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 polyethyleneglycol (PEG)-N^6-(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 amounts[2]. 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.

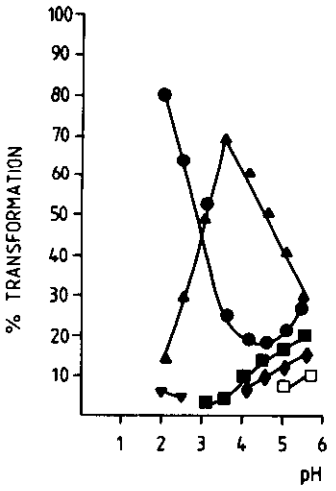


FIG 1: OPTIMIZATION OF THE ALKYLATION OF NAD WITH ETHYLENEIMINE TO N(1)-(2-AMINOETHYL)-NAD WITH RESPECT TO pH
CONDITIONS. 0,5M NAD, 1M ETHYLENEIMINE IN AQUEOUS SOLUTION (20°C)
REACTIONTIME 250h

- NAD
- N(1)-(2-AE)-NAD
- N(1)-[N-(2-AMINOETHYL)AMINOETHYL]-NAD
- OLIGOETHYLENEIMINE-N(1)-ALKYLATED NAD
- UNDEFINED BYPRODUCT IN pH-RANGE 2-2 5
- UNDEFINED BYPRODUCT IN pH-RANGE 5-5 5

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 (± 0.05)	NAD (%)	N(1)-(2-AE)-NAD (%)	N(1)-NAD byproducts (%)	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 choosen 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)-N^6-(2-aminoethyl)-NADH[3].

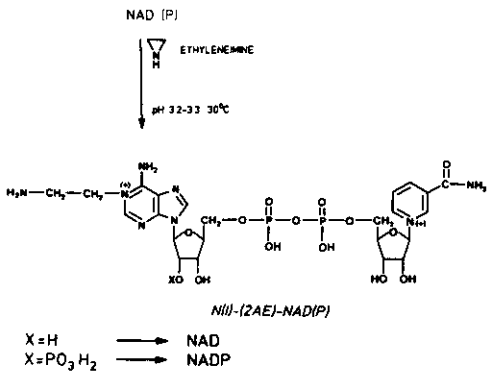
If pure N(1)-(2-aminoethyl)-NAD is the final aim the reaction time should be longer (IV[b]).

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



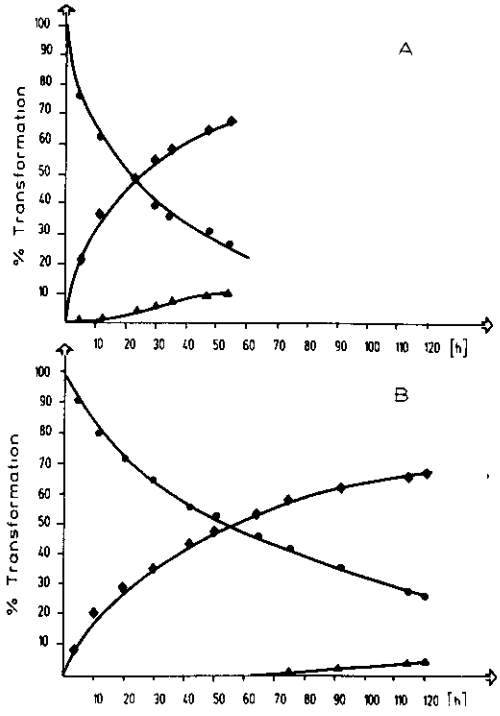


Figure 2: Reaction scheme and composition of the reaction mixture (%) as a function of time (h) for the alkylation of NAD (A) and NADP (B) with ethyleneimine under optimized conditions (see Experimental). (●) NAD(P); (◆) N(1)-(2-aminoethyl)-NAD(P); (▲) N(1)-NAD(P) byproducts; pH 3.25 ± 0.05.

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 N^6-(2-aminoethyl)-NAD(P) and tricyclic 1,N^6-(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 $60F_{254}$ (Merck, 0.2 mm) in isobutyric acid/25 % aqueous NH_3/H_2O (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, di-sodium 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 % $HClO_4$.

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).

REFERENCES

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