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Free radicals in chemistry, biology and medicine: contribution of radiation chemistry

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Abstract The scope of this article is limited to the concept of free radical and its historical background and a brief introduction to time-resolved techniques (pulse radiolysis, laser flash photolysis), which allowed direct observation of free radicals on real time. The selected contributions of pulse radiolysis to better understanding the role of free radical reactions in chemistry, biology and medicine are presented and some selected future research needs and opportunities in radiation chemistry are briefly addressed.

Key words free radicals • pulse radiolysis • oxidative stress • ionic liquids • long range electron transfer • β-amyloid peptide

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Introduction

Free radical chemistry is one of the topics to which radiation chemistry has made many important and essential contributions. Research on these usually short-lived transients has been particularly enhanced since the development of time-resolved techniques such as pulse radiolysis (in radiation chemistry) [34, 64] and laser flash photolysis (in photochemistry) [11], which allowed direct observation of free radicals on real time. There is a continuing need for basic research in this area due to the relevance of free radical reactions in many fields of chemistry [41], biology [49, 88], and medicine [49]. Radiation chemistry is a very valuable and powerful tool for solving fundamental and technological problems connected with atmospheric and environmental chemistry, organic synthesis, the polymer and paint industry, processes occurring in nonstandard environments such as supercritical media, ionic liquids, interfacial and heterogeneous systems [41]. Important applications of radiation chemistry are also connected with understanding of radical processes that are of particular interest in biology and medicine. Relevant examples include radical processes connected with a damage of biological material [32, 87], oxidative stress [70, 87], repairing and protective mechanisms [49, 71], aging [26, 31, 100], inflammation processes [92], and various diseases including cancer, and neurodegenerative diseases [31, 49, 87]. This article is not and cannot be an attempt to review important all experiments that have been conducted over the years by radiation chemists. There are numerous excellent and comprehensive

reviews [6, 9, 10, 51, 58] and books [11, 37, 65, 88, 97] which present and discuss these problems in a more detailed manner. One of the main purpose of the article is to familiarize those readers, who are not working in the free radical chemistry field, with the concept of free radical and with its brief historical overview which starts with the discovery of the first free radical 105 years ago. An equally important purpose is showing why radiation chemistry, pulse radiolysis in particular, has turned out to be so useful and successful in free radical research and is no longer just of specialized interest. A few selected important achievements emerging in this field will be highlighted, some future perspectives as far as new topics and development of new techniques are concerned will be addressed, and a recently installed at the Institute of Nuclear Chemistry and Technology in Warsaw nanosecond pulse radiolysis facility will be presented.

Historical background [40, 73]

Historically, the triphenylmethyl radical ($Ph_3C^{\bullet}$) discovered by Moses Gomberg in 1900 was the first known organic free radical. In fact, Gomberg's results demonstrated for the first time trivalency for carbon atoms, however, they did not provide the first example of what we might currently call as a stable organic free radical. Paneth and Hofeditz presented the first conclusive demonstration of free radicals ($^{\bullet}CH_3$), as reactive intermediates, in 1929 in their studies of the pyrrolysis of tetramethyl lead. The involvement of free radicals in solution chemistry was established in 1937, simultaneously by Kharasch and Flory. Kharasch interpreted addition of hydrogen bromide to alkenes, and Flory vinyl polymerization in terms of radical chain mechanism. Ironically, the II World War was an important stimulus for the development of radical chemistry when US chemists were called upon to find substitutes for natural rubber. In 1942–1945 Mayo, Walling and Lewis established the rules of free radical polymerization and copolymerization and developed kinetic laws. New synthetic methods have been developed about 1970 by various researchers including Barton, Cadogan, Julia, Giese and others. In recent years, synthetic strategies based on radical reactions have been more frequently considered since a great progress has been accomplished in the stereoselective control of radical reactions. The contribution of free radicals in chemical processes that occur in biological and living systems has been recognized in 1950. Interestingly, the free radical theory of ageing proposed nearly 50 years ago (in 1956) by Harman [50], arosed in part from radiation-chemical studies. There is a general agreement nowadays that in biological systems at the molecular level the majority of oxidative events is associated with an increase in free radical concentration.

