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Oxidative stress, free radicals and protein peroxides

Janusz M. Gebicki

Department of Biological Sciences, Macquarie University, Sydney, Australia

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ABSTRACT

Primary free radicals generated under oxidative stress in cells and tissues produce a cascade of reactive secondary radicals, which attack biomolecules with efficiency determined by the reaction rate constants and target concentration. Proteins are prominent targets because they constitute the bulk of the organic content of cells and tissues and react readily with many of the secondary radicals. The reactions commonly lead to the formation of carbon-centered radicals, which generally convert in vivo to peroxyl radicals and finally to semistable hydroperoxides. All of these intermediates can initiate biological damage. This article outlines the advantages of the application of ionizing radiations to studies of radicals, with particular reference to the generation of desired radicals, studies of the kinetics of their reactions and correlating the results with events in biological systems. In one such application, formation of protein hydroperoxides in irradiated cells was inhibited by the intracellular ascorbate and glutathione.

Contents

1. Introduction

The widely quoted 1979 review by Chance, Sies and Boveris [1] provided not only a summary of the contemporary knowledge of the formation and metabolism of H_2O_2 but also a farsighted prediction of the broader significance of research into the biology of peroxides. This prediction has been spectacularly fulfilled in recent years, with a multitude of key roles discovered especially for H_2O_2 in normal and abnormal functions of living organisms (reviewed in Sies [2]). A major role of H_2O_2 currently under intensive study is the part it plays in cell metabolism and signalling, prompting Sies to revise his original definition of oxidative stress [3] by inclusion of the phrase "disruption of redox signaling" [2]. The qualifying addition recognizes the dual damaging and beneficial roles of radicals, H_2O_2 and other partly reduced oxygen species (PROS) in vivo under different circumstances [2,4,5].

On the damaging role, the initial definition of oxidative stress emphasized the deleterious aspects of actions of H_2O_2 and other PROS in living organisms. This notion is supported by a vast literature demonstrating that the formation of radicals and other strong oxidants in excess of the antioxidant capacity of the organism is a

E-mail address: jan.gebicki@mq.edu.au.

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deleterious event [5]. In the case of the rather unreactive H_2O_2, its major damaging role lies in the potential conversion to the powerful oxidizing hydroxyl radical (HO•) in reactions involving transition ions [6,7]. Perhaps surprisingly, rather less knowledge has accumulated on the formation and biological roles of organic peroxides, although already 30 years ago Willson identified their precursor peroxyl radicals as likely ultimate agents of oxygen toxicity in vivo [8]. This view has received support more recently by the expanded knowledge of the identities, formation and properties of reactive species capable of generating organic free radicals in vivo. Such radicals, when located on carbon atoms, tend to form peroxyl radicals in the presence of dioxygen, which in tissues initiate the formation of new PROS or are reduced to semistable hydroperoxides.

This article briefly reviews the current evidence for the formation of protein hydroperoxides by radicals under conditions relevant to biological systems. The rationale for this interest is based on the high probability of the formation of protein C-centered radicals by radicals such as hydroxyl (HO•), thiyl (RS•), alkoxyl (RO•) and peroxyl (ROO•), and on the detection of protein hydroperoxides and their derivatives under physiological conditions. A fuller account of the discovery and properties of protein hydroperoxides has been published [9].

2. Radicals and targets

2.1. Radicals in simple solutions

The most extensive knowledge of the formation and basic properties of radicals relevant to the biological context was derived from studies of well-defined aqueous solutions of pure chemicals. Here the crucial contribution was made by radiation chemistry, with most of the knowledge of the identities, properties and reactions of radicals derived from experiments using sparsely ionizing radiations, such as X or γ rays, and high-energy electrons [10]. In dilute aqueous solutions, virtually all of the absorbed radiation energy causes decomposition of the water, present at around 50 M concentration. The following primary PROS are then generated, with the yields in units of 10^{-7} mol J^{-1} (G values) shown in brackets: HO• (2.8), e^-_{aq} (2.8), H• (0.6) and H_2O_2 (0.7) [11]. The energy absorbed by the solution can be easily determined, giving precise concentrations of the primary PROS. In addition, manipulation of the composition and pH of the solutions allows the choice of primary radicals by selective scavenging of the remaining PROS; in neutral solutions H• dissociates to H^+ and $e_{aq}{}^-$, HO• can be converted to relatively inert radicals by solutes such as *t*-butanol, e^-_{aq} converted quantitatively to HO• by N_2O, while H_2O_2 can often be removed enzymatically. It is also possible to change the reactivity of the primary radicals by converting them to secondary species. Thus, the strongly oxidizing HO• can be scavenged by solutes such as azide or bromide, forming the weaker oxidants N_3• or $Br_2^{\bullet -}$ [10]. An example of such manipulation in radical–protein reactions is the conversion of HO•, capable of oxidizing any amino acid, to N_3• which reacts only with the Trp residues [[12], Figs. 1 and 2]. Overall therefore, radiation-generated radicals allow studies of the reactions of specified radicals with chosen solutes, often allowing determination of the mechanism of the initial and subsequent reactions.

