

The Underrated Carbonate Radical ($\text{CO}_3^{\bullet-}$) – Detoxification and Reduced Formation by Melatonin

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Abstract

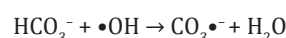
Carbonate radicals are frequently overlooked or underrated oxidants that have a considerably larger range of action than the short-lived hydroxyl radicals. They possess sufficient reactivity for abstracting electrons or hydrogen atoms from many biomolecules. Carbonate radicals are formed in several reactions, e.g., via hydrogen abstraction from bicarbonate by a hydroxyl radical, through interaction of a formate radical with bicarbonate and, most importantly, from the peroxyxynitrite-carbon dioxide adduct. All these pathways of formation indicate an important role of carbon dioxide and bicarbonate levels for the generation of carbonate radicals and, therefore, many oxidizing reactions with aromates can be considerably enhanced by adding bicarbonate. Under physiological and, even more, pathophysiological conditions, high $\text{CO}_2/\text{HCO}_3^-$ concentrations are found in mitochondria and in situations of under-perfusion or ischemia. Increased levels of nitric oxide considerably increase via generation of peroxyxynitrite the formation of carbonate radicals, effects of relevance with regard to inflammation, Alzheimer's disease, stroke, and all pathologies related to mitochondrial dysfunction. Melatonin counteracts both the formation and persistence of carbonate radicals, by down regulating inducible and neuronal NO synthases, by scavenging carbonate radicals, and by protecting mitochondria. By interaction with carbonate radicals, melatonin is converted to *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK), another protective compound, which is metabolized to *N*¹-acetyl-5-methoxykynuramine (AMK). AMK also down regulates inducible and neuronal NO synthases and scavenges carbonate radicals as well as various reactive oxygen species.

Keywords: Carbonate Radical; Ischemia; Mitochondria; Peroxyxynitrite; Tyrosine Nitration

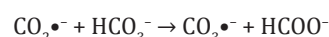
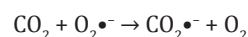
Introduction

Damage to biomolecules by reactive oxygen species is often attributed to the hydroxyl radical, because of its particularly high reactivity that enables this intermediate to interact with countless other compounds. However, the high reactivity leads to the consequence of a very short half-life of about a nanosecond and, thus, of an extremely short range of action. The carbonate radical ($\text{CO}_3^{\bullet-}$) differs considerably in this regard. Despite its lower reactivity, it is still capable of abstracting electrons or hydrogen atoms from the same molecules that would undergo these reactions with hydroxyl radicals. While the awareness of damage by hydroxyl radicals is widely present in the community, this is not the case with regard to carbonate radicals. However, it seems important to direct researchers' attention to the pathophysiological relevance of $\text{CO}_3^{\bullet-}$, especially because of the fact that this symmetric radical, by virtue of resonance stabilization, has a much longer lifetime and, thus, a more far-reaching action [1]. This property makes $\text{CO}_3^{\bullet-}$ particularly dangerous with regard damage of important biomolecules, including nucleic acids and proteins [2,3].

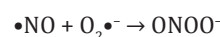
$\text{CO}_3^{\bullet-}$ can be formed in different ways. One possibility consists in the abstraction of a hydrogen atom from bicarbonate by a hydroxyl radical:



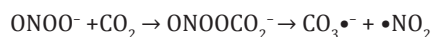
In this case, $\text{CO}_3^{\bullet-}$ is a more far-reaching mediator of the hydroxyl radical. Another pathway is based on the interaction of CO_2 with the superoxide anion, which represents the most abundant free radical in biological material. The formate radical ($\text{CO}_2^{\bullet-}$) generated by electron transfer further reacts with bicarbonate, to give a formate anion [4]:



Most importantly, $\text{CO}_3^{\bullet-}$ can derive from peroxyxynitrite (ONOO^-) [3,5-7], a particularly damaging intermediate that is formed by combination of nitric oxide with superoxide:



