Tyrosine nitration and peroxonitrite (peroxynitrite) isomerisation: ^{15}N CIDNP NMR studies

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In the presence of tyrosine, peroxonitrous acid forms nitrite, nitrate and 3-nitrotyrosine by radical processes.

One of the most interesting developments in our understanding of the role of nitric oxide in animal physiology is the emergence of peroxonitrite ($ONOO^-$, sometimes called peroxynitrite), formed by reaction of nitric oxide with superoxide, as an important cytotoxic agent.[1] Also, peroxonitrite appears to bring about tyrosine nitration[2] which may have consequences for the process of tyrosine phosphorylation. The presence of nitrated tyrosine residues has been suggested as a marker for peroxonitrite activity.[3] The formation of NO_2^+ has been proposed as an essential step in tyrosine nitration by peroxonitrite[4] but, for a substrate as activated towards electrophiles as tyrosine, there are well established alternative pathways. For example, we have observed that the nitration of *N*-acetyltyrosine by dilute nitric acid is a nitrous acid catalysed process and must occur either by nitrosation followed by oxidation or by a radical mechanism. The use of the CIDNP has proved very useful in probing further the mechanisms of such reactions.[5] Nitration of tyrosine with ^{15}N-labelled nitric acid containing catalytic quantities of nitrite, and examination of the ^{15}N NMR spectrum immediately after reaction, gave a very large inverted signal for 3-nitrotyrosine, which slowly changed to the normal signal. It is possible to make a number of mechanistic deductions from this observation but it is sufficient, in this preliminary communication, to note that there must be a substantial radical pathway in the formation of 3-nitrotyrosine. We now turn to an examination of nitration by peroxonitrite where both radical and ionic pathways have been suggested.

^{15}N-labelled peroxonitrite in alkaline solution was prepared by the method of Leis *et al.*[6] and allowed to react with tyrosine at 25 °C and pH 12. EDTA was present to prevent complications due to a metal-catalysed decomposition pathway.[7] The reaction was followed from changes in the ^{15}N NMR spectrum using a 500 MHz Varian Unity instrument. The quoted chemical shifts are downfield from liquid ammonia at 25 °C and were measured relative to [^{15}N]nitrobenzene (δ_N 370.3) as an external standard. The identification of the signals was carried out by spiking with authentic samples of ^{15}N-labelled material.

The initial signal from peroxonitrite ions at δ_N 554.6 decreased exponentially with time, showing no sign of nuclear polarisation, and two product signals appeared: one from nitrite ions at δ_N 609.1 in emission and one from nitrate ions at δ_N 375.9 showing evidence of enhanced absorption (Fig. 1). The UV–VIS spectra of the solutions showed the formation of 3-nitrotyrosine; this absorbs at δ_N 375.3 (very close to nitrate ions) under these conditions but the amount formed is insufficient to contribute significantly to that signal. When tyrosine is absent or replaced by *O*-methyltyrosine, the only reaction observed is the slow conversion of peroxonitrite ions to nitrate ions without any evidence of nuclear polarisation.

The main features of these observations can be explained by the reaction pathway shown in Scheme 1. The absence of nuclear polarisation in the peroxonitrite ions suggests that the initial homolysis of peroxonitrite is essentially irreversible; this also accords with AMI calculations on the relative energies of the possible transition states. The fact that the nitrate and nitrite ions show nuclear polarisation of opposite phase indicates that one product is derived from reaction within the $NO_2^{\bullet}$–$^{\bullet}OH$ encounter pair and the other from reaction after dissociation of this radical pair. From Kaptein's rules[8] as applied to the ^{15}N nucleus[9] and from the *g*-values of the $NO_2^{\bullet}$ and $^{\bullet}OH$ radicals,[10] it follows that the reaction within the encounter pair should give an enhanced absorption signal and that from the dissociated radicals should give an emission signal; this accords with the observed spectra of the nitrate and nitrite ions. Tyrosine appears to be involved in two ways: first, the conjugate base of tyrosine reduces the dissociated radicals by an electron transfer reaction and, secondly, the tyrosyl radical so formed reacts directly with the $NO_2^{\bullet}$ and $^{\bullet}OH$ radicals. The relatively low yield of 3-nitrotyrosine may be a consequence of the high reactivity of $^{\bullet}OH$ radicals towards tyrosyl radicals. In the absence of tyrosine, the dissociated $NO_2^{\bullet}$ and $^{\bullet}OH$ radicals are considered to recombine to form nitrate ions and, since only one product is formed, nuclear polarisation would not be expected.

The scheme is similar to that proposed by Pryor and Squadrito.[11] Nitrophenols are known to be formed by the reaction of phenoxy radicals with nitrogen dioxide[12] and aromatic hydroxylation by peroxonitrite ions has been des-

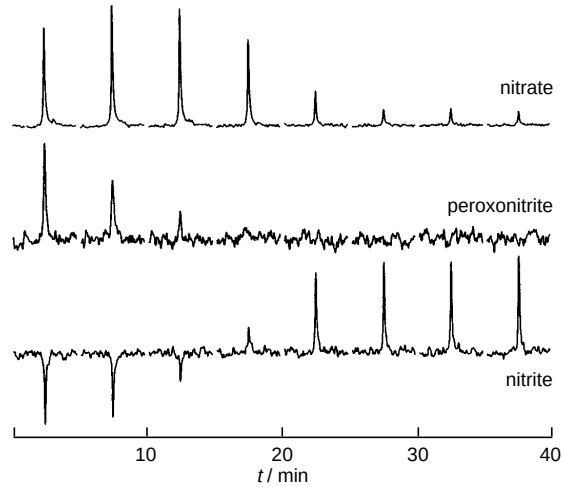
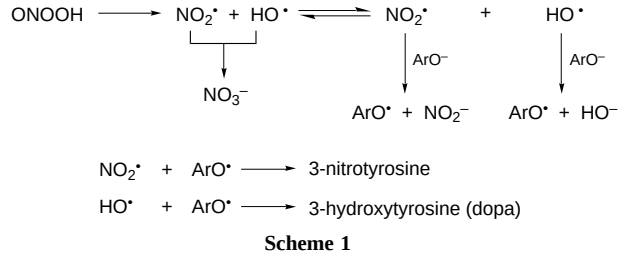


Fig. 1 Variation of signals with time in the ^{15}N NMR spectrum for the reaction of peroxonitrite (0.1 mol dm^{-3}) with l-tyrosine (0.025 mol dm^{-3}). [NaOH] = 0.15 mol dm^{-3}.



Scheme 1

cribed previously.[13] It is clear from our observations that one pathway, perhaps the only pathway, in the reaction of peroxonitrite ions with tyrosine in alkaline solution is a radical one. The small concentration of nitrotyrosine formed unfortunately precludes any observation of nuclear polarisation in this product but it is reasonable to infer that this nitration is also a radical reaction.

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Footnote

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