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Interaction of biomolecules with qinghaosu (artemisinin) and its derivatives in the presence of ferrous ion—an exploration of antimalarial mechanism*

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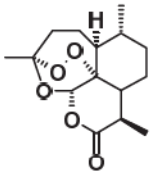
Qinghaosu (artemisinin) is the antimalarial principle of the Chinese traditional herb qinghao (*Artemisia annua* L.). Now qinghaosu and its derivatives have distinguished themselves as a new generation of antimalarial drugs, especially in the treatment of multidrug-resistant cases. Qinghaosu has a unique carbon framework containing a special endoperoxy bridge, which is entirely different from that of all previous antimalarial agents and hence makes a novel antimalarial mechanism possible. Up to now there is still no clear picture about this mechanism at the molecular lever. However, the ferric/ferrous ions that are abundant in the red cell in which the parasite lives, is likely the most important co-factor for the antimalarial process of qinghaosu (Scheme 1).



Qinghaosu (Artemisinin, 青蒿素)

Scheme 1

As a continuation of a long-term research project [1] of qinghaosu since the 1970s, we have systematically studied the free radical reaction of qinghaosu with ferrous ion. A series of qinghaosu derivatives were used as the substrate and all major products were isolated and identified. On the basis of all available data, a unified mechanism framework for the Fe(II)-induced cleavage of qinghaosu and derivatives/analogs has been postulated [2] (Scheme 2). The formation of two carbon-centered free radicals were trapped and identified by EPR [2,3], and proposed to be responsible for the antimalarial activity. Then we considered that the radicals might eventually attack the DNA of the parasite, similar to the damage of DNA with Fenton reagent [4] to some extent. There is no nuclei in the normal mature red cell, so qinghaosu is not toxic to them. And there is no hemin (or Fe^{2+}) in other cells, so qinghaosu is also safe for them. In our laboratory, it was found that in the presence of DNA this free radical process can really cleave the DNA strand [5]. For DNA pUC18, calf thymus DNA, and salmon DNA, a fragment with about 100 base pairs was detected due to this process (Fig. 1). However, for DNA isolated from *Plasmodium berghei*, the DNA spot just disappeared and the similar fragment couldn't be detected by agarose electrophoresis. It is going to be studied if the DNA cleavage in this free radical process shows

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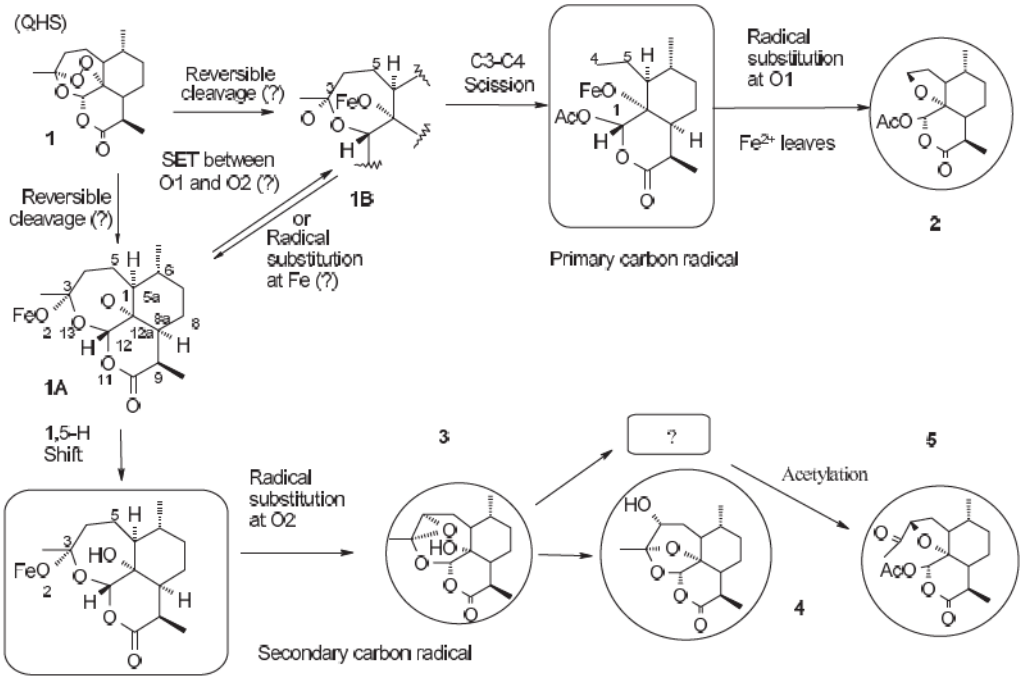


