沒食子酸相關化合物在含氧自由基形成的氧化還原角色

The redox role of gallate-related compound in formation of oxygen radicals

中文摘要

山茶素單寧 A〈以下簡稱山茶素〉是由細葉山茶中純化而得，含有八個沒食子酸。最近的研究顯示，多酚類單寧酸和金屬離子嵌合後會產生自由基使去氧核醣核酸的斷裂。本研究，探討山茶素與鐵和銅兩種金屬離子反應產生自由基的能力，以及觀察超螺旋質體 pBR322〈以下簡稱 SC〉受斷裂的型態分佈改變。同時我們以黃嘌呤氧化　與黃嘌呤的超氧自由基反應探討山茶素對於超氧自由基的清除能力，當中以 INT 與自由基之呈色反應，作為超氧自由基產量的指標。山茶素與金屬離子的反應結果顯示，在山茶素/金屬濃度比小於 1:1 時，無論加入銅或鐵離子，其 SC 斷裂程度與山茶素量成正比。當山茶素/鐵離子的濃度比超過 1:1 時，其斷裂程度反而受到抑制，但在與銅離子反應下，並無此抑制現象。觀察 SC 的斷裂與沒食子酸單元間的關係，結果顯示，鐵離子存在下並無反應，而銅離子存在下沒食子酸對 SC 的斷裂與濃度成正比。焦性沒食子酸與金屬離子的反應，在低濃度時，不論在銅或鐵離子的去氧核醣核酸損害均明顯增加，但當焦性沒食子酸與鐵離子在比例為 8:1 時，去氧核醣核酸達到最大傷害，因此此種去氧核醣核酸的損害，應是金屬離子和焦性沒食子酸加成自體氧化作用，或是金屬離子增加了自體氧化效率而得。我們加入雙氧水，藉由 Fenton 和 Haber-Weiss 的反應產生大量的含氧自由基，以觀察山茶素在自由基反應中伴演的角色。隨著山茶素、沒食子酸與焦性沒食子酸的濃度增加，我們發現去氧核醣核酸損害的程度也越來越小。可見山茶素有其清除自由基而保護去氧核醣核酸的能力。乙二銨四乙酸是一種金屬離子的嵌合物，在氫氧自由基生成反應中，加速氫氧自由基生成。由電子自旋共振光譜中得知，隨著山茶素的濃度增加，在鐵離子的存在下，氫氧自由基的產量都增加。相較之下，山茶素不但能與金屬結合，亦具氧化還原能力。在山茶素對超氧自由基產生的抑制上，隨著山茶素濃度的增加，INT 的呈色速率有明顯的受到抑制，此抑制的反應有兩種不同斜率的線性曲線。當山茶素濃度介於 0.04~0.16mM 之間,此抑制作用為一斜率為 53.66　IR/mM 良好線性。而當濃度大於 0.32mM 後，斜率成為 25.14　IR/mM。此結果形成了明顯的兩相性，成因應是由於山茶素同時具清除超氧自由基及抑制黃嘌呤氧化　活性所造成。

英文摘要

Gallic acid which commonly exists as the derivatives of many tannins, such as camelliin A and tannic acid, usually decarboxylate to form pyrogallol. In this thesis, we demonstrated that the redox roles of Camelliin A (CA), gallic acid and pyrogallol on formation of oxygen radicals, reactive to cause DNA damage. Metal ions in low concentration, such as ferrous (0.1mM) and cupric (0.4mM) ions, are harmless to DNA; however, usually mediates the formation of radicals by interacting with those polyhydroxyl group. Results show that CA causes DNA damage via dose-dependent manner when the ratio of CA and metals is under 1. The damage can be mostly recovered once addition of catalase indicating that hydroxyl radical was produced to break DNA strands. Therefore, the hydroxyl radical signals were trapped and measured by ESR spectroscopy. The intensities of DMPO-OH at 3430-3440 guass linearly in dose-dependence of CA. The role of CA in generation of hydroxyl radicals is probably to substitute EDTA on chelation of ferrous and cupric ions where EDTA is highly selective. When the ratio of CA and metals is above 1, DNA was gradually protected from the cleavage, indicating that excess of CA reserve the structure able to scavenge hydroxyl radicals. CA also show a dual effects on superoxide scavenger activity and xanthine oxidase inhibition. Although superoxide anion found have little effect on CA-mediated DNA breakage. CA-mediated DNA cleavage was also found in both of gallate- and pyrogallol-induced DNA cleavage, indicating that the cleavage mechanism is probably via the trihydroxyl group on the aromatic ring.