RADICAL MECHANISMS OF CEPHALOSPORINS: A PULSE RADIOLYSIS STUDY

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Abstract—Radiosterilization induces radicals, and it is very important to describe radical mechanisms before the possible use of cephalosporins gamma sterilization. Moreover, physiological or radiotherapeutically induced free radicals also initiate radical mechanisms. For this study, pulse radiolysis was used. This method permits to avoid in vivo direct study difficulties of biradical processes and gives quantitative data. Reactions of solvated electron (e−aq), hydroxyl radical (·OH), azide radical (N3−), dibromine radical anions (Br2−), oxygen, and superoxide radical (O2−) with three cephalosporins have been studied. Absorption spectra and rate constants have been determined. It has been found that both e−aq and ·OH quickly react (k = 1010 mol−1 L s−1) with the molecules to give radicals with similar absorption spectra. N3− gives an absorption spectra that has been attributed to an electron transfer, whereas a part of ·OH and Br2− could add themselves to an unsaturated bond.

Keywords—Pulse radiolysis, Cephalosporins, Radical mechanisms, Rate constants, Free radicals

INTRODUCTION

Cephalosporins are β-lactam antibiotics that are the most frequently prescribed antibacterial drugs.1 Commercially available as powder for injections, a sterilization is required. Because of their degradation, especially at high temperature, which eliminates conventional sterilization methods such as autoclaving, a costly sterilizing filtration in aseptic conditions is used. Therefore, the gamma radiosterilization could be a better process: the packed drug is sterilized and undergoes very little degradation.2 Moreover, the sterility control can be easily made by measuring the absorbed dose (chemical dosimetry). However, the ionizing treatment leads to new substances produced by radical mechanisms.3 Thus, it is essential to study this radical mechanism by methods like electron spin resonance (ESR) in solid state1 or pulse radiolysis in liquid state (this study). ESR measurements give qualitative features only in favorable studies like the radiolysis of solid cefazidim, where the ESR spectra have been recorded during the warming up of the irradiated samples.4 ESR study of the two other cephalosporins is in progress.

Moreover, the use of quantitative data obtained in vitro by pulse radiolysis could be of fundamental interest in vivo and, perhaps, could give new views on drugs metabolisms: free radicals as hydroxyl radical, superoxide radical, and so forth, are physiological products and can react to administered cephalosporins.5

Pulse radiolysis is a method commonly used to study the radicals formed by irradiation of dissolved molecules or ions. The electron pulse produces a high initial concentration of reactive intermediates with lifetimes of microseconds or longer. The change in concentration of these species can be followed by the changes in optical density of the irradiated solutions. Spectroscopic and kinetic data about the transient species formed by the attack of different free radicals (e−aq, ·OH, N3−, Br2−) on three cephalosporins—sodium cefotaxim, sodium cefuroxim, and pentoxychlorinated cefazidim (Fig. 1)—are described.

There are very few publications on the radiolysis of cephalosporins. Most of them deal with the loss of antibacterial activity under gamma irradiation, but no mechanism and no rate constant are given.6–8 The only one that uses pulse radiolysis gives the rate constant of
hydrated electron with cephalosporin C, cephaloridine, and cephalothin to study the interaction of these compounds with serum albumine.

MATERIALS AND METHODS

Sodium cefotaxim was obtained from Hoechst, sodium ceftiroxime and hydrated ceftazidim were obtained from Glaxo. Ceftazidim contains Na₂CO₃, CO₂, and N₂. Terbutanol, sodium azide, and potassium bromide pro analysis were used without further purification. All solutions were prepared with tri-distilled water.

Pulse radiolysis experiments were performed using a modified Febetron 707, which delivers electron pulse of 1.8 MeV during 15 ns. The irradiation cell in quartz had an optical path length of 2.5 cm. The solution inside the cell was changed after each pulse. The variation of dose from pulse to pulse was monitored by measuring the total pulse charge with a charge integrating current. The doses were calibrated with Fe (CN)₆³⁻/Fe (CN)₆⁴⁻, ɛ₂₅₀ = 1040 M⁻¹cm⁻¹ at room temperature, and G = 5.8.10⁻⁷ mol J⁻¹. The fast spectrophotometric detection system has been described in earlier publications.¹⁰¹¹

RESULTS

It is known that water radiolysis leads to radical (e⁻, 'OH, H⁺) and molecular (H₂O₂, H₂) products. To simplify the radical mechanisms, some of these radicals were selected by using different working conditions.¹²

1. Reducing conditions (cₕₑ⁻)—Aqueous solutions were degassed with argon, and terbutanol was added to convert hydroxyl radicals into nonreactive terbutanol radicals.

```
'OH + (CH₃)₃COH → H₂O + (CH₃)₃CH - COH
```

In such solutions, the total radiolytic yield (G) of e⁻ is 2.9.10⁻⁷ mol J⁻¹.

