Presented at „Oxidants and Antioxidants in Biology" Cadiz, Spain, February 6-9, 2003

In vitro antioxidative properties of reduced nicotinamide adenin dinucleotide

K. Oettl, J. Greilberger, G. Juergens and G. Reibnegger

Institute for Medical Chemistry and Pregl Laboratory,
Karl Franzens Universität Graz, Harrachgasse 21, A-8010 Graz, Austria

INTRODUCTION

NADH is a strong reducing agent and has been suggested to act indirectly as an antioxidant. One such possibility is via the reduction of GSSG to GSH. Only recently the activity of NADH as a directly acting antioxidant has been reported and NADH was proposed to be of major importance as antioxidant in mitochondria. In the presence of transition metals, however, reducing agents may become prooxidative. Therefore we also investigated a potential prooxidant property of NADH.

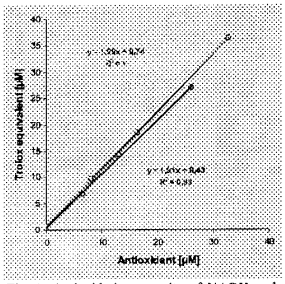


Fig. 1: Antioxidative capacity of NADH and ascorbic acid using the ABTS⁺-assay. Trolox equivalents are shown.

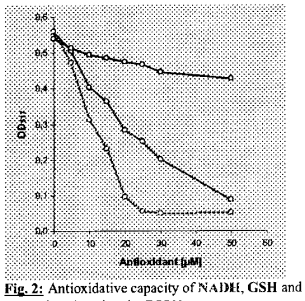


Fig. 2: Antioxidative capacity of NADH, GSH and ascorbic acid using the DPPH-assay.

METHODS

Reaction of NADH with nitrogen centered radicals was performed using a commercial testkit based on the formation of $ABTS^+$ (Randox) as a kinetic assay measuring the lag time of radical formation and the quenching of the stable radical DPPH, respectively. The concentration of these radicals can be measured photometrically.

Reaction with superoxide was measured with a device allowing photochemical generation of superoxide and luminometric measurement of its formation (Photochem-system, Analytik Jena).

Oxidation of LDL was performed by incubating LDL (0.3 mg/ml) in the presence of 2 mM AAPH, 10 µM EDTA and in the presence or absence of 50 µM NADH or ascorbic acid at 37 °C. Oxidation was monitored by measurement of diene formation (OD_{234}).

The prooxidative potential was measured by investigating the influence of NADH on hydroxylation of salicylic acid in the presence or absence of copper ions in comparison to ascorbic acid. NADH, ascorbic acid and the hydroxylation product of salicylic acid were quantitated by HPLC with electrochemical detection.

ABBREVIATIONS

ABTS, 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]; **AAPH**, 2,2'-azobis-(2-amidinopropane) hydrochloride; **DHBA**, dihydroxybenzoic acid; **DPPH**, diphenylpicrylhydrazyl; **LDL**, low density lipoprotein; **PBS**, phosphate buffered saline.

RESULTS

NADH reacts with $ABTS^+$ radicals and shows distinct lag times in the kinetic assay. Its capacity to scavenge $ABTS^+$ is identical to ascorbic acid and trolox (Fig. 1).

NADH also reacts with DPPH (Fig. 2). The concentration for half maximal scavenging (IC_{50}) of 50 µM DPPH is 20 µM. For GSH and ascorbic acid we found IC_{50}-values of 120 and 8 µM, respectively.

On the other hand, NADH is not a scavenger of superoxide (Fig. 3). While increasing concentrations of ascorbic acid give increasing lag times of radical formation, NADH even at a ten fold concentration shows no lag time.

When LDL is oxidized in vitro induced by peroxyl radicals NADH reveals an antioxidant effect identical to ascorbic acid during the first 90 minutes (Fig. 4). However, after 90 min ascorbic acid has no more effect while NADH is still antioxidative.

When NADH is incubated in the presence of salicylic acid in PBS, no hydroxylation of salicylic acid is observed and only 12 % of NADH is lost after 9 h (Fig. 5). After addition of copper ions NADH is consumed rapidly and some hydroxylation occurs. On the other hand, when ascorbic acid is used instead of NADH, hydroxylation products are found even without additon of copper ions and in the presence of Cu^{2+} hydroxylation is enhanced dramatically.

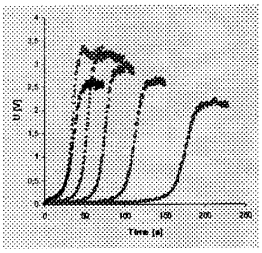


Fig. 3: Antioxidative effect of NADH (20 nmol) and ascorbic acid (0.25, 0.5, 1 and 2 nmol) against superoxide (blank is shown in green).

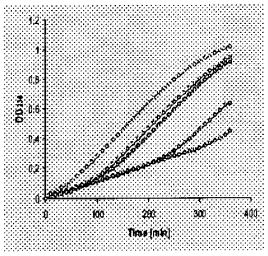
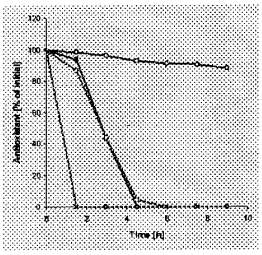


Fig. 4: Diene formation during LDL oxidation in the presence or absence of NADH (10, 30, 50 µM) and ascorbic acid (30 µM).



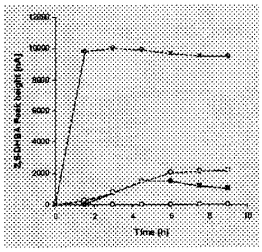


Fig. 5: Incubation of salicylic acid with NADH or ascorbic acid (500 µM) in the absence (open symbols) or presence (full symbols) of Cu^{2+} (10 µM). Consumption of antioxidant (left) and formation of hydroxylation product 2,5-DHBA (right) are shown.

SUMMARY

- ★ NADH acts as scavenger of nitrogen centered radicals often used in antioxidant assays, but it is not a scavenger of superoxide.
- ★ NADH is an antioxidant in LDL oxidation induced by peroxyl radicals.
- ★ NADH is much less prooxidative in the presence of transition metals compared to ascorbic acid.