Free-radical concept [8, 40]

A free radical is defined as any atom or molecule that has a single unpaired electron on one of its orbitals. Free radicals can have an anionic, cationic, or neutral character. The singly occupied molecular orbital (SOMO) is of special importance to free radical chemistry. For example, addition of a single electron to a neutral molecule generates unique chemical species called radical anion. The electronic structure of the radical anion can be simply illustrated as the entry of an electron into the lowest-energy unoccupied molecular orbital (LUMO) of a neutral precursor. The LUMO of the neutral then becomes the SOMO of the radical anion. On the other hand, ionization of a neutral molecule (ejection of an electron) generates a chemical species known as a radical cation. The electron is usually removed from the highest-energy occupied molecular orbital (HOMO) of a neutral molecule, which then becomes the SOMO of the radical cation. The presence of unpaired electrons has a very important consequence, namely the generally very high reactivity of such species. Accordingly, most radical-radical or radical-molecule reactions occur in diffusion-controlled processes. The high rate at which most of these processes usually occur constitutes the basic problem in studies of free radical reactions. One intrinsic parameter that lowers this value is an internal stabilization of the free radical due to delocalization of spin density into existing bonds or atoms of particular high electron affinity or to adjacent aromatic π-systems. A second parameter that influences the rate of radical reactions is the activation energy, i.e. the energy difference between the reagents and the highest-energy transition state [40].

Selected examples of most important radicals

The most abundant and important radicals are those located on carbon, oxygen [1], sulfur [3], and nitrogen atoms [2]. The carbon-centered radicals are formed mostly as a result of hydrogen atom abstraction from the respective organic molecules. Alkyl ($RC^{\bullet}HR'$), hydroxyalkyl ($RC^{\bullet}HOH$), acyl ($RC^{\bullet}=O$), α-(alkylthio)-alkyl ($RSC^{\bullet}HR'$) radicals are given as selected examples. Most of the oxygen-centered radicals are either derived from or associated with the presence of molecular oxygen. The most important and particularly relevant radicals are: hydroxyl ($^{\bullet}OH$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), phenoxyl ($ArO^{\bullet}$), and semiquinone ($HO\text{-}ArO^{\bullet}$) radicals, and a superoxide radical anion ($O_2^{\bullet-}$). While the $^{\bullet}OH$ is the most reactive among the O-centered radicals, $ROO^{\bullet}$ are probably the most abundant O-centered radicals in biological systems. All of these radicals are either strong ($^{\bullet}OH$) or moderately good oxidants except $O_2^{\bullet-}$ which itself is a moderate reductant. In recent years, S-centered radicals have attracted considerable attention in view of the very interesting redox chemistry [5, 79, 89]. One of the most important sulfur-centered radicals is the thiyl radical, $RS^{\bullet}$, which is the one-electron redox intermediate between thiols (RSH) and disulfides (RSSR). Some reactions of sulfur radical cations ($R_2S^{\bullet+}$, $(R_2SSR_2)^{\bullet+}$, $(RSSR)^{\bullet+}$, $Ar_2S^{\bullet+}$) have attracted interest for their application in organic synthesis and as intermediates in biological redox systems [42]. As an example of the novel reaction behaviors, is the propensity of alkyl sulfide radical cations ($R_2S^{\bullet+}$)