2. Oxidizing conditions ('OH)—Solutions were saturated with nitrous oxide (N₂O) to convert e⁻ into hydroxyl radicals.

```
N₂O + cₜₑ⁻ → N₂ + OH⁻ + 'OH
```

\[ G('OH) = 5.8.10^{-7} \text{ mol J}^{-1} \]

3. Azide radical (N₃⁻) or dibromine radical anion (Br₂⁻) selection—In N₂O saturated solutions, high concentrations of NaN₃ or KBr were added to transform quantitatively 'OH radicals into N₃⁻ or Br₂⁻, respectively.

![Differential absorption spectra](image-url)

Fig. 2. Differential absorption spectra of 4 × 10⁻³ M cefotaxim (--), ceftiroxime (•••), and ceftazidim (•••) in terbutanol 1% aqueous solutions, argon-saturated.
Fig. 3. Differential absorption spectra of $1 \times 10^{-2}$ M cefotaxim (---), cefuroxim (-----), and ceftazidim (-----) in aqueous solutions, N$_2$O-saturated.

\[ ^{-} \text{OH} + N_{2}\text{O} \rightarrow \text{OH}^- + N_{2} \quad G(N_{2}) = 5.8 \times 10^{-7} \text{ mol J}^{-1} \]

or

\[ ^{-} \text{OH} + \text{Br}^- \rightarrow \text{OH}^- + \text{Br}^- \]

\[ \text{Br}^- + \text{Br}^- \rightarrow \text{Br}_2^- \quad G(\text{Br}_2^-) = 5.8 \times 10^{-7} \text{ mol J}^{-1} \]

**Absorption spectra**

Absorption spectra, recorded 200 ns after the pulse (2 ms for the radicals from KBr solutions), were determined for the three studied cephalosporins (Figs. 2–4). The different working conditions described before were used. The molar extinction coefficients were calculated after determination of the absorbed doses and by using the radiochemical yields mentioned previously.

Fig. 4. Differential absorption spectra of $1 \times 10^{-2}$ M cefotaxim in aqueous solution (---), in 0.1 M Na$_2$N$_2$O aqueous solution (-----), and in 0.2 M KBr aqueous solution (-----). All these solutions were N$_2$O-saturated.

Table 1. Rate Constants of the Solvated Electron Reactions on Cephalosporins in Terbutanol 1% Aqueous Solutions, Argon-Saturated

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_1$ (mol$^{-1}$ L s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxim</td>
<td>$9.8 \times 10^6 \pm 0.5 \times 10^6$</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>$9.9 \times 10^6 \pm 0.5 \times 10^6$</td>
</tr>
<tr>
<td>Ceftazidim</td>
<td>$1 \times 10^4 \pm 0.05 \times 10^3$</td>
</tr>
</tbody>
</table>

**Kinetic analysis of radicals formation**

**Reaction of the solvated electron on cephalosporins (RH).** The hydrated electron acts as a nucleophile. This reaction can be written:

\[ e_{aq} + \text{RH} \rightarrow \text{radical anion} \quad (\text{reaction } 1) \]

This reaction was followed by monitoring the decay of the solvated electron at 700 nm. Due to the high concentration of the solute (RH) in comparison with the initial $e_{aq}$ concentration, the hydrated electrons decay with a pseudo first-order kinetic with a rate constant $k = k_1 [\text{RH}]$. A plot of $k$ against the solute concentration gives a straight line with a slope equal to $k_1$. Table 1 summarizes the results obtained for the different solutes studied. The rate constants for the three cephalosporins are very close to the value obtained for cephalosporin C, cephaloridine, and cephalothin.

**Reaction of hydroxyl radical.** The hydroxyl radical can add itself to unsaturated bonds, abstract hydrogen atoms, or transfer an electron.\textsuperscript{13} These reactions can be symbolized:

\[ ^{-} \text{OH} + \text{RH} \rightarrow \text{ROH} \quad (\text{reactions 2}) \]

\[ \text{R} + \text{H}_2\text{O} \]

\[ ^{-}\text{OH} + \text{H}_2\text{O} \]

Because the hydroxyl radical absorbs only weakly in the U.V., we observed the growth of the oxidated specie(s) to deduce the bimolecular rate constant $k_2$ of reactions 2. For the same reasons as before, the reaction followed a pseudo first-order kinetic. Table 2 summarizes the results obtained.

