

应用电喷雾质谱快速鉴定谷胱甘肽与 茶多酚化合物EGCG的反应产物

曹冬^{1,2}, 张养军¹, 钱小红^{1,2}

(1. 北京放射医学研究所, 北京蛋白质组研究中心, 蛋白质组学国家重点实验室, 北京 102206;

2. 北京理工大学, 北京 100081)

Rapid Identification of Reaction Products of Glutathione with Tea Polyphenol (-)-Epigallocatechin-3-Gallate Using Direct Injection Electrospray Ionization Mass Spectrometry

CAO Dong^{1,2}, ZHANG Yang-jun¹, QIAN Xiao-hong^{1,2}

(1. State Key Laboratory of Proteomics, Beijing Proteome Research Center,

Beijing Institute of Radiation Medicine, Beijing 102206, China;

2. Department of Life Science and technology, University of Beijing Institute of Technology, Beijing 100081, China)

Abstract: (-)-Epigallocatechin gallate (EGCG) is a major bioactive component in leaves of green tea, and has been widely investigated by scientists for its various physiological activities. In this work it was found for the first time that EGCG can react with glutathione (GSH) to form GSH conjugates of EGCG in potassium phosphate buffer, without any peroxidase/hydrogen peroxide existence. Using direct injection electrospray ionization tandem mass spectrometry, we rapidly identified the reaction products of GSH with EGCG. The MS/MS spectra of GSH conjugate with EGCG provided enough information to confirm that GSH is covalently bound to the 2'- or 6'-position of EGCG. Finally, the reaction mechanism of EGCG with GSH in potassium phosphate buffer without any peroxidase/hydrogen peroxide existence was proposed.

Key words: (-)-Epigallocatechin gallate; glutathione; quinone; mass spectrometry

中图分类号: O 657.63 文献标识码: A 文章编号: 1004-2997 (2008) 增刊-200-02

茶多酚化合物EGCG^[1]作为茶叶中的主要成分, 已经被证明具有广泛的生理活性, 如抗氧化、抗肿瘤、抗菌等活性^[2], 然而EGCG在生物体内发挥作用的机理以及作用的靶标目前还不清楚。有文献报道EGCG在过氧化酶的催化作用下, 能与还原型谷胱甘肽(GSH)发生加成反应, 这可能是EGCG在体内发生作用的机理之一。而在本研究中发现, 在没有任何过氧化酶的催化作用下, 仅在pH 7.4 (生理pH条件)的磷酸二氢钾缓冲溶液中, EGCG仍能与GSH发生加成反应。应用Q-TOF-MS电喷雾质谱技术, EGCG与GSH的加成产物 m/z 764被成功捕获, 结果示于图1。应用串联质谱技术, 得到上述加成产物 m/z 764的二级质谱图(如图2A所示), 经分析, 可以确定GSH作为亲核试剂结合到EGCG的B环2'-或6'-的位置(如图2B所示)。结合已有的文献报道^[3], 推测EGCG与GSH发生反应的

基金项目: 国家重点基础研究发展规划项目(No. 2007CB914104, 2004CB518707, 2006AA02A308)资助

作者简介: 曹冬(1980~), 男(汉族), 河北唐山人, 博士研究生, 从事蛋白质组学新技术新方法研究。E-mail: cao.dong1980@163.com

通信作者: 钱小红(1955~), 女(汉族), 江苏人, 研究员, 博士生导师, 从事蛋白质组学研究。Email: qianxh@nic.bmi.ac.cn

机理为: 在pH 7.4的偏碱性条件下, EGCG作为多酚羟基化合物很不稳定, 易被水中溶解的氧在微量金属离子的催化条件下氧化成相应的醌式结构, 类似于 α , β -不饱和酮类化合物的结构, 容易与亲核试剂发生加成反应; 同时GSH在这中条件下能形成GS⁻阴离子, 此离子可以作为亲核试剂与EGCG的氧化醌式结构发生加成反应, 形成EGCG的B环结合一个GS⁻的加成产物。

