Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Immuno-spin trapping from biochemistry to medicine: Advances, challenges, and pitfalls. Focus on protein-centered radicals ☆

Sandra E. Gomez-Mejiba [a], Zili Zhai [b], Maria C. Della-Vedova [a], Marcos D. Muñoz [a], Saurabh Chatterjee [c], Rheal A. Towner [d], Kenneth Hensley [e], Robert A. Floyd [f], Ronald P. Mason [g], Dario C. Ramirez [a,h,*,1]

[a] Laboratory of Experimental Medicine and Therapeutics, Institute Multidisciplinary of Biological Investigations-San Luis (IMIBIO-SL), National Bureau of Science and Technology (CONICET) and National University of San Luis, San Luis, 5700 San Luis, Argentina
[b] Section of Gastroenterology, Department of Gastroenterology, The University of Chicago, IL 60637, USA
[c] Environmental Health & Disease Laboratory, Department of Environmental Health Sciences, University of South Carolina, Columbia, 29208 SC, USA
[d] Advanced Magnetic Resonance Center, Oklahoma Medical Research Foundation, Oklahoma City, 73104 OK, USA
[e] Department of Pathology, University of Toledo Medical Center, Toledo, 43614 OH, USA
[f] Laboratory of Experimental Therapeutics, Oklahoma Medical Research Foundation, Oklahoma City, 74104 OK, USA
[g] Laboratory of Pharmacology and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, 23709 NC, USA
[h] Molecular Physiology, Molecular Biology Section, Department of Biochemistry and Biological Sciences, National University of San Luis, 5700 San Luis, San Luis, Argentina

ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 24 April 2013
Accepted 27 April 2013
Available online xxxx

Keywords:
Reactive chemical species
Protein radical
Spin trap
Anti-DMPO
Immuno-spin trapping
Disease/exposure model

ABSTRACT

Background: Immuno-spin trapping (IST) is based on the reaction of a spin trap with a free radical to form a stable nitrone adduct, followed by the use of antibodies, rather than traditional electron paramagnetic resonance spectroscopy, to detect the nitrone adduct. IST has been successfully applied to mechanistic *in vitro* studies, and recently, macromolecule-centered radicals have been detected in models of drug-induced agranulocytosis, hepatotoxicity, cardiotoxicity, and ischemia/reperfusion, as well as in models of neurological, metabolic and immunological diseases.
Scope of the review: To critically evaluate advances, challenges, and pitfalls as well as the scientific opportunities of IST as applied to the study of protein-centered free radicals generated in stressed organelles, cells, tissues and animal models of disease and exposure.
Major conclusions: Because the spin trap has to be present at high enough concentrations in the microenvironment where the radical is formed, the possible effects of the spin trap on gene expression, metabolism and cell physiology have to be considered in the use of IST and in the interpretation of results. These factors have not yet been thoroughly dealt with in the literature.
General significance: The identification of radicalized proteins during cell/tissue response to stressors will help define their role in the complex cellular response to stressors and pathogenesis; however, the fidelity of spin trapping/immuno-detection and the effects of the spin trap on the biological system should be considered.
This article is part of a Special Issue entitled Current methods to study reactive oxygen species — pros and cons.

1. Introduction

The "*gold-standard*" technique that allows the unambiguous detection of free radicals is electron spin resonance spectroscopy [ESR, also called electron paramagnetic resonance (EPR)] because this technique is based on fundamental physics and makes no assumptions [1–4]. There is no doubt that ESR has a number of undisputed advantages over other methods of studying free radicals [5]. However, the greatest limitation of ESR in the study of free radicals in cells and tissues is its poor sensitivity in relation to the steady-state concentrations of free radicals under physiological conditions, or even in the response to severe stress. Typically, the steady-state concentration of free radicals under normal conditions is less than 1 nM or even 1 pM, which is far below the best sensitivity of ESR of 3 nM. These limitations of ESR

Abbreviations: AG, aminoglutethimide; BSA, bovine serum albumin; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; ESR, electron spin resonance; HPLC, high performance liquid chromatography; hoMb, horse myglobin; huHb/Mb, human hemoglobin/myoglobin; I/R, ischemia/reperfusion; IST, immuno-spin trapping; LC, liquid chromatography; LPS, lipopolysaccharide; MRI, magnetic resonance imaging; MS, mass spectrometry; OA, octanoic acid; SOD, superoxide dismutase; TPO, thyroid peroxidase

☆ This article is part of a Special Issue entitled Current methods to study reactive oxygen species — pros and cons.

