Novel peptoids for the detection and suppression of reactive oxygen and nitrogen species

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Abstract

Novel peptoids useful for the detection and suppression of various components contributing to oxidative stress and for elucidation of the interplay between these species are presented. Oxidative stress involves redox-active metal ion activation/generation of RONS (reactive oxygen and nitrogen species). For detection of RONS, the peptoid probes consist of a conjugate designed to (1) complex redox-active and non-redox-active metal ions, and (2) differentiate between RONS based upon the reaction products following RONS attack on the probe. For suppression of RONS, subtle modifications in peptoid structure impart catalase and superoxide dismutase activities to the peptoids upon ferric or cupric ion complexation.

Introduction

Oxidative stress is a major component of inflammation and causes immense damage to biological systems, and thus perpetuates incurable autoimmune diseases such as rheumatoid arthritis and Alzheimer’s disease [1]. These species are also proposed to have a fundamental role in aging [2]. Termed RONS (reactive oxygen and nitrogen species), they comprise superoxide (O$_2^•−$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (•OH) and peroxynitrite (ONOO$^{−}$), among others. Numerous studies have demonstrated that RONS initiate and/or perpetuate the lipid peroxidation process, degrade DNA, destroy endothelial cells and induce increased vascular permeability [1].

Research into oxidative damage can be broken down into four major categories: (i) formation of RONS, (ii) detection of RONS, (iii) suppression of RONS and (iv) study of RONS-mediated damage [1,3,4]. The focus of this paper will be on the detection and suppression of RONS.

Current trends within the field indicate that the highly reactive species *OH and ONOO$^{−}$ are the principal agents that cause oxidative damage [5]. Other RONS such as O$_2$•−, H$_2$O$_2$ and NO* can be controlled by regulation of their production and/or by antioxidant enzymes [1]. Thus assessment of levels of the species *OH and ONOO$^{−}$ is of particular interest. A comprehensive understanding of the mechanisms of their generation and their mediation of oxidative damage is required for their optimal detection.

In general, detection of RONS is problematic due to their short-lived and reactive nature. Methods of assessment of RONS can be separated into direct detection, analysis of RONS via their interaction with a chemical probe, analysis of endogenous biochemical markers of oxidative damage, and measurement of total antioxidant activity.

A major requirement for the successful determination of RONS is to maximize their interaction with the chemical probe. Competitive scavenging by endogenous biomolecules leads to major imprecision in current assays. Current research is revealing important roles for redox-active metal ions in mediating oxidative damage via the highly reactive *OH and ONOO$^{−}$ species [6,7]. Critically, metal ions generate *OH from O$_2$•− and H$_2$O$_2$ by the Haber–Weiss and Fenton reactions respectively. In addition, they enhance ONOO$^{−}$-mediated damage, resulting in enhanced nitration of aromatics [8]. Metal ion-activated decomposition of ONOO$^{−}$ is condition-specific and can generate varying amounts of *OH and nitrated aromatics [9].

Owing to their high reactivity, it is essential to co-localize the probe with redox-active metal ions to optimize RONS capture for detection and suppression. The presence of a metal ion enhances sensitivity by minimizing loss via scavenging by endogenous molecules. Current methods of detection of the various components of oxidative stress are quite diverse, and standardization of all of the different methods is impracticable. No suitable single assay is available for either a broad range of RONS activities or the consequent multiple oxidation products derived from them.

Simultaneous detection of multiple RONS

The assay is based on two key aspects. (1) By binding low-molecular-mass metal ions selectively, it is not damaging to metalloproteins, but directs the metal ion to the vicinity of the antioxidant to improve efficacy. (2) It scavenges RONS to give specific products, which can be assessed in order to monitor levels of ONOO$^{−}$, *OH and H$_2$O$_2$ simultaneously. These characteristics allow the assay to be employed to simultaneously detect different RONS that cause oxidative stress (Scheme 1), thus yielding information to aid in the development of improved therapies.
Assay for $\text{H}_2\text{O}_2$ [10]

In the presence of a reduced metal complex of ET$_2$ [EDTA bis(methyl tyrosinate)], $\text{H}_2\text{O}_2$ can be assayed owing to the formation of •OH via Fenton chemistry. The reactive •OH seek electron-rich moieties in order to pair off the unpaired electron. This generates hydroxylation products of the aromatic moiety. Thus, for example, phenylalanine moieties are hydroxylated to become tyrosine isomers, and tyrosines become catechols upon one hydroxylation (Scheme 1). These hydroxylation products are detectable by NMR spectroscopy, MS and HPLC with commercial compounds as references.