Protonation of ONOO⁻ leads to a decay into •OH and •NO₂, but the damage in biological material by oxidation and nitration is rather limited [7]. However, an alternate possibility of generating free radicals from ONOO⁻ leads to higher rates of oxidation and nitration [7,8], in which CO₃•⁻ is involved:



The formation of the peroxyxynitrite-CO₂ adduct is decisive for CO₃•⁻ at more substantial rates relative to the previously discussed reactions leading to this radical [3,5-9]. In particular, the combination of CO₃•⁻ and •NO₂ is a highly effective nitrating mixture for phenolic and indolic compounds that is based on the combination of hydrogen abstraction and •NO₂ addition and, notably, differs profoundly from the classic, non-radical nitration reaction of aromates [8]. This should deserve more future consideration when discussing nitration of tyrosyl residues in proteins.

Damage by Free Radicals in Relation to CO₂ Abundance

The enhanced formation of CO₃•⁻ at high CO₂ and HCO₃⁻ concentrations should direct researchers' attention to the pathophysiologically relevant conditions and sites. In chemical systems, many oxidation reactions can be considerably enhanced by adding HCO₃⁻, under avoidance of pH changes [9]. Increased levels of 3-nitrotyrosyl residues in atherosclerotic plaques have been repeatedly described, e.g., in refs. [10-13]. These findings have been largely related to inflammatory processes, which is not wrong, since inflammation is associated with formation of both •NO and O₂•⁻, the reactants that combine to peroxyxynitrite. However, the role of CO₂, which is in equilibrium with HCO₃⁻ and which leads to the adduct ONOOCO₂⁻, has usually not been considered. As components of the most important buffer in the blood, CO₂ and its product HCO₃⁻ are abundantly available in the circulation. Under conditions of hypo-perfusion, e.g., by atherosclerosis, their local concentrations are elevated and favor CO₃•⁻ formation. This is even more the case in ischemia, also directly after reperfusion. This is also evident from data on increased 3-nitrotyrosine [14-16]. Notably, tyrosine nitration does not only affect proteins in the circulation, thereby, contributing to protease-resistant, poorly removable plaques, but also causes dysfunctionality of cell biologically important molecules. For example, under conditions of ischemia/reperfusion, voltage-dependent anion channels [17] and the H₂O₂-eliminating thioredoxin [18] were inactivated in the heart by tyrosine nitration. In endothelial cells, tyrosine nitration of the antioxidant regulator Keap1 (kelch-like ECH-associated protein 1) stimulated its transfer into the nucleus [19]. A full record of such effects would exceed the scope of this article. Finally, increased peroxyxynitrite formation and tyrosine nitration in neurodegenerative processes, in particular, Alzheimer's disease, should be mentioned [20-22]. In particular, tyrosine nitration of Aβ peptides was reported to increase aggregation and plaque formation [22].

In mitochondria, peroxyxynitrite formation and damage by its derivatives, CO₃•⁻ and •NO₂, is of utmost importance, since this causes interruptions of electron flux, increased electron leakage, oxidative/nitrative stress to the entire cell and, at a certain degree

of severity, cell death. Details of these processes and consequences have been summarized elsewhere [23-29]. Recurring to the role of CO₂ as a component of the CO₃•⁻-generating peroxyxynitrite adduct, it is important to remember that the Krebs cycle continuously generates CO₂ and, therefore, steadily provides high levels to enhance adduct formation and its decomposition products, CO₃•⁻ and •NO₂. In addition to the generation of O₂•⁻ by electron leakage and the existence of a mitochondrial inducible NO synthase [30], the high availability of CO₂ makes mitochondria particularly vulnerable to damage by CO₃•⁻.