* Corresponding author at: Laboratory of Experimental Medicine and Therapeutics-IMIBIO-SL-CONICET-UNSL, Suite 13X, First floor, Chacabuco y Pedernera, San Luis, 5700 San Luis, Argentina. Tel.: +54 9 266 4207483.
E-mail address: ramirezlabimibiosl@ymail.com (D.C. Ramirez).
[1] DCR is an Affiliated Guest Researcher at the Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, NIH, USDHHS, USA.

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.04.039

have led to an intensive search for alternatives for the investigation of free radicals in biological systems.

The limitations of direct ESR in the study of short-lived free radicals led, in the 1960s, to the development of spin trapping [6], in which a free radical adds to the carbon end of the nitrone function of a spin trap (Fig. 1). This reaction produces a much more stable free radical, a nitroxide radical adduct or radical adduct that can be seen by ESR spectroscopy [6]. The greatest advantage of using spin trapping in the study of free radicals is the increased stability leading to increased radical adduct concentrations, which are often above the 3 nM limit of ESR detection [2]. This has led to a renaissance of ESR-spin trapping in the study of oxidative processes in biological systems [7,8]. Indeed, a number of free radical metabolites, proteins and nucleic acids have been detected by ESR-spin trapping both *in vitro* and *in vivo* [2].

Many spin traps, such as nitroso and nitrone compounds, have been used to study free radicals in biological systems [7,9]. Because of their physico-chemical properties, membrane permeability [10,11], effectiveness at trapping free radicals [9], and low toxicity [12], nitrone spin traps have been employed both as reagents to detect radicals using ESR spectroscopy [7,9] and as pharmacological agents against oxidative stress-mediated injury [13,14]. The most popular of these spin traps is 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) [15], which has been cited in Medline over 1300 times. In a radicalized macromolecule, for example a radicalized protein, the spin trap adds to an atom in a solvent-exposed site with high electron spin density [6]. The greater the stability of the radical adduct, the higher the concentration of the radical adduct for a given rate of free radical generation [7]. The lifetime of the radical adduct is usually the most important factor in deciding the success of an ESR-spin-trapping experiment [2]. In addition, it is important to note that unlike direct ESR, the spin-trapping methodology depends on the absolute fidelity of the spin-trapping reaction [16]. Importantly, nitrone spin traps are known to react with free radicals and non-radicals *via* electrophilic and nucleophilic addition reactions [17]. Two alternate mechanisms of radical adduct artifacts with DMPO have been recently investigated and discussed [16,18]: inverted spin trapping (one-electron oxidation of the spin trap) and the Forrester–Hepburn (nucleophilic addition of the spin trap) mechanisms. In biological systems the Forrester–Hepburn mechanism, which is initiated by a nucleophilic addition of a nucleophile to the spin trap, would be the major mechanism of generation of potential artifactual DMPO-molecule adducts. See [16,18] for a comprehensive chemical discussion of these two sources of artifacts in spin trapping.

The specificity of the reactions of nitrone spin traps with free radicals has already made spin trapping with ESR detection the most universal and specific tool for the detection of free radicals in biochemical systems as well as in cells, tissues and animals [19]. Unfortunately, ESR-spin trapping of protein radicals *in vivo* has severe limitations. Some of them are: 1) the cost of acquiring the instrument; 2) the instability of radical adducts in tissues, which may compromise reproducibility; 3) the lossiness of the dielectric sample, and 4) the low radical adduct concentration due to the presence of antioxidants that can compete with DMPO.

