学校编码：10384　　分类号_____密级_____
学号：24520091153027　　UDC _____

厦门大学

硕　士　学　位　论　文

虾青素抗氧化分子机制研究及纳米混悬剂的制备

The molecular mechanism of antioxidant effect of astaxanthin and preparation of its nanosuspensions

梁信芳

指导教师姓名：朱铉 副教授
专　业　名　称：药理学

论文提交日期：2012 年 4 月
论文答辩时间：2012 年 5 月
学位授予日期：2012 年　月

答辩委员会主席：_____
评　　阅　　人：_____

2012 年 5 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下，独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为（　　）课题（组）的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打"√"或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

摘 要

虾青素(astaxanthin，3,3'-二羟基-4,4'-二酮-β,β' 胡萝卜素) 属于叶黄素类 β-胡萝卜素。研究发现虾青素对人类健康很有益处，对许多疾病都有很好的治疗保护作用。它主要的药理作用包括：抗氧化、抗肿瘤、抗炎、对心血管保护作用及神经保护作用等。

前期研究表明，虾青素的抗溃疡作用与氧化应激密切相关，但未在分子水平进行深入研究。因此，本课题以胃癌细胞为研究对象，采用 Western blot 法检测虾青素刺激后氧化还原信号通路关键蛋白的激活情况，通过 DCFH-DA 法检测细胞内 ROS 的改变，分析他们之间的相关性。结果显示，ERK5 的激活与细胞内 ROS 的量之间有一定的相关性，我们通过 PEI 法建立 ERK5 过表达细胞，采用 MTT 法检测 ERK5 对细胞增殖的影响。结果表明，ERK5 能够促进细胞增殖。

采用 MTT 法观察细胞损伤模型的建立和虾青素的保护作用。通过显微镜下观察细胞形态，Hoechst33342 染色观察细胞核的情况，Annexin V/PI 双染并经流式细胞仪检测相结合的方法，研究证实 AST 可以有效减少 H_2O_2 诱导的细胞凋亡。最后，我们检测了细胞内 ROS 含量的变化，以及 ERK5 激活情况。结果表明，ERK5 与虾青素抗氧化作用具有相关性。

另一方面，与大多数 β 胡萝卜素一样，虾青素的生物利用度与结构有很大的关系，而水溶性差、性质不稳定都使它的应用受到限制。我们通过乳化-溶剂蒸发法制备虾青素纳米混悬剂。通过单因素分析优化了制备工艺，得到平均粒径 7.8nm，水中分散性良好的虾青素纳米混悬剂。建立了虾青素的 HPLC 分析方法对虾青素含量进行定量分析。采用透射电镜拍摄了虾青素纳米分散体的形态，初步考察了制剂于 4℃放置 3 个月的稳定性、细胞摄取和对 MGC-803 细胞中 ERK5 的激活情况。结果表明制备的虾青素纳米混悬剂有效提高了虾青素在水中的溶解度及化学稳定性。

关键词：虾青素；ERK5；抗氧化；纳米混悬剂

Abstract

Astaxanthin, 3,3'-dihydroxy-β,β'-carotene-4,4'-dione, belongs to the family of xanthophylls. Astaxanthin has considerably potential and promising applications in human health and nutrition,and has been attributed with extraordinary potential for protecting the organism against a wide range of diseases. Most significant activities of astaxanthin including its antioxidative, anticancer, antidiabetic, and anti-inflammatory properties, its protective effects on stomach, liver, the heart and the blood vessels, the nervous system, the eye, the skin, and other activities.

Above all, we noted that all of the therapeutical studies of astaxanthin on gastric ulcer, mentioning the ability of the regulation of antioxidant enzymes, but dosen't make further explanation about the molecular mechanism. Therefore, human gastric carcinoma cell line MGC-803 was choosen to study the antioxidant effect of astaxanthin. Western blot was performed to test the key proteins of some redox-signalings followed by astaxanthin treatment. Changes of intracellular ROS were detected through the DCFH-DA method, then the correlationship were analysised between them. It was found that activation of ERK5 is involved with the generation of ROS in MGC-803 cells. ERK5 overexpression cells were established by PEI assay, then MTT assay was used to exam the effect of ERK5 on cell proliferation. The results show that ERK5 can promote cell proliferation.

We hypothesized that ERK5 is involved in the protect progress of cell damage caused by H_2O_2. First of all, cell damage model and protect effect were tested by MTT assay and inverted microscope observation. We combined Hoechst33342 staining and Annexin-V/PI staining assays to investigate whether astaxanthin could protect cells from apoptosis induced by H_2O_2 . By use of fluorescent microscopy and flow cytometry respectively, we were happy to find that AST cound protect cells from oxidant damage. Finally, we tested the cells ROS level and the changes of the ERK5 activation. Results showed that ERK5 is important in the antioxidant activity of astaxanthin.

On the other hand， like other carotenoids， bioavailability of astaxanthin is also depends on its structure; insolublity in water and poor stability of astaxanthin limit its application. Astaxanthin nanosuspensions were prepared through emulsification-

solvent evaporation method. Single factor analysis was used to optimize the preparation technology, the resulting nanoparticles were well dispersible in water and with a mean particle size of 7.8nm. HPLC analysis method of astaxanthin was established, and transmission electron microscopy was used to observe the microstructure of the nanosized particles. Preliminary examed the stability of AST nanoparticles after stored at 4℃ for 3 months. Cellular uptake of AST in MGC-803 cells was tested and activation of ERK5 was also examed by western blot. All of the results show that astaxanthin nanodispersions effectively improved the water solubility of AST, and the chemical stability was very good during the time period of the experiment.

Key words: astaxanthin；ERK5；antioxidant；nanosuspensions

目 录

Table of contents