to bond to electron rich centers (S, O, N, Cl, Br, I, P) to form 2c-3e bonds [4, 5]. N-centered radicals have importance in all fields of chemistry starting with upper atmosphere chemistry, chemical synhesis, and ending with metabolism processes in biology. The most commonly encountered N-centered radicals are $^{\bullet}NO_x$ radicals ($^{\bullet}NO$, $^{\bullet}NO_2$ and $^{\bullet}NO_3$). $^{\bullet}NO$ is involved in many important physiological functions, including neurotransmission. It is, however, believed that fraction of $^{\bullet}NO$ activity is partly due to the peroxynitrite $ONOO^-$, formed on coupling with $O_2^{\bullet-}$ [43] and $^{\bullet}NO_2$ which is an intermediate in the reaction between $^{\bullet}NO$ and O_2 [90]. $^{\bullet}NO$ react with many other radicals, for example with peroxyl ($ROO^{\bullet}$), tyrosyl ($TyrO^{\bullet}$), and tryptophyl ($TrpN^{\bullet}$) radicals [49]. Other examples of N-centered radicals of biological relevance are, in particular: aminyl $^{\bullet}NH$-R [4] and indolyl radicals ($IndN^{\bullet}$) (Ind = fused benzene and pyrrole rings) [27].

Time-resolved techniques (pulse radiolysis *vs.* laser flash photolysis)

Any technique for a direct study of fast free radical reactions must be characterized by a short time of generation of free radicals and simultaneously comprise of a correspondingly fast detection system. It is highly desirable that the formation of the radical is completed within a time period that is short compared to the lifetime of the radical. Furthermore, the radical must exhibit a "detectable property". In this respect, the most frequently used "properties" in measurements are: optical absorbance (in UV, Vis and IR regions), conductance, electron paramagnetic resonance, resonance Raman scattering, polarography and microwave absorption. The invention and development of the fast time-resolved techniques of pulse radiolysis and laser flash photolysis have provided a powerful means of generating and studying a wide range of free radicals, especially in solution [25]. Due to the instability of radicals in solution, fast time-resolved techniques (pulse radiolysis in particular) would remain a premiere tool for obtaining thermodynamic properties such as solvation energies or standard free energies of formation for radicals [41]. The principle idea is the same for both methods and is briefly described below. Generation of radicals is achieved by admitting short pulses of either high-energy electrons (MeV range) generated in accelerators (pulse radiolysis, PR), or photons from lasers (laser flash photolysis, LFP). In PR and LFP with optical detection the formation of the radicals and their subsequent reactions are then monitored by the change in the transmitted light intensity through the reaction cell as a function of time using monochromator and suitable photodectors. The detector converts changes in the analyzing light intensity into electrical analog signals. These signals are digitized, displayed and stored in the digitized oscilloscope, and are subsequently transferred to the computer for further processing. Despite the principle similarities of these two techniques, one important feature differring them must be pointed out. It is caused by the fact that the energy of "accelerated" electrons is significantly higher compared to that of laser photons. As a consequence, the high-energy electrons while traversing through matter do not distinguish between the various molecules (solvent and solute molecules) since they interact with the coulombic field of any atomic electron or nucleus they pass by. Therefore, at least for dilute solutions, the respective energy deposits are preferentially located in the solvent molecules rather than near solute molecules. In consequence, it leads primarily to solvent-derived radicals that can subsequently react with the solute forming solute-drived radicals. On the other hand, the laser photons may interact directly with solute molecules in a process that leads either to their excitation or ionization. The solute has to absorb light with a sufficiently high extinction coefficient at the wavelength that corresponds to the energy of incident photons, which is also sufficient to initiate the desired process. Numerous valuable data, such as the nature and properties of radical reaction products including: (i) absolute rate constants for reactions between selected radicals and compounds and mutual reactions between radicals, spectroscopic parameters (absorption and emission spectra) and thermodynamic (redox potentials, acid-base equilibria) have been obtained by means of these two, in fact, complementary techniques [57]. Two potential advantages of PR over LFP have to be mentioned. Pulse radiolysis permits: (i) initiation of reactions in systems that do not contain a chromophore or an excited state that would be available via photolysis, and (ii) exclusive generation of either oxidizing or reductive equivalents in the same chemical system [94].

Nanosecond pulse radiolysis facility at the INCT (Warszawa) [67]

The nanosecond pulse radiolysis facility based on the electron linear accelerator installed at the INCT, Warszawa was constructed in 1999 [101]. The LAE 10 has been solely dedicated to pulse radiolysis experiments with the following nominal parameters: pulse duration (4–10 ns, 100 ns), electron energy (10 MeV), pulse current (1 Å), and beam power (0.2 kW) (Fig. 1). The LAE 10 accelerator and the experimental room

Fig. 1. General view of the linear electron accelerator (LAE 10) installed in the INCT.