2. Principle of immuno-spin trapping

To an ESR spectroscopist, the conservation of the unpaired electron is the most important aspect of the reaction of a protein-centered radical with a spin trap to form a radical adduct. To an organic chemist, the most unique feature of the reaction is the formation of a new covalent bond between the DMPO and the free radical in a reaction that is specific for free radicals. To an immunologist, the reaction of a free radical with a spin trap marks the creation of a novel epitope; and to a biochemist this is a novel way to identify a protein target of oxidation in states of stress that may lead to an understanding of the chemical basis of a free radical process in biologically-relevant scenarios.

DMPO is very stable and nearly redox inert, being reduced to the hydroxylamine only at the very low potential of −1.68 V and oxidized only at the very high potential of 1.87 V *versus* normal hydrogen electrode. Once formed DMPO–protein adducts can exist in three redox forms: (1) the nitroxide radical adduct, (2) the corresponding hydroxylamine formed by a one-electron reduction of the radical adduct, and (3) the corresponding nitrone formed by a one-electron oxidation of the radical adduct [20] (see Fig. 1). The nitroxide radical adducts and their corresponding hydroxylamine adducts are all unstable and decay over time. The nitrone adduct is the most thermodynamically stable product of the reaction of a free radical with a nitrone spin trap that can be studied in tissues excised from an experimental animal treated with the spin trap.

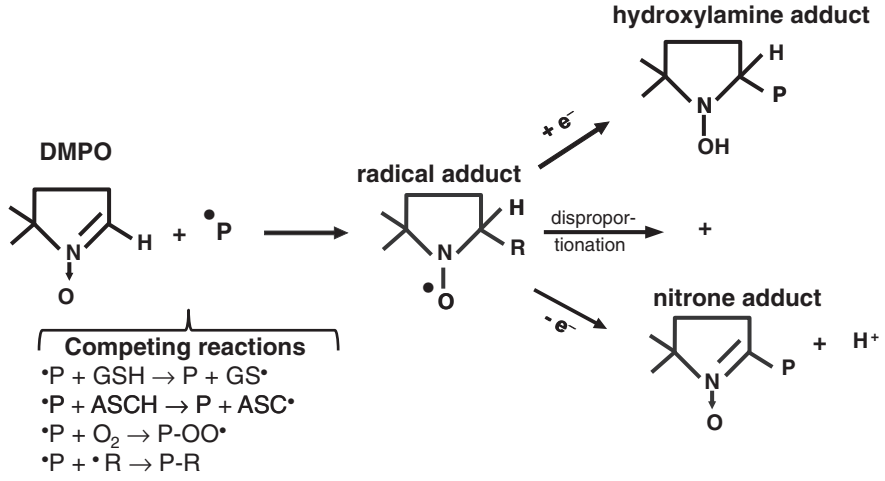

Fig. 1. Spin trapping and fate of protein–DMPO adducts. A protein radical (a radical site in a protein) reacts with DMPO to form a radical adduct. Depending on microenvironment conditions and structural characteristics of the target protein, the radical adduct can be reduced to hydroxylamine or oxidized to a stable nitrone adduct. It can also disproportionate to generate both the hydroxylamine and nitrone adducts. In cells and *in vivo* there are a number of competing reactions that can affect the yield of DMPO–protein adducts. Reduced glutathione (GSH) and L-ascorbate (ASCH) [28] can react with protein radicals faster than the rate of reaction with DMPO, resulting in reduced yield of protein–DMPO nitrone adducts, a repaired protein and a less reactive radical (*i.e.*, GS• and ASC•). Oxygen, the best spin trap in nature, can also react with protein radicals to form protein-peroxyl radicals, which very slowly react with DMPO. Protein radicals can react with other radical sites in the same or different proteins to form cross links (*e.g.*, Tyr-Tyr, His-His or Trp-Trp). Protein radicals can also react with lipid radicals, $•NO_2$/•NO or drug/toxicant radicals, thus resulting in protein-lipid or protein-drug/toxicant adducts (P-R).

Please cite this article as: S.E. Gomez-Mejiba, et al., Immuno-spin trapping from biochemistry to medicine: Advances, challenges, and pitfalls. Focus on protein-centered radicals, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbagen.2013.04.039