**Reactions of azide radical and dibromine radical anion on sodium cefotaxim.** N$_2^-$ (ref. 14) and Br$_2^-$ (ref. 15) can transfer an electron and Br$_2^-$ can also add itself to unsaturated bonds. These reactions can be symbolized:
Table 2. Rate Constants of the Hydroxyl Radical Reactions on \(10^{-3}\) M Cephalosporins in Aqueous Solutions, N\(_2\)O-Saturated

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\lambda) (nm)</th>
<th>(k_3) (mol(^{-1}) L s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxim</td>
<td>450</td>
<td>(9.8 \times 10^9 \pm 0.9 \times 10^9)</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>350</td>
<td>(1.3 \times 10^{10} \pm 0.1 \times 10^{10})</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>(1.5 \times 10^{10} \pm 0.1 \times 10^{10})</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>(7.5 \times 10^9 \pm 0.7 \times 10^9)</td>
</tr>
<tr>
<td>Cefazidim</td>
<td>385</td>
<td>(7.9 \times 10^9 \pm 0.8 \times 10^9)</td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>(6.5 \times 10^9 \pm 0.6 \times 10^9)</td>
</tr>
</tbody>
</table>

\[ N_i + RH \rightarrow \ \bullet\bullet RH + N_2 \]  \(k_1\)  (reaction 3)

\[ Br_2^- + RH \rightarrow \ \bullet\bullet RH + 2 Br^- \]  \(k_4\)  (reactions 4)

\[ RHBr + Br^- \]

The bimolecular rate constants \((k_1\) and \(k_4\)) were obtained by the same method as those for the hydroxyl radicals reactions. Tables 3a and 3b summarize the results obtained for the reactions of \(N_i\) and \(Br_2^-\) on \(10^{-2}\) M sodium cefotaxim. Other concentrations were used \((5 \times 10^{-3}\) M in 0.1 M NaN\(_2\) solution, \(10^{-3}\) M in 0.02 M NaN\(_2\) solution and \(4 \times 10^{-3}\) M in 0.1 M KBr solution), but the same values of the rate constants were found.

Kinetic of the decay phases of the radical products. The rate constants of the disappearance of the transient species from reactions 1, 2, 3, and 4 were determined. The decay showed a second-order kinetic law. The rate constants were measured from the slope of the straight line when the inverse of optical density was plotted as a function of time. The slope is equal to \(\frac{2k}{e_1}\). These rate constants are summarized in Tables 4a, 4b, and 4c.

**Oxygen and superoxide radical reactions on radicals.**

\[ O_2 + \text{Ce} \rightarrow RO_2 \]

\[ R^* + R^* \rightarrow R_2 \]

Those rate constants are summarized in Table 5.

The experimental results of the radical decay from aerated \(10^{-2}\) M sodium cefotaxim solution and oxygen-saturated \(10^{-2}\) M sodium cefotaxim solution are shown in Figures 5a and 5b, respectively. Simulated results are reported on the same graphs. The correlation is very good for \(k(R^* + O_2)\) equal to \(6.10^9\) mol\(^{-1}\)Ls\(^{-1}\).

**Superoxide radical.** The same experiments were realised at lower cephalosporins concentrations \((10^{-3}\) M instead of \(10^{-2}\) M). In this case, most of the e\(_m\) react on oxygen to form superoxide radical.

\[ e_m + O_2 \rightarrow O_2^- k_6 = 2 \times 10^{10} \text{ mol}^{-1} \text{ L s}^{-1}\]

The superoxide radical can react on the radicals formed by reactions 2 of hydroxyl radicals on the solutes.

\[ O_2^- + P^* \rightarrow \]

where P* are the radicals formed by reactions 2.

The rate constants \(k_7\) of this reaction were determined and are summarized in Table 6.

**DISCUSSION**

**Absorption spectra**

All the radicals produced from the three cephalosporins had similar absorption spectra—a flat absorption...
band near 500 nm and an intense one at lower wavelength. Radicals formed from sodium cefuroxim showed quite different spectra.

The same spectrum was observed after the hydroxyl radicals reaction on 10^{-3} M or 10^{-2} M cefotaxim aqueous solutions (see Figs. 3, 4)—all hydroxyl radicals reacted on the solute. The same effect was observed for azide radicals.