Multiple Levels of Protection by Melatonin Against Damage by CO₃•⁻

Melatonin is known to interact with various free radicals [31], but most studies have focused on hydroxyl radicals. It seems important to underline the capacity of melatonin to also scavenge the carbonate radical [1,32,33], which is frequently underrated and may contribute more strongly than previously believed to damage of DNA, proteins, organelles, in particular, mitochondria and other cell constituents. The earliest report [32] stated that, contrary to expectation, no stable nitrated products were obtained with melatonin. Instead, the main product was a product formed by pyrrole ring cleavage, N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK) [1], a meanwhile frequently investigated metabolite. In the reaction system used, a substantial participation of hydroxyl radicals that might have been generated from a concurrent decay of ONOOH was excluded, since the reaction also took place in the presence of the •OH scavenger dimethyl sulfoxide [1]. Moreover, the formation of AFMK was concluded to result from sequential actions of CO₃•⁻ and O₂•⁻, which would well conform to the relatively high cellular and organellar abundance of O₂•⁻. Direct scavenging of the CO₃•⁻ precursor peroxyxynitrite by melatonin, which has been repeatedly claimed in the literature, will not be discussed here, because this possibility is difficult to distinguish from actions of its radical derivatives in biological material. Concerning the quantitative contribution of scavenging by melatonin to overall detoxification, its low amounts have to be considered. In this regard, a substantial role can only be present in cells or organelles that produce or accumulate melatonin. Notably, melatonin is not only synthesized in the pineal gland, but also in numerous other tissues and cells [34]. Accumulation of melatonin has been described in mitochondria [29,35]. However, even in those organs, compartments or body fluids in which melatonin levels are low, the formation of AFMK is possible. This is insofar important as AFMK also represents a protective compound [36], which is especially generated under inflammatory conditions [29,37] and has been shown, in patients with viral meningitis, to attain in the cerebrospinal fluid levels by orders of magnitude higher than melatonin [38]. At least in part, protection by AFMK may be attributed to its deformedylated metabolite AMK (N¹-acetyl-5-methoxykynuramine), which is formed by arylamine formamidase or hemoperoxidases [37]. AMK is also a potent scavenger of various reactive oxygen species and carbonate radicals [37,39], and also of •NO and other reactive nitrogen species [8,9,40,41]. Nitration by the peroxyxynitrite-derived radicals CO₃•⁻ and •NO₂ has been specifically demonstrated [8].

Contrary to melatonin, which also scavenges •NO, AMK does not re-donate •NO, but rather forms a stable product, 3-acetamidomethyl-6-methoxycinnolinone [8,40].

Both melatonin and AMK possess additional, regulatory properties that reduce the formation of peroxynitrite and $\text{CO}_3^{\bullet-}$. Melatonin protects mitochondria in manifold ways and prevents excessive electron leakage, thereby reducing the levels of $\text{O}_2^{\bullet-}$, effects that are accompanied by additional upregulations of antioxidant enzymes including MnSOD [9,24,25,28,29,31]. Moreover, inducible NO synthase (iNOS) is downregulated and neuronal NO synthase (nNOS) inhibited at low concentrations of melatonin [9,25,28-31] or AMK [9,25,29,37]. The concomitant reduction of $\text{O}_2^{\bullet-}$ and •NO sets limits to the formation of ONOO⁻ and, therefore, its products ONOOCO₂⁻, CO₃^{•-} and •NO₂, conclusions that are well in accordance with the observed attenuation of tyrosine nitration by melatonin [19,30,42,43].

Conclusion

Carbonate radicals should be considered much more in the future as damaging intermediates, which undergo reactions similar to those of hydroxyl radicals. Compared to •OH, the lower reactivity of $\text{CO}_3^{\bullet-}$ is outweighed by its longer lifetime and more far-reaching action. The contribution of CO_2 levels to their formation deserves further attention in the pathologies related to oxidative damage, mitochondrial dysfunction and tyrosine nitration. Melatonin, in conjunction with its metabolites AFMK and AMK, represents a promising option for counteracting damage by $\text{CO}_3^{\bullet-}$.

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