

# SDS-PAGE 和 HPLC-荧光检测分离鉴定 恩施高硒区大豆中含硒蛋白\*

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**摘要** 采用凝胶电泳技术分离恩施高硒地区大豆中的含硒蛋白组分, 分辨出 27 条蛋白条带。用高效液相色谱-荧光测定法对这些条带分别进行硒含量测定并进行空白扣除, 发现其中有 13 条带含硒。用标准分子量蛋白标定含硒蛋白组分分子量分别为: 58.1—60.3; 52.5—53.7; 46.8—50.1; 29.5—30.9; 28.8; 25.1—25.7; 24.3; 19.7—20.9; 18.4—18.6; 16.8—17.9; 16.1—16.2; 15.2—15.8 和 14.3—14.8 kDa。

**关键词** 凝胶电泳, 高效液相色谱, 荧光检测, 含硒蛋白。

研究硒的形态分布可以了解不同硒含量生态环境中硒中毒和硒缺乏所导致的健康问题的机理。植物性食物处在食物链的中间环节, 其硒形态分布的工作已相继有所报道<sup>[1-3]</sup>, 其中大量的报道研究了硒代氨基酸的形式, 关于硒结合蛋白的形式的报道并不多见。Mason<sup>[4]</sup>、Sathe<sup>[5]</sup>以大豆为材料作了一些工作, 结果表明大豆中硒的优势形态均为蛋白结合态。因此, 对其所含硒的形态研究应重点放在不同含硒蛋白的进一步分离鉴定上。

要对不同来源蛋白中不同含硒组分进一步分离鉴定, SDS-PAGE 是最有效的分离技术。本工作结合高效液相色谱-荧光检测测定方法和 SDS-PAGE 分离技术, 用于研究恩施高硒地区含硒蛋白组分, 得到了满意的结果。

## 1 实验方法

恩施地区大豆凉干后磨粉, 过 100 目筛得大豆粉(总硒含量为 5.036 mg/kg)。称取 0.3 g 大豆粉, 加入 3 ml 缓冲液(0.0625 mol/L, Tris/HCl, pH = 8.6, 4% SDS, 5%  $\alpha$ -巯基乙醇, 10% 蔗糖, 0.002% 溴酚兰)匀浆。匀浆后在 5000 r/min 离心 30 min 后取上清液备用, 加样前煮沸 3 min 制成样品液。

SDS-PAGE 分离方法参照 Laemmli 配方<sup>[7]</sup>。样品胶采用 12% 浓度, 浓缩胶采用 5% 浓度。在 200 × 150 × 3 胶室内制成带 12 个样品槽的凝胶, 其中 1—5 槽作为空白, 6 槽加入标准蛋白混

合溶液(Pharmacia Fine Chemical 公司产品)10  $\mu$ l, 7—12 槽各加 10  $\mu$ l 样品液。电泳装置采用北京六一仪器厂的 DYY II 型稳压稳流电泳仪, 在 20 mA 稳流下进行电泳。电泳结束后用 0.1% 考马斯亮兰+7% 冰醋酸+30% 甲醇的溶液中染色, 在 7% 冰醋酸中脱色。

蛋白条带中硒的测定方法参照文献<sup>\*\*</sup>, 将脱色后的 8—12 槽蛋白条带一切下, 同时切下 1—5 槽中的相应位置的条带作为实验空白。切割的条带分别移入  $\varnothing 20 \times 200$  mm 消化管, 加入硝酸:高氯酸混合液 2 ml (5:2, V/V), 放入加热器中, 先在 150 °C 下低温消化, 再在 300 °C 下高温消化至冒白烟。10 min 后取下, 加 1 ml 2.5% 盐酸羟胺溶液, 100 °C 保持 2 min 后加 2 ml 6 mol/L 盐酸, 100 °C 下还原 5 min, 冷却后用浓氨水调 pH 值到 1—2 之间, 加 0.9% EDTA 二钠溶液 0.5 ml, 0.01% DAN(2,3-二氨基萘)溶液 1 ml, 50 °C 恒温 30 min 后加 0.5 ml 环己烷振荡萃取 5 min, 取环己烷相用于测定。测定在 Waters Model 510 型 HPLC 配置 Shimadzu RF-535 Fluorescence Monitor 上进行, 进样量 100  $\mu$ l。

## 2 结论与讨论

凝胶过滤色谱、离子交换色谱等能较粗地分

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\*\* 王子健, 博士论文, 中国科学院生态环境研究中心, 1991。

离硒形态组分,一般能得到几个包含相近分子量或相近性质的含硒组分的峰<sup>[9]</sup>,但这些方法仅能粗略观察硒分布的大致趋势,能够区分的组分较少,难于给出分子量的精确估算。例如,可能有些无机硒或低分子量硒与蛋白松散连结而不被洗脱,造成此蛋白被误认为含硒蛋白。SDS-PAGE方法分离要精细得多,能给出较精确的分子量,不存在无机硒或低分子量硒与蛋白松散结合的问题,但 SDS 是很强的二硫键破坏剂,所以蛋白高分子电泳后得到的往往是其亚结构单位。Behne<sup>[6]</sup>用此方法从大鼠组织中分离得到的 13 种蛋白就包含有含硒的蛋白亚单位,一些分子量体现的也只是蛋白亚单位的信息。Sathe<sup>[4]</sup>等把此方法用于大豆蛋白的分离,同时结合凝胶过滤色谱、离子交换色谱把大豆蛋白分离为 11S、7S、乳清蛋白等后再进行电泳,得到了精细的蛋白条带谱,并给出了条带在 11S、7S、乳清蛋白等各类组分上的归类信息,表明 7S 包含的多是分子量较高的条带,11S 包含的多是分子量较低的条带,清蛋白包含的是分子量更低的条带。图 1 下部是本次实验得到的典型 SDS-PAGE 条带谱,底部为标准分子量蛋白条带(6 槽),分别为 67.0、43.0、25.0 和 16.7kDa。其余 7—11 槽为同一大豆样品的蛋白或多肽条带。电泳后,可以区分出 27 种不同分子量的蛋白或多肽。从上往下依次编号为 A—Z、AA。将本实验结果与已述工作比较发现,所得谱带与 Sathe 得到的条带谱很相似。

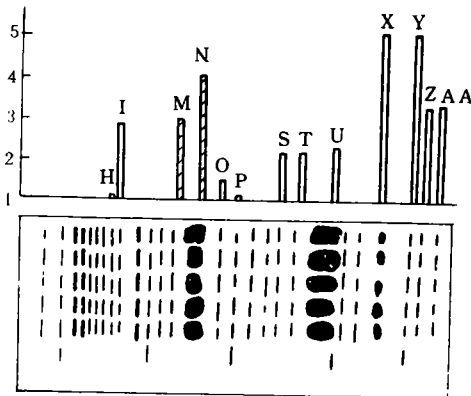


图 1 SDS-PAGE 条带谱(下部)及含硒条带(上部)

检测上述蛋白条带中的可能硒含量是一个

难点。从所报道的工作来看,大都采用了同位素标记,但<sup>75</sup>Se 标记方法所得到的结果并不一定与自然状态硒的形态分布真实情况相吻合,例如 Whanger<sup>\*\*\*</sup>最近的工作表明硒形态分布很大程度上取决于摄入硒的形态。本实验室采用 HPLC-荧光检测方法后,上述蛋白组分的含硒量测定结果如图上部所示。图 1 中的纵坐标为相对硒含量,横坐标为蛋白组分电泳过程中的移动距离