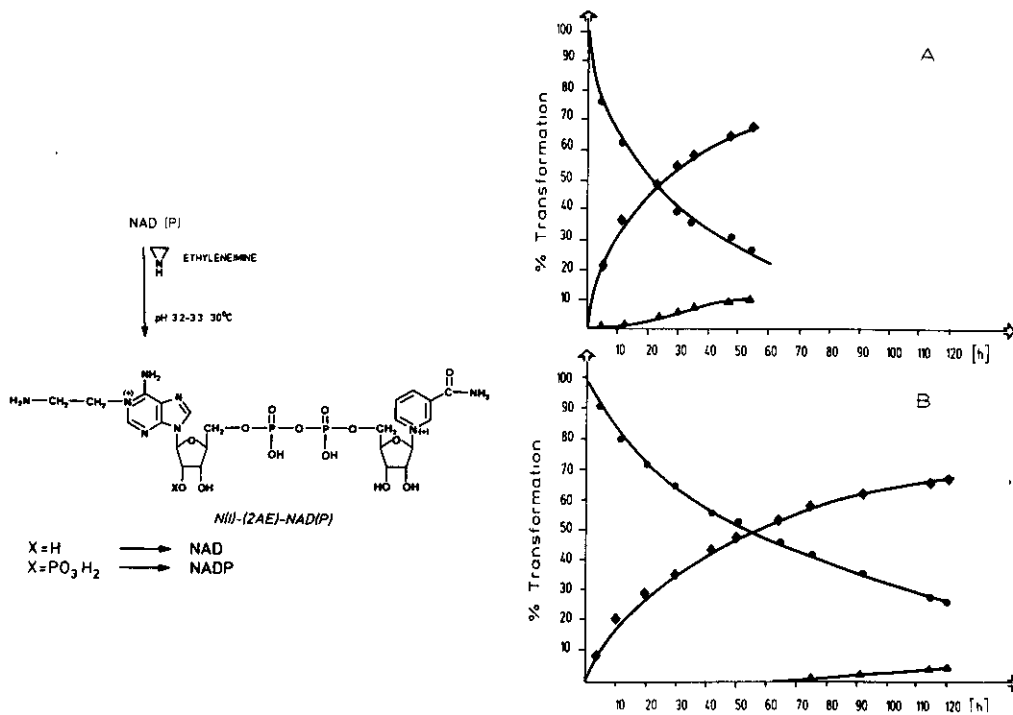


Figure 2: Reaction scheme and composition of the reaction mixture (%) as a function of time (h) for the alkylation of NAD (A) and NADP (B) with ethyleneimine under optimized conditions (see Experimental). (●) NAD(P); (◆) N(1)-(2-aminoethyl)-NAD(P); (▲) N(1)-NAD(P) byproducts; pH 3.25 \pm 0.05.

The reaction pathway of the conversion of NAD(P) in N(1)-(2-aminoethyl)NAD(P) and the course of the N(1)-alkylation of NAD and NADP on a preparative scale, suppressing the formation of the N(1)-(NAD(P)) byproducts, is outlined in Fig. 2.

Both N(1)-(2-aminoethyl)-NAD and -NADP can be purified by cation exchange chromatography with overall yields approaching 50 % due to the unexpected simultaneous formation of N⁶-(2-aminoethyl)-NAD(P) and tricyclic 1,N⁶-(ethano-adenine)-NAD(P) from N(1)-(2-aminoethyl)-NAD(P) under the mild purification conditions amounting up to 20 %.⁴

EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out on silica gel 60F₂₅₄ (Merck, 0.2 mm) in isobutyric acid/25 % aqueous NH₃/H₂O (66/1/33, V/V/V), pH 3.7. Quantitative visualization was performed by scanning at 259 nm with a Shimadzu CS-920 high-speed TLC scanner.

The data of figure 1 were obtained by dissolving NAD (2 g, 3 mmol, acid form, Oriental Yeast, Japan) together with ethyleneimine (0.3 ml, 6 mmol, Serva, Heidelberg, FRG) in distilled water up to 6 ml (20 °C).

The results summarized in Table 1 were achieved by dissolving NAD (2g, 3 mmol) together with varying amounts of ethyleneimine (0.375 ml, 7.5 mmol, exp. I and II; 0.425 ml, 8.5 mmol, exp. III; 0.5 ml, 10 mmol, exp. IV^a and IV^b) in distilled water up to 6 ml (30 °C).

The results of Table 2 were achieved by dissolving NADP (7.5 g, 9.52 mmol, disodium salt, Boehringer, Mannheim, FRG) together with ethyleneimine (1.4 ml, 28 mmol) in distilled water up to 25 ml (exp. I) and 16 ml (exp. II) (30 °C).

The data of figure 2 were obtained by dissolving (A) NAD (200 g, 300 mmol) together with ethyleneimine (42.5 ml, 850 mmol) in distilled water up to 650 ml and (B) NADP according to exp. I of table 2 (30 °C).

In all cases the appropriate pH was maintained by adding 70 % HClO₄.

The R_f values of the cofactor (derivatives) for the TLC system used are:

NAD (0.35), N(1)-(2-aminoethyl)-NAD (0.075),

N(1)-(N-(2-aminoethyl)aminoethyl)-NAD (0.025),

N(1)-(oligoethyleneimine)-NAD (0.01), NADP (0.21),

N(1)-(2-aminoethyl)-NADP (0.06), N(1)-NADP byproducts (0).

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