

行政院國家科學委員會專題研究計畫 成果報告

以奈米技術開發茶葉黑色素的新功能

計畫類別：個別型計畫

計畫編號：NSC94-2320-B-009-003-

執行期間：94年08月01日至95年07月31日

執行單位：國立交通大學奈米科技研究所

計畫主持人：黃國華

報告類型：精簡報告

處理方式：本計畫可公開查詢

中 華 民 國 95 年 10 月 1 日

行政院國家科學委員會補助專題研究計畫 成果報告
 期中進度報告

以奈米技術開發茶葉黑色素的新功能

Developing novel function of tea melanin using nanotechnology

計畫類別： 個別型計畫 整合型計畫

計畫編號：NSC 94-2320-B-009-003-

執行期間：94 年 8 月 1 日至 95 年 7 月 31 日

計畫主持人：黃國華

共同主持人：

計畫參與人員：

成果報告類型(依經費核定清單規定繳交)： 精簡報告 完整報告

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中 華 民 國 年 月 日

Melanin(黑色素)

Melanin belongs to a category of phenolic polymers of black pigments from plant, animal, to microbes. Melanin is commonly divided into two types: black eumelanins and reddish-brown pheomelanins. Eumelanins are composed of indolic units derived from the oxidation of tyrosine. Pheomelanins are composed of benzothiazine derivatives from the oxidation of cysteinyldopa molecules. The primary function of melanin is to protect life from direct sun radiation. Melanin is not just a color. It is a strong antioxidant with free radical scavenging activity, a strong heavy metal chelator, a strong toxic organics absorber, and is non-toxic and bio-absorbable itself; thus it serves as a natural guardian against hostile chemical and heavy-metal environments.

Recently we have disclosed a biopolymer of melanin nature in tea (Sava, Galkin et al. 2001; Sava, Yang et al. 2001). The extracted original melanin from tea represents an abundantly available resource with a rather high yield (Sava, Galkin et al. 2001; Sava, Yang et al. 2001). According to our procedure the average yield of crude product was 3.7%. Tea melanin displayed all the physical and chemical properties common to natural melanin (Sava, Galkin et al. 2001; Sava, Yang et al. 2001).

Tea melanin, unlike animal melanin, is homo-polymer with single molecular weight of 14 kD. We disclose, for the first time, the micro-scaled superstructure and nano-structure of self assembled tea melanin using scanning electron microscopy (SEM), and also by AFM. The unexpected superstructure of self-assembled melanin indicates its biological properties are yet to be explored. We also obtained crystal of tea melanin which not only indicates the possibility of obtaining detailed structure at atomic scale (Figure 2), but also indicates the possibility of exploring nano-particle of melanin. Two distinctive forms of nano-particles of melanin can be generated: self-assembled rod-like melanin and nano-crystals of melanin. It is promising, yet to be proven, that nano-particle of melanin might present novel structural and biological properties.

Publication (2006):

1. **Huang GS*** Wang MT, Weng-Hong Shaw (2006). A versatile QCM matrix for online and high throughput bio-sensing. *The Analyst* 131:382-387. (SCI, IF 2.783)
2. Yao-Ching Hung, Ann Chen, Meng-Yen Hong, and **G. Steve Huang*** (2006). Cholesterol Loading