The formation of primary radicals in irradiated aqueous solutions occurs in nanoseconds, requiring methods capable of fast delivery of energy and rapid detection. The most commonly used techniques are pulse radiolysis and flash photolysis. The subsequent radical reactions usually take place in milliseconds, allowing the additional application of stopped flow kinetic measurements. These methods have been described in numerous publications

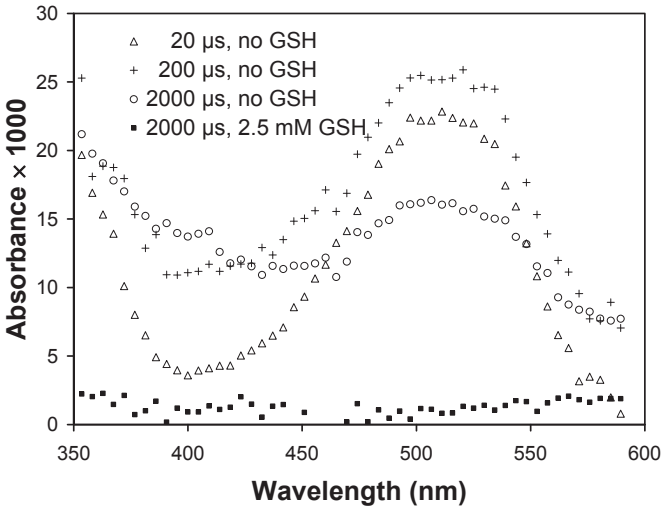

Fig. 1. Time-resolved spectra of lysozyme radicals. Lysozyme 0.14 mM solution was irradiated with 50 ns pulse of 2 MeV electrons. The solution was saturated with N_2O at pH 7.4. The 510 mn and 400 nm peaks are characteristic of the LZTrp• and LZTyrO• radicals respectively.

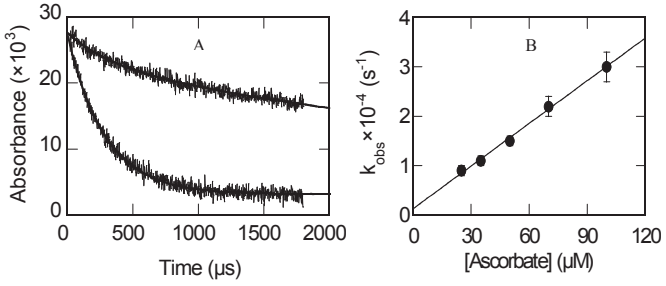

Fig. 2. Formation and reaction of insulin TyrO•. 2A. Pulse-irradiated 1 mM solution of insulin and 0.1 M NaN_3, pH 7.4 saturated with N_2O. Top curve: spontaneous decay of the Ins-TyrO• at 405 nm. Lower curve: decay in the presence of 100 μM ascorbate. 2B. Dependence of the decay rate constant on ascorbate concentration [20]. Continuous lines were generated by computer analysis of the data fit [19].

[such as [8,13,14]]. Formation and reactions of the radicals are studied on specialized equipment, usually by measuring rapid changes in optical absorptions characteristic of the radicals and products, although other fast kinetic methods are also employed. Pulse radiolysis produces the primary water radicals by high energy electrons, typically in the 1–10 MeV range. Inclusion of various solutes and manipulation of their concentrations, energy doses, pH, temperature and other conditions can give the reaction order and rate constant of radical-solute or radical–radical reactions, radical reduction potentials, activation energies, equilibrium constants and other parameters. Much of this information relating to thousands of radical processes is listed in databanks [15–18]. Existence of this data is crucial for studies of solutions of compounds potentially competing for the radical, because rate constants and solute concentrations determine which reactions will occur and which will not. This is especially important for complex physiological systems, where reactions highly unlikely on kinetic grounds are sometimes proposed to explain experimental results.

Examples of the power of pulse radiolysis to provide kinetic and mechanistic details of radical reactions are given in Figs. 1–3.

Fig. 1 shows the formation of Trp radical (Trp•) in lysozyme with absorbance peak at 510 nm immediately after a 20 ns 2 MeV electron pulse delivered to a 0.14 mM solution of the protein at