The absorption spectrum of the radicals produced by N³ reaction can be attributed to an electron transfer reaction\(^{14}\) (see Fig. 4). The reactions of the other oxidizing radicals (‘OH and Br²⁻) formed radicals showing different absorption spectra (see Fig. 4)—the decrease of the absorption band centered at 360 nm and an increase at higher wavelength. The addition of OH and Br²⁻ to an unsaturated bond in competition with an electron transfer could explain these differences.

**Reduction by the solvated electron**

The electron reaction on the solutes is fast, and it is likely that e^{-} adds itself to similar positions of the three cephalosporins. The sulphur center of the 7-aminocephalosporanic acid (see Fig. 1) could be proposed because a fast reaction (k_s \approx 10^{10} mol^{-1} L s^{-1}; see Table 1) and a similar absorption spectra were observed for the three compounds. But, a mixture of radicals was probably observed because the rate constant of recombination of the radical anions from cephalosporins depended on the wavelength (see Table 4a).

**Oxidization by ‘OH, N³, and Br²⁻**

The hydroxyl radicals can react by hydrogen abstraction, addition on a double bond, or electron transfer.\(^{15}\) The hydroxyl radical is a stronger oxidant than N³ (E⁺OH/OH⁻ = 1.91 V (ref. 17), E⁺N³/N³⁻ = 1.35 V

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**Table 4a. Disappearance Rate Constants of the Transient Species Formed From Cephalosporins in 1% Aqueous Solutions, Argon-Saturated**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (M)</th>
<th>(\lambda) (nm)</th>
<th>(\epsilon) (mol^{-1} L cm^{-1})</th>
<th>(2k) (mol^{-1} L s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxim</td>
<td>(4 \times 10^{-3})</td>
<td>450</td>
<td>100</td>
<td>(3.1 \times 10^{9} \pm 0.3 \times 10^{9})</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>(4 \times 10^{-2})</td>
<td>500</td>
<td>400</td>
<td>(1.1 \times 10^{9} \pm 0.1 \times 10^{9})</td>
</tr>
<tr>
<td>Ceftazidim</td>
<td>(4 \times 10^{-3})</td>
<td>390</td>
<td>1900</td>
<td>(2.8 \times 10^{9} \pm 0.3 \times 10^{9})</td>
</tr>
</tbody>
</table>

**Table 4b. Disappearance Rate Constants of the Transient Species Formed From Cephalosporins in N₂O-Saturated Solutions**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (M)</th>
<th>(\lambda) (nm)</th>
<th>(\epsilon) (mol^{-1} L cm^{-1})</th>
<th>(2k) (mol^{-1} L s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxim</td>
<td>(1 \times 10^{-2})</td>
<td>450</td>
<td>2400</td>
<td>(2.1 \times 10^{9} \pm 0.2 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>1100</td>
<td></td>
<td>(3.4 \times 10^{9} \pm 0.3 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1100</td>
<td></td>
<td>(4.2 \times 10^{9} \pm 0.4 \times 10^{9})</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>(1 \times 10^{-3})</td>
<td>450</td>
<td>1200</td>
<td>(2.8 \times 10^{9} \pm 0.3 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>1300</td>
<td></td>
<td>(9.5 \times 10^{9} \pm 0.9 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>300</td>
<td></td>
<td>(5.5 \times 10^{9} \pm 0.6 \times 10^{9})</td>
</tr>
<tr>
<td>Ceftazidim</td>
<td>(1 \times 10^{-3})</td>
<td>385</td>
<td>2200</td>
<td>(1.6 \times 10^{9} \pm 0.2 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>1200</td>
<td></td>
<td>(2.0 \times 10^{9} \pm 0.2 \times 10^{9})</td>
</tr>
</tbody>
</table>

**Table 4c. Disappearance Rate Constants of the Transient Species Formed From Sodium Cefotaxim in 0.1 M NaN₃, Aqueous Solution N₂O Saturated and in 0.2 M KBr Solutions N₂O-Saturated**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Concentration (M)</th>
<th>(\lambda) (nm)</th>
<th>(\epsilon) (mol^{-1} L cm^{-1})</th>
<th>(2k) (mol^{-1} L s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M NaN₃ solution N₂O-saturated</td>
<td>(1 \times 10^{-2})</td>
<td>450</td>
<td>700</td>
<td>(1.1 \times 10^{9} \pm 0.1 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>(5 \times 10^{-3})</td>
<td>390</td>
<td>4300</td>
<td>(3.4 \times 10^{9} \pm 0.3 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>500</td>
<td></td>
<td>(1.6 \times 10^{9} \pm 0.2 \times 10^{9})</td>
</tr>
<tr>
<td>0.2 M KBr solution N₂O-saturated</td>
<td>(1 \times 10^{-2})</td>
<td>390</td>
<td>1800</td>
<td>(9.5 \times 10^{9} \pm 0.9 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>1100</td>
<td></td>
<td>(1.7 \times 10^{9} \pm 0.2 \times 10^{9})</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1100</td>
<td></td>
<td>(2.7 \times 10^{9} \pm 0.3 \times 10^{9})</td>
</tr>
</tbody>
</table>