Fig. 2. General view of the measurement room (left) and the operating room (right).

(Fig. 2, left) is located in a specially developed building equipped with adequately shielded rooms and with independent shielding and grounding systems to avoid distortion produced by high power and high voltage modulators. The pulse radiolysis setup consists of the fast digital storage oscilloscope (DSO) (LeCroy 9354AL) which produces a sufficient number of time points that multiple time scales can be generated by the computer from a single kinetic trace originating from DSO. With the shortest time-base, one can, in principle, with a single kinetic trace, resolve time constants from a few nanoseconds to tens, or even hundreds of microseconds. The simultaneous recording transient absorption data on multiple time scales is valuable for saving both experimental time and the amount of valuable samples. Transients are detected by UV/VIS absorption spectroscopy using two kinds of xenon lamps as sources of analytical light. The data-acquisition subsystem also includes a Spectra Pro-275 monochromator (Stanford Reseach Instruments), a 5-stage photomultiplier tube (Hamamatsu R-955) with a wide spectral response (160–900 nm) powered with the HV Power Supply PS310 (Stanford Research Instruments) and a PC computer (Fig. 2, right). The program controls most of the peripherals over the GPIB (IEEE488) and RS-232 and RS-485 lines. The software was written using Delphi 3 (Borland) within Windows 9x/NT/ME. The new pulse radiolysis set-up due to its modular structure and applied programming tools is very flexible, adopted easily to all changes and friendly for users. Schematic diagram of the PR system operated currently in the INCT is presented in Fig. 3.

Selected important achievements in free radical studies

Chemistry

Ionic liquids (IL) are a class of novel solvents with very interesting properties, and thus can be applied in catalytic, organic and electrochemical reactions. One important feature of IL is the possibility of tuning their physical and chemical properties by varying the nature of the anion and cation [29]. Moreover, IL are excellent media for the generation of radical ions since radiolysis generates high yield of electrons and holes, which are further trapped by cations and anions [61, 95]. Recent pulse radiolysis studies have shown that the rate constants for the ET reactions in IL are generally lower than those in water and in organic solvents, the activation parameters are closer to those measured in aqueous solution than in alcohols, poor correlation of the rate constants with typical solvent polarity parameters, however, a reasonable good one with hydrogen-bond donor acidity and with anion-solvation tendency parameters [45–48]. The dynamics of very fast processes such as solvation and diffusion of radicals can be also studied by mans of pulse radiolysis in order to explore charge transfer phenomena in IL for a wide range of compositions and viscosities [96].

One of the classic reaction pathways available to free radicals is bimolecular homolytic substitution (S_H2) [40]. S_H2 reactions involving organic molecules with hypervalent atoms have been studied extensively in the gas phase. On the other hand, in solution, S_H2 reactions with the simplest free radical containing nucleus, $^{\bullet}H$ atom, are almost nonexistent. Pulse radiolysis studies with time-resolved ESR detection of aqueous solutions containing α-(methylthio)acetamide ($H_3C\text{-}S\text{-}CH_2\text{-}C(=O)NH_2$) prove that S_H2 of the acetamide radical fragment ($^{\bullet}CH_2\text{-}C(=O)NH_2$) by an H atom is the most likely reaction pathway. It is driven by the relatively strong S-H bond formation while only breaking a weaker C-S bond [98].