Assay for peroxynitrite [8]

Addition of ONOO$^-$ to the ferric or cupric complexes of ET$_2$ results in the development of mono-, di-, tri- or tetra-nitrotyrosines. These adducts can be assessed by MS, NMR spectroscopy and electronic absorption spectroscopy (Scheme 1) [8]. Thus this assay can be used to quantify ONOO$^-$.

Assay for dityrosine formation as an indicator of hydroxyl radical generation

Proteins subjected to oxidative stress form dityrosine adducts that can be identified by fluorescence spectroscopy [11]. A chelator/scavenger containing two or more tyrosine moieties in suitable geometry to allow formation of dityrosine will enable assessment of oxidative stress within a biological system (Figure 1).

Establishment of the role of redox metal ions in oxidative stress [8]

Most events contributing to oxidative stress can be mediated by redox-active transition metal ions such as copper and iron ions [12]. For example, a stable metal complex of the selected chelator/scavenger could be prepared such that the metal ion is not redox-active and thus would not participate in oxidative stress. In addition, a sister complex could be prepared containing a redox-active metal complex of the chelator/scavenger. Thus comparison of the various scavenged species in the presence and absence of either redox-active or non-redox-active metal ions will provide information on the role played by metal ions in various systems undergoing oxidative stress.

An example of this approach is the study of peptoid nitration in the presence of redox-active metal ions. In an in vitro system, rates of tyrosine nitration by peroxynitrite were greatly enhanced after formation of either cupric or ferric complexes [8]. In this example the capture rates of peroxynitrite as nitrated tyrosine were 36% for Fe(III)–ET$_2$, 25% for Cu(II)–ET$_2$ and 6% for ET$_2$. Clearly, the inclusion of a redox-active metal within the probe greatly enhanced its ability to detect peroxynitrite as the nitration product. Given the high reactivity of ONOO$^-$, these results demonstrate the benefits of including a redox-active metal ion to enhance detection and suppression.

Figure 1 | Fluorescence detection of the ONOO$^-$-mediated dimerization of ET$_2$

The spectra demonstrate that dityrosine is generated inversely to nitrotyrosine [9]. This agrees with kinetic models which predict that, at low concentrations of ONOO$^-$, dityrosine is the favoured product resulting from the generation of •OH.
Suppression of RONS

Recent results show that subtle changes to the base peptoid structure result in a switch between scavenging and catalytic decomposition of RONS. Thus peptoid analogues may be applied to both the suppression and the detection of RONS. Analogues of EDTA with two tyrosine moieties had virtually no catalase or SOD (superoxide dismutase) activities when co-ordinated to cupric or ferric ions. However, the peptoid analogue containing only one tyrosine conjugated to EDTA {ET₁ [EDTA mono(methyl tyrosinate)]} exhibited substantial SOD activities in comparison with bovine erythrocyte SOD. At a concentration of 1 µM, SOD activity equivalent to 2.3 units was exhibited by the ET₁–Cu(II) complex, with a value of 0.24 unit for the ET₁–Fe(III) complex. Optimum catalase activities were exhibited by the ET₁–Fe(III) complex (11% compared with bovine liver catalase). Further studies are under way in an attempt to enhance this activity.

Conclusions

These novel peptoids provide a RONS assay with potential for the simultaneous detection of multiple RONS and the clarification of the roles of redox-active metal ions in RONS activation or generation. In addition, peptide analogues exhibiting SOD and catalase activities are dually beneficial in that they reverse the pro-oxidant nature of the low-molecular-mass redox-active metal ions to become catalytic antioxidants.

This work is supported by the EPSRC and the University of Brighton.

References


Received 1 July 2003