**Table 5. Rate Constants of the Oxygen Reaction on the Transient Species Formed From Cephalosporins**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\lambda) (nm)</th>
<th>(k_s) (mol^{-1} L s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxim</td>
<td>450</td>
<td>(6 \times 10^{6} \pm 2 \times 10^{6})</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>500</td>
<td>(5 \times 10^{6} \pm 2 \times 10^{6})</td>
</tr>
<tr>
<td>Ceftazidim</td>
<td>385</td>
<td>(8 \times 10^{6} \pm 2 \times 10^{6})</td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>(1 \times 10^{6} \pm 2 \times 10^{6})</td>
</tr>
</tbody>
</table>
It was not possible to distinguish among these radicals experimentally because the reactions of \( \text{e}_\text{aq}^+ \) and 'OH gave the same absorption spectra. The rate constant of this reaction was quite slow \((10^9 - 10^7 \text{ mol}^{-1} \text{ L s}^{-1})\) (see Table 5). In \(10^{-3}\) M cephalosporins solutions, superoxide radicals were formed and could react on the radicals formed by the solute oxidation. The observed rate constant was faster \((10^6 \text{ mol}^{-1} \text{ L s}^{-1})\) (see Table 6).

\[
\text{RH}^- + \text{O}_2 \rightarrow \text{slow} \quad k \approx 10^6 - 10^7 \text{ mol}^{-1} \text{ L s}^{-1}
\]

\[
\text{P}^- + \text{O}_2 \rightarrow \text{slow} \quad k \approx 10^6 - 10^7 \text{ mol}^{-1} \text{ L s}^{-1}
\]

\[
\text{P}^- + \text{O}_2^- \rightarrow \text{fast} \quad k \approx 10^9 \text{ mol}^{-1} \text{ L s}^{-1}
\]

The decay of the radicals followed a second-order kinetic as expected because the concentration of the radicals and of \(\text{O}_2^-\) were equal. On the other hand, the recombination of the radicals with themselves was one order of magnitude slower than the reaction with \(\text{O}_2^-\). There was no experimental evidence that \(\text{O}_2^-\) could react with cephalosporins at least under the pulse radiolysis experimental conditions. In the same way, in pulse radiolysis, the rate of the reaction between the radicals and \(\text{O}_2^-\) was faster than the rate with \(\text{O}_2\). The reverse situation would be observed in gamma radiolysis, because the concentration of the radicals would be several orders of magnitude lower.

**CONCLUSION**

Radicals such as 'OH and \(\text{O}_2^-\), physiologically or radiotherapeutically produced, could react on administrated cephalosporins. Moreover, radiation processing like radiosterilization also led to the formation of free radicals. Pulse radiolysis combined with kinetic spectroscopy is well appropriated to gather basic informations about such radical reactions. This technique gives free radicals in homogeneous solution in a determined amount and allows the direct observation of their reaction with substrates. Quantitative data like rate constant values can be obtained.

This study reveals that in aqueous solutions, both

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**Reactions of oxygen and superoxide radicals**

The solute concentration determines the proportion of \(\text{e}_\text{aq}^+\) that reacts on the oxygen.

\[
\text{e}_\text{aq}^+ + \text{RH} \rightarrow \text{R}^- \\
\text{e}_\text{aq}^+ + \text{O}_2 \rightarrow \text{O}_2^- \\
[\text{R}^-] = k_1[\text{RH}] \\
[\text{O}_2^-] = k_2[\text{O}_2]
\]

In \(10^{-2}\) M cephalosporins solutions, it was mainly the oxygen that reacted on the transient organic radicals.
the hydroxyl radical and the hydrated electron react fast with the cephalosporins to form free radicals. The reaction of the radicals formed either by oxidation with hydroxyl radicals or by reduction by hydrated electrons with molecular oxygen is comparatively slow, but they react fast with the superoxide ion. To study the mechanism of reaction, the analysis of the stable final products from radical–radical combination reaction will be undertaken. These final products will be compared with those obtained after the irradiation of the solid cephalosporins.

REFERENCES