Recent investigations of free radical chemistry in supercritical fluids (SCF) have shown that physical and chemical properties of free radicals are extremely useful as mechanistic probes of SCF solvation and solvent effects. SCF viscosity is one of the bulk physical properties that may be continously tuned with changes in temperature and pressure. Three general categories of free radical reactivity have been considered: (i) diffusion-controlled reactions, (ii) solvent cage effects and (iii) activated processes [28]. SCF have also been exploited for environmental remediation purposes. Moreover, super-critical-water-cooled reactor is a prime candidate for developing a new generation of reactors because of the increased thermal efficiency. Therefore, from both technological and scientific perspectives

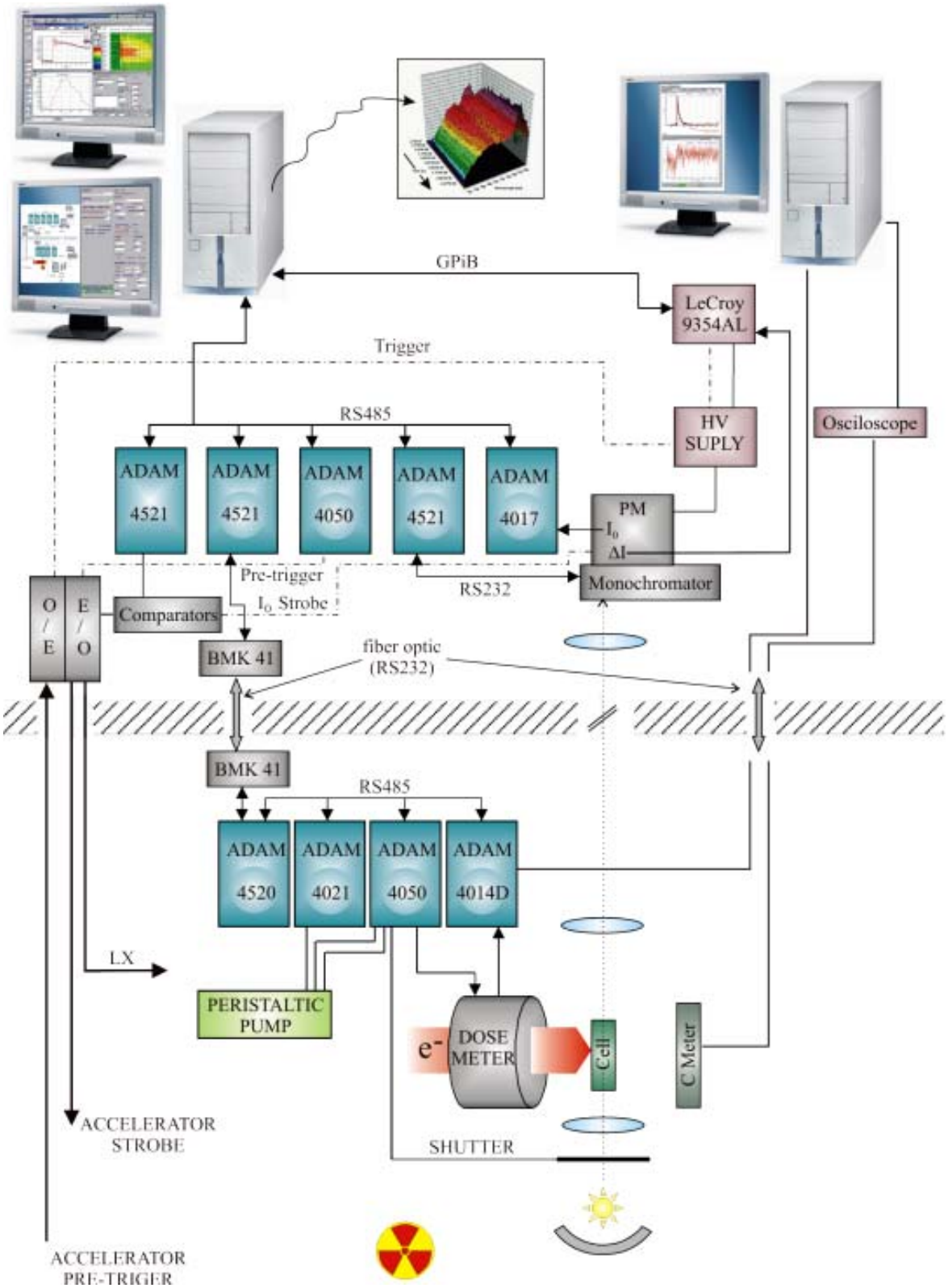

Fig. 3. Design of the experimental control functions (computer and control circuits).

supercritical water provides a convenient medium for altering systematically water properties important in radiolysis (dielectric constants, solvent structure, and dissociation of water) as a function of density without changing phase [41]. In recent three years, a series of papers have been published that addressed spectral, thermodynamic and kinetic properties of hydrated electrons and hydrogen atoms formed during radiolysis of water at supercritical temperatures and pressures [7, 30, 62, 63].