Scheme 2

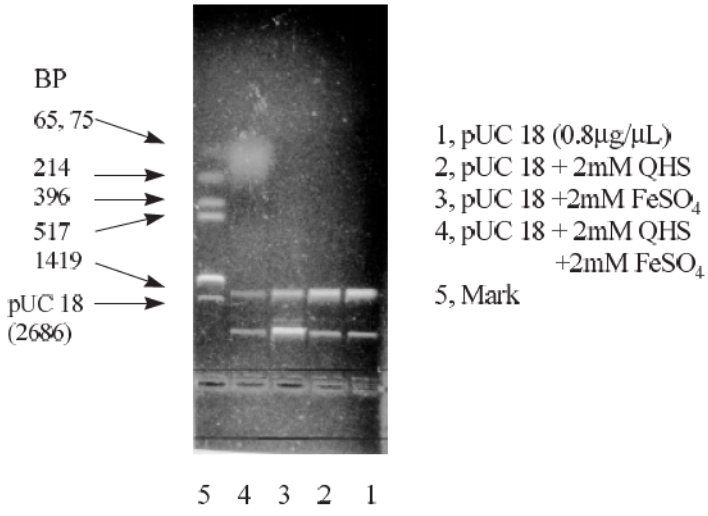
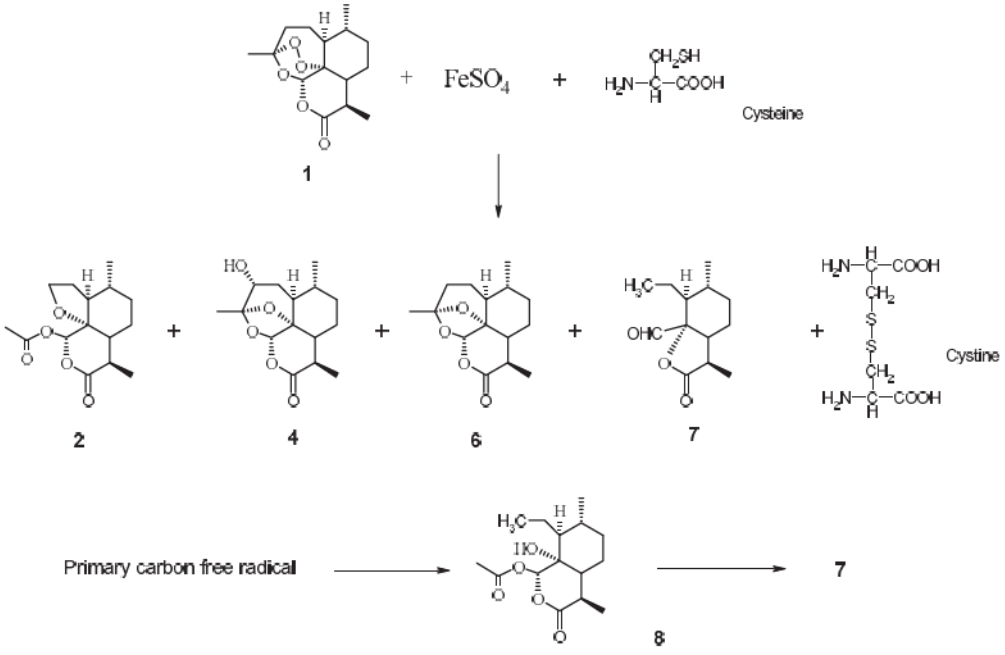


Fig. 1

the sequence selectivity. There is no reaction of the four bases (adenine, guanine, thymidine, and cytosine) and nucleosides (deoxyadenosine, deoxygunosine, thymidine, and deoxycitidine) with qinghaosu-ferrous ion system. Deoxynucleotides, except 2′-deoxygunosine-5′monophasphate (d-GMP), do not reacted with qinghaosu-ferrous ion system, either. The reaction products of d-GMP in this radical system are without UV absorption and are currently under isolation and identification.