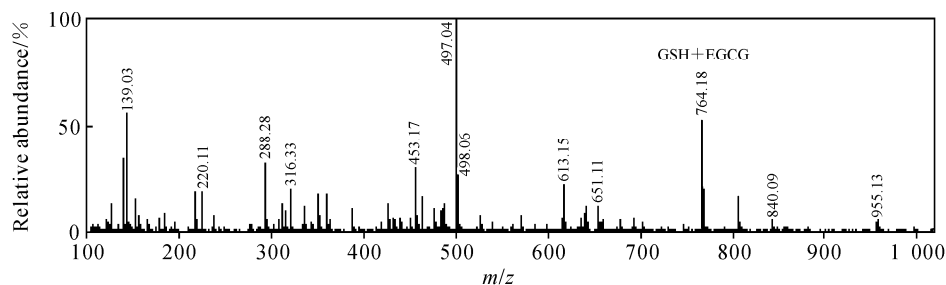


图1 EGCG与GSH反应产物的一级质谱图

Fig. 1 The MS of the reaction product of EGCG with GSH

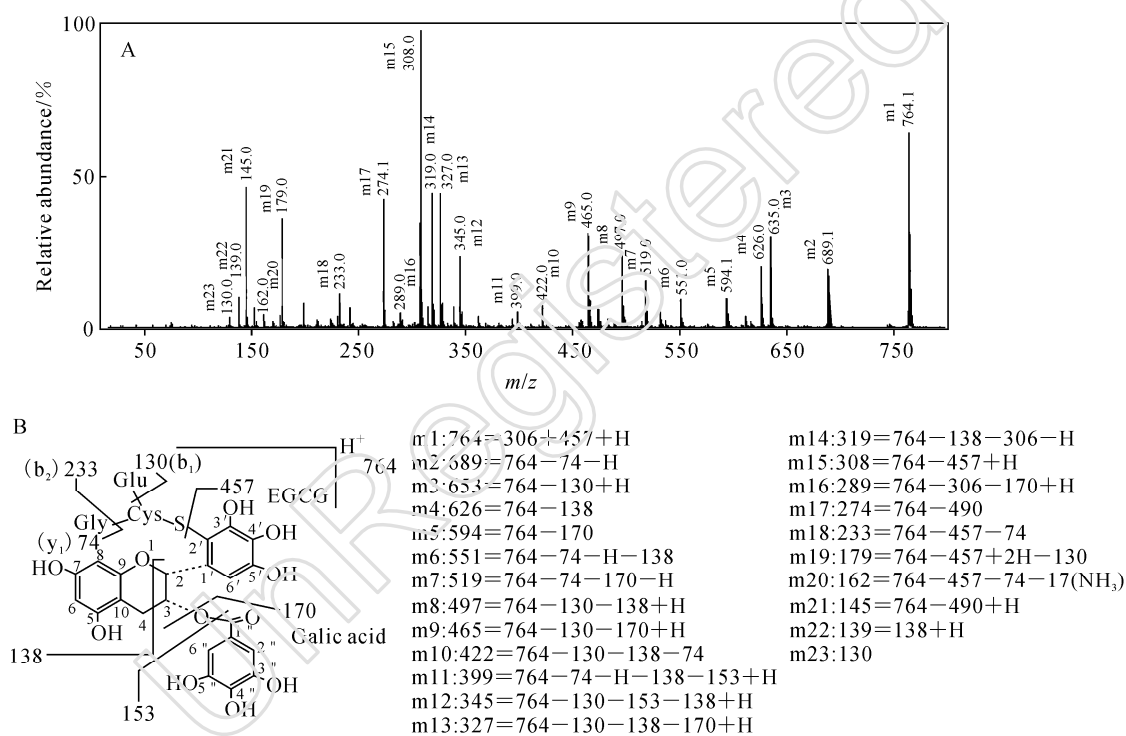


图2 EGCG与GSH反应产物的二级质谱图(A), EGCG与GSH反应产物的二级碎片离子的归属(B)

Fig.2 MS/MS spectra of conjugate of GSH and EGCG at m/z 764 (A), the contribution of Product ions of the GSH conjugates of EGCG(B)

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