Cell-Protective and Antioxidant Activity of Two Groups of Synthetic Amphiphilic Compounds – Phenolics and Amine N-Oxides

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Received 14 August 2007
Revised version 29 October 2007

ABSTRACT. Two classes of newly synthesized amphiphilic compounds, phenolic antioxidants ("phenolics") and N-oxides exert \textit{in vivo} antioxidant effects on live \textit{S. cerevisiae} cells. Both groups have low toxicity, phenolics being more toxic than N-oxides and compounds with a longer alkyl chain having higher toxicity than those with a shorter alkyl chain. Phenolic antioxidants protect yeast cells exposed to the superoxide producer paraquat and peroxyl generator tert-butylhydroperoxide better than N-oxides at 3-fold higher concentration. Both types of antioxidants enhance the survival of pro-oxidant-exposed cells of \textit{S. cerevisiae} mutants deficient in cytosolic and/or mitochondrial superoxide dismutase and could be good compounds which mimic the role of superoxide dismutases. The results of measurement of antioxidant activity in an \textit{in vitro} chemiluminescence test differ from the results obtained \textit{in vivo} with \textit{S. cerevisiae} superoxide dismutase mutants. In contrast to their action on live cells, phenolics are less effective than N-oxides in preventing lipid peroxidation of an emulsion of lipids isolated from \textit{S. cerevisiae} membranes.

Abbreviations

AAPH 1,1´-azobis(3-aminopropane) dihydrochloride
IC\textsubscript{50} inhibitory concentration (50 % reduction of growth)
LD\textsubscript{50} lethal dose (50 % killing)
MC\textsubscript{crit} critical micelle concentration
NBD-PE \textit{N}-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (‘nitrobenzoxadiazole dipalmitoylphosphatidylethanolamine’)
PDR pleiotropic drug resistance
ROS reactive oxygen species
SOD superoxide dismutase (EC 1.15.1.1)
TBHP tert-butylhydroperoxide
TBRS thiobarbituric acid-reactive substances
TMA-DPH \textit{N},\textit{N},\textit{N}-trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium 4-toluenesulfonate (‘trimethylammonio-diphenylhexatriene’)

CONTENTS

1 INTRODUCTION 585
2 Synthetic amphiphilic antioxidants 586
  2.1 Phenolics 587
  2.2 N-Oxides 587
3 Effect of antioxidants on \textit{S. cerevisiae} parent strains 587
  3.1 Protection of yeast cells against hydrophilic and amphiphilic oxidants 588
4 Incorporation of synthetic antioxidants into membranes 589
5 Antioxidant activity \textit{in vitro} determined by chemical methods 589
6 Conclusions 591
References 591

1 INTRODUCTION

The properties of ROS are crucial in the type, course and outcome of oxidative stress. Generally, the prime agents responsible for causing oxidative stress are primary and secondary ROS. Primary oxidants formed as products of energy metabolism include the superoxide anion, hydrogen peroxide and hydroxy radical.
Secondary oxidants are generated as products of reactions of primary ROS with major cell constituents – DNA, lipids and proteins. In living cells, production of ROS and induction of oxidative stress can be triggered by many physical and chemical factors (radiation, ‘heavy metals’, etc.) (Sigler et al. 1999).

To keep optimum oxidative–antioxidative balance vis à vis oxidative assault, cells possess a number of enzymic and non-enzymic antioxidative systems whose functions are frequently very close or overlap. Thus, although the superoxide anion is not an especially potent oxidant, it is neutralized by two or three types of superoxide dismutases; this reflects its harmful potential for the cell that is due to a number of reactions of superoxide that can yield other, highly deleterious ROS. Thus, in a reaction with hydrogen peroxide, superoxide anion produces a very active hydroxy radical that is capable of oxidizing almost all cellular constituents and can cause, e.g., peroxidation of lipids that is highly harmful for the cell (Sigler et al. 1999).

In situations involving pathological conditions or massive oxidative assaults, the activity of natural antioxidants in cells may not be sufficient to neutralize the free radical attack; synthetic antioxidants are therefore very useful in these cases. In addition to having strong antioxidative properties and being able to scavenge a wide range of reactive species, these antioxidants possess a number of other features, such as non-toxicity and activity in both lipophilic and hydrophilic compartments, and the ability to function in both intracellular and extracellular environments. Natural antioxidants, such as β-carotene, sometimes exert a pro-oxidative activity at high concentrations (Łukaszewicz et al. 2004). It is therefore important to have antioxidants free of pro-oxidative properties and possessing antioxidant effects at safely low concentrations. Amphiphilic properties have been recognized to be important for antioxidants that can act, e.g., on different regions in biological membranes (Beckman and Ames 1998).

The reactions which maintain the oxidative–antioxidative balance in the cell are often interdependent. It is therefore not always simple to investigate these processes in live cells. The currently available methods for quantification of ROS production using spectroscopic and/or fluorometric probes in cellular systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006).

The yeast _Saccharomyces cerevisiae_ represents a eukaryotic system highly suitable for testing the activity of antioxidants. It is a standard object of genetical manipulations and expression of proteins and, unlike with animals, work with it does not involve ethical issues.

Natural phenolic antioxidants (“phenolics”) have long been known to be efficient in protecting cells from oxidative stress, especially from lipid (L) peroxidation or the harmful influence of superoxide anion. Tocopherol (T; vitamin E) is one of the best known natural phenolic antioxidants that terminates the chain peroxyl radical as a donor in the reaction (1):

\[
\text{LOO}^* + \text{TOH} = \text{LOOH} + \text{TO}^* \tag{1}
\]

Tocopherol is a good chain-breaking antioxidant, because the TO• radical formed in the reaction with hydroperoxides is too weak to initiate reductive reactions (Buettner 1993). Moreover, tocopherol can undergo reactivation in a reaction with ascorbate (Packer et al. 1979; Hiramoto et al. 2002). Due to its hydrophobic properties tocopherol does not react with superoxide immediately at the site of its generation in the aqueous cytosol, but it can prevent membranes from the action of free radicals produced in reactions of superoxide with other cell molecules.

Nitric oxide •NO is a natural sacrificial antioxidant which is utilized in reaction (2):

\[
\text{LOO}^* + \cdot\text{NO} = \text{LOONO} \tag{2}
\]

2 SYNTHETIC AMPHIPHILIC ANTIOXIDANTS

Synthetic nitroxides bearing an unpaired electron on the –NO group included in an aliphatic or aromatic ring system are good antioxidants that react with a wide range of free radicals (alkyl, peroxyl, alkoxy, superoxide, etc.) (Stipa 1997; Damiani et al. 1999, 2001). The best known described nitroxides are the cyclic synthetic compounds Tempo (2,2,6,6-tetramethylpiperidine-1-oxyl) and Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) (Samuni and Barenholtz 2003).