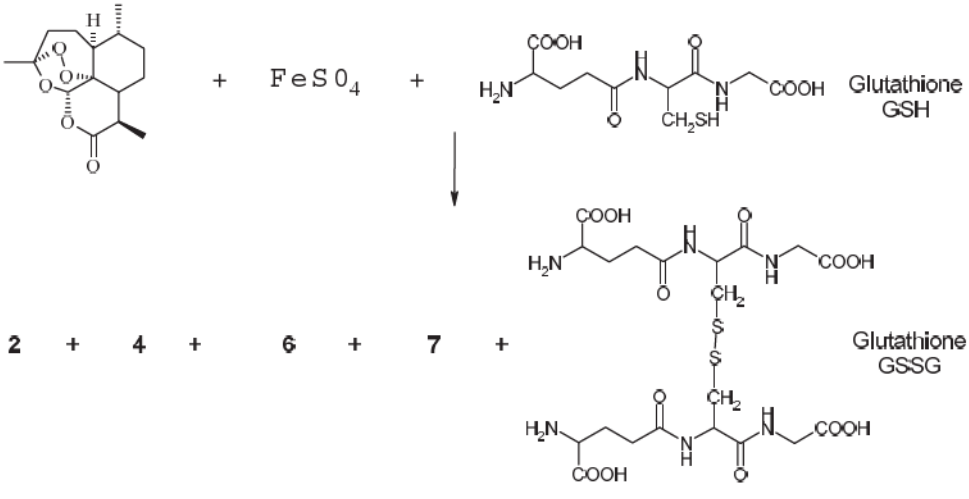
On the other hand, we also have paid the attention to the reaction of protein, peptide, and amino acid with qinghaosu–ferrous ion system. As the first step, the reaction of QHS-Fe(II) and cysteine in aqueous acetonitrile (1:1) was studied at 37 °C under nitrogen. Besides the precipitate cystine, four products could be isolated from the organic extract of the reaction mixture (Scheme 3). The tetrahydofuran and hydoxydeoxyqinghaosu products (**2** and **4**) are the normal products in the reaction of qinghaosu and ferrous ion. Deoxyqinghaosu (**6**) is obviously obtained from the secondary carbon free radical abstracting



Scheme 3

a proton from cysteine. In the same way, the primary carbon free radical abstracts a proton to form an unstable acetal acetate (**8**), which then rearranges to an aldehyde lactone (**7**). Thus, the identification of product **6** and **7** confirms once again the presence of two carbon-centered free radicals during the reaction of qinghaosu and ferrous ion. It is also worth to be mentioned that this reaction could take place even in the presence of catalytic quantity of ferrous or ferric ion as low as 100 p.p.m.

Glutathione is an important intracell agent for maintaining the normal cell cycle. The inhibition of glutathione reductase or the deficiency of glutathione reduced form (GSH) will induce the cell to a situation called ′oxidative stress', and may be useful for antimalarial or anti-cancer. [6] At the same reaction condition the reduced form of glutathione (GSH) was oxidized to the oxidized form (GSSG) with QHS-Fe(II) and four compounds **2**, **4**, **6**, **7** derived from qinghaosu were also isolated. However the reaction with qinghaosu-ferrous ion was substantially slower than that of cystein (Scheme 4).



Scheme 4

In summary, along with the reaction of qinghaosu and its derivatives/analogs with ferrous ion, DNA damage, different reactivity of nucleotide, glutathione, cysteine have been observed. These observation may be heuristic for understanding the antimalarial activity and/or other bio-activities of these compounds.

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