- Augments Oxidative Stress in Macrophages. *FEBS Letters* 580:849-861. (SCI, IF 3.843)
3. Yao-Ching Hung, **G. Steven Huang***, Vasyl M. Sava, S.Y. Makan, and Meng-Yen Hong. Camellia sinensis tea melanin suppresses transformation of the aryl hydrocarbon receptor causing protection against dioxin in mice *Int. J. Food Science and Technology* (accepted, SCI, IF 0.97).
 4. Y. C. Hung, **G. S. Huang***, V. M. Sava, V.A. Blagodarsky, and M.-Y. Hong, Dual protection of tea melanin against 2,3,7,8-tetrachlorodibenzo-p-dioxin. Antioxidant activity and suppressive effect on the aryl hydrocarbon receptor. *Biol Phar Bul* (accepted, SCI, IF 1.317).
 5. **Huang GS***, Chen YS, and Lin YN, Measuring the flexibility of immunoglobulin by gold nanoparticles *Nano Letters* (accepted, SCI, IF 9.847, 1/27).
 6. Hung YC, **Huang GS***, Lin LW, Hong MY, and Se PS, Protection of tea melanin against cisplatin-induced nephropathy in mice *Food Chemistry Toxicology* (revision) (SCI, IF 2.086).
 7. Liu Y.C., **Huang G.S.**, Wu M.C., Hong M.Y., Hsiung K.P. (2006). Detection of foot and mouth disease and porcine reproductive and respiratory syndrome viral genes using microarray chip. *Vet Res Commun.*, 30, 191-204. (SCI, IF 0.269)
 8. **Huang GS*** and Hong MY (2006), Functional and Molecular Fingerprint of Damp-Obstruction in Rats. *American J. Chinese Med.* 34 (2):323-340 (SCI, IF 0.593)

In the year 2006 we have 7 papers published or accepted and 1 in revision. We have manuscript published in esteemed journals (9.847, 3.843). This has been a very productive year. Our results can be highlighted into Microarray analysis/Melanin biochemistry and Nanotechnology.

Microarray analysis/Melanin biochemistry

Cholesterol Loading Augments Oxidative Stress in Macrophages *FEBS Letters* 580:849-861.

Foam cells are hallmark of atherosclerosis (Ross 1990; Berliner, Navab et al. 1995). Oxidized LDL promotes formation of foam cells, which accelerate the progression of atherosclerotic lesions. OxLDL is known to induce gene expression of many systems, such as inflammatory response, immune response, and apoptosis. The foam cell system serves as a cellular system to investigate the molecular response of antioxidant.

To investigate the molecular consequence of loading free cholesterol into macrophages, we conducted a large-scale gene expression study to analyze acetylated-LDL-laden foam cells (AFC) and oxidized-LDL-laden foam cells (OFC) induced from human THP-1 cell lines. From a microarray containing 9600 genes, we identified 97 commonly up-regulated genes and 54 commonly down-regulated genes in AFC and OFC. Functional annotation of the differentially expressed genes indicated that several biological functions were deregulated, including oxidative stress, the steroid hormone receptor signaling pathway, extra-cellular remodeling, the pyrimidine deoxyribonucleotide metabolism, and apoptosis. We performed a cluster analysis based on the differentially expressed genes and identified 159 genes that distinguished AFC from OFC. These genes are associated with cellular functions such as hormone activity, growth control, homeostasis, and signal transduction. We also performed a cluster analysis employing the genes related to oxidative stress, but we were unable to distinguish AFC from OFC in this manner. We performed real-time RT-PCR on foam cells to examine the transcripts of interleukin 1 beta (IL1B). IL1B was rapidly induced in foam cells, but its level of the expression dropped immediately and was attenuated for AFC. We performed hydroethidine staining and observed high levels of superoxide anion in the foam cells. We conclude that loading free cholesterol induces high levels of the superoxide anion and oxidative stress and triggers a transient inflammatory response in macrophages.

Nanotechnology

Measuring the flexibility of immunoglobulin by gold nanoparticles *Nano Letters* (accepted, SCI, IF 9.847, 1/27)

Nanotechnology is obviously the technology of the next generation. Future trend for nanoelectronics is not clear at the present time, but the application of nanomaterial and nanobiotechnology is comprehended. We have applied gold nanoparticles to measure the flexibility of immunoglobulin. This paper presented for the first time a simple but delicate platform to study the most fundamental interaction in Immunology-antigen recognition. The hypothesis underlined this work is novel and the conclusion is solid, thus was accepted and will be published in October issue.