Biology

The potential role of pulse radiolysis for studying biological redox processes, particularly of macromolecules, has been recognized rather early. Initially, pulse radiolysis was employed for investigating radiation-induced damage in peptides, proteins, lipids, sugars, nucleobases (pyrimidines and purines), nucleosides, and nucleotides, poly-U and poly-A, and DNA oligomers [84, 88]. In most cases, pulse radiolysis method has been valuable in identification of radicals, establishing their structures and exploring their reactivities. One of the recent results concerns $^{\bullet}$OH-induced oxidation of glycine, the simplest amino acid. Two main radical products $^{+}H_2N^{\bullet}$-CH_2-CO_2^{-} and aminyl radicals $HN^{\bullet}$-CH_2-CO_2^{-} have been identified and their subsequent reaction pathways including decarboxylation with parallel formation of $^{\bullet}CH_2NH_2$ [23] and β-fragmentation into the respective imine and $CO_2^{\bullet-}$ respectively [21] have been characterized by means of pulse radiolysis.

Long range electron transfer (LRET) in biological systems (synthetic peptides, proteins and DNA) constitutes a worthwhile challenge to radiation chemistry [12, 36, 54, 66, 72, 91]. Most of these ET processes proceed on ground-state potential energy surfaces, making pulse radiolysis an effective and truly unique tool for these studies. The typical LRET pulse radiolysis experiment begins with the rapid selective oxidation or reduction of one redox site on a macromolecule (formation of the donor-acceptor complex) followed by the intra-molecular ET. The advantage of pulse radiolysis is that using either an oxidizing or a reducing radical can generate the donor-acceptor complex. For the very rapid LRET reactions limitation of the technique is the pseudo-first order reaction of the formation of the donor-acceptor complex. In a series of elegant experiments, Isied, Wishart and coworkers examined the role of the distance, standard free energy change, and reorganization energy by studying electron transfer across polypeptides between metal binuclear complexes (Ru, Co, Os) [52–54]. Influence of secondary structural features of the peptide bridge has been probed by applying flexible oligoglycine bridges, conformationally more rigid oligoproline bridges and helical bridges. Intramolecular ET involving radicals located on the side chains of aromatic (tryptophan, tyrosine, histidine) and sulfur (methionine, cysteine) have been extensively in several laboratories. Pulse radiolysis studies of simple model synthetic peptides with flexible and rigid oligopeptides including helical bridges have demonstrated radical transformations: $Trp/N^{\bullet} \rightarrow Tyr/O^{\bullet}$ [15, 17, 18, 33, 35, 36, 60, 78], $Met/S\therefore Br \rightarrow Tyr/O^{\bullet}$ [20, 76], $Met/S\therefore Br \rightarrow Trp/N^{\bullet}$ [19], $CysS^{\bullet} \rightarrow Tyr/O^{\bullet}$ [77], and $His/N^{+\bullet} \rightarrow Tyr/O^{\bullet}$ [86]. Elaboration of LRET mechanism by resolving the parameters that determine specific rates of LRET has stimulated pulse radiolysis studies in proteins. Examples include generation of metastable stable electron donor and acceptor complexes in (i) native and mutant proteins [16, 76, 85], (ii) proteins with the directed single-site specific mutations [38, 39], (iii) native and mutant multi-site redox proteins [59], (iv) proteins with the site specific modification with transition metal complexes covalently attached to a specific surface aminoacid residues [68] or with site specific modifications of tyrosine residues [44].

The reactions of superoxide radical anions ($O_2^{\bullet-}$) with sulfide radical cation complexes might represent an important and efficient reaction pathway for the formation of sulfoxides in peptides and proteins containing methionine residues. Absolute rate constants for two sulfide radical cation complexes $(S\therefore S)^{+}$ and $(S\therefore N)^{+}$ with $O_2^{\bullet-}$ were measured using pulse radiolysis [22]. The rate constant for the reaction of $O_2^{\bullet-}$ with the $(S\therefore N)^{+}$ complex was found to be *ca*. 3-fold slower as compared to that of the reaction with the $(S\therefore S)^{+}$ complex. This drop in reactivity may, in part, reflect the lower probability of $O_2^{\bullet-}$ to encounter S atom in the $(S\therefore N)^{+}$ complex as compared to the symmetrical $(S\therefore S)^{+}$ complex. It is important to note that the reactions of $O_2^{\bullet-}$ with the sulfide radical cation complexes proceed 2.5 to 8-fold faster than the reaction of $O_2^{\bullet-}$ with superoxide dismutase. From a biological point of view, it means that sulfide radical cation-$O_2^{\bullet-}$ reactions might represent a potential source for sulfoxide formation when system is exposed to high concentrations of reactive oxygen species.

Medicine

The pathogenesis of Alzheimer's disease is strongly associated with the formation and deposition of β-amyloid peptide (β-AP) in the brain. This peptide contains a methionine (Met^{35}) residue in the C-terminal domain, which is important for its neurotoxicity and its propensity to reduce transition metals (Cu^{II}) and to form reactive oxygen species [24, 74]. Stoichiometrically, the reduction of Cu^{II} to Cu^{I} requires the one-electron oxidation of Met to a Met radical cation ($MetS^{+\bullet}$) [80, 81]. Neighboring group effects play a significant role in product formation [75, 83]. In order to define the potential reactions of $MetS^{+\bullet}$ in β-AP, pulse radiolysis studies with UV/Vis spectrophotometrical and conductometric detection have been performed in small model peptides, N-Ac-Gly-$(Gly)_{n-1}$-Met-$(Gly)_n$ [82]. They show for the first time that (i) $MetS^{+\bullet}$ in peptides can be stabilized through bond formation with either the oxygen or the nitrogen atoms of adjacent peptide bonds; (ii) the formation of transients with sulfur-oxygen bonds is kinetically preferred, but on longer time scales transients with sulfur-oxygen bonds convert into transients with sulfur-nitrogen bonds in a pH dependent manner; (iii) ultimately transients with sulfur-nitrogen bonds transform intramolecularly into carbon-centered radicals located on the $^{\alpha}C$ moiety of the peptide backbone. Another type of C-centered radicals, located in the side chain of Met residue, could be formed via deprotonation of $MetS^{+\bullet}$. Carbon-centered radicals are precursors for peroxyl radicals that might be involved in chain reactions of peptide and/or protein oxidation.

It has been recently shown that Met^{35} is rapidly oxidized to methionine sulfoxide on the addition of Cu^{II} to the β-AP(1-39) solution. In the structure of β-AP, $Met^{35}S^{+\bullet}$ formation may be facilitated by a preexisting close sulfur-oxygen (S-O) interaction between the Met^{35}

sulfur and the carbonyl oxygen of the peptide bond C-terminal to Ile^{31} that might lower 1e reduction potential of $MetS^{+\bullet}$/Met couple. One-electron oxidation of β-AP(1-40) using azide radicals ($^{\bullet}N_3$) produced by pulse radiolysis have shown that Met^{35} is the target in β-AP(1-40) oxidation. Conversely, the oxidation of β-AP(40-1) with a reversed sequence of aminoacids have shown that Tyr^{10} is the target of $^{\bullet}N_3$ radicals [56]. Thermodynamic considerations indicate that $^{\bullet}N_3$ should not oxidized Met residues unless the one-electron reduction potential of Met is lowered because of favorable environment. These observations are the first experimental evidences that: (i) Met^{35} in β-AP(1-40) is more easily oxidized than in other peptides or proteins, (ii) a change in a primary sequence affects drastically one-electron reduction potential of Met, even in small peptides. Such observtions seems to be indeed relevant to explain specificity of the β-AP in the development of Alzheimer's disease.

The pathogenesis of another well-known neurodegenerative disease (Jacob-Creutzfeld's) seems to be strongly linked to the presence of prion proteins in the brain. These macromolecules contain multiple Met residues [99]. The interaction with particular protein domains involving nucleophilic functionalities in side chains of aminoacids residues (Asp, Glu, Lys, Val, Thr), thioether moiety (from Met) or in peptide bonds present in the vicinity could be vital in stabilizing $MetS^{+\bullet}$. Intramolecular stabilization of $MetS^{+\bullet}$ as $[>S\therefore S<]^{+}$ complexes has been already characterized by means of pulse radiolysis in linear oligopeptides containing multiple methionine residues [14]. Since weak intramolecular non-bonded S•••O and S•••N interactions have been recently suggested in proteins [55], stabilization of $MetS^{+\bullet}$ through formation of S∴N and/or S∴O-bonded radicals might potentially accelerate oxidation processes in proteins. The first experimental evidence for the formation of $MetS^{+\bullet}$ which complex to adjacent amide groups was obtained during one-electron oxidation of calmodulin ($CaM\text{-}Ca_4$, wild type), studied on the microsecond time domain by pulse radiolysis [69]. The structure of $CaM\text{-}Ca_4$ reveals that all Met sulfur atoms are located in close vicinity to at least one peptide bond amide and/or carbonyl function. Stabilization of $MetS^{+\bullet}$ by peptide bonds might be a general phenomenon in proteins.

Future perspectives

Topics

The number of scientific topics for applying radiation chemistry in various fields of physics, chemistry, biology, and medicine is very large. There is neither need nor possibility addressing them all in this article. Few selected examples below will only illustrate potential research opportunities, and new challenges and needs in the field. Electron driven processes in aqueous environment are important for the understanding of the impact of radiation exposure on biological systems and advancing the fields of nuclear medicine and radiation therapy. An understanding of relaxation and reaction processes occurring under highly non-equilibrium conditions is needed. This might be essential in all aspects of nuclear energy production. Supra-molecular chemistry is a rapidly expanding area and can also benefit from the work of radiation chemists. Important future applications of radiation chemistry include electron transfer processes through unusual media such as membranes, reactive solvents, and molecular bridges. Radiation chemical methods can implement free-radical based methods for organic synthesis. Radiation-initiated living radical polymerization that provides new strategies for the design of block copolymeric materials is one of technologically significant applications. Heterogeneous systems involving constrained environments are of increasing importance; metal and semiconductor nanoparticles, microporous and mesoporous materials, microelectronics material are just few examples. Environmental remediation using radiation chemistry is one of the most promising Advanced Oxidation Processes (AOP). Understanding of the basic mechanisms of AOP will allow predicting, optimizing and controlling of the technological processes in heterogeneous environmental systems.

Instrumentation

There is also a strong need to develop experimental capabilities for pulse radiolysis to push the forefront of radiation research. This involves RF photocathode electron gun based linear accelerators, which allow for synchronization of the electron pulse and a femtosecond light pulse [93]. Recent advances in accelerator technology are enabling generation of subpicosecond electron pulses. The next generation of time-resolved radiolysis might be accomplished without conventional accelerators. A novel source for radiolysis such as table top laser generation of electron beams is just one of the examples. Efforts should be put into developing new detection methods with IR, Raman, neutron and X-ray scattering, as examples.

The state of the art and future scientific topics and advances in the field of radiation chemistry were the issues of two workshops organized by the Division of Chemical Sciences of the US DOE in Chesterton, Indiana on April 19–22, 1998 [*Research needs and opportunities on radiation chemistry workshop, US DOE Final Report*], and in Richland, WA on September 26–28, 2002 [41]. Recently, the current and new research activities that have been undertaken by radiation chemists in Poland have been also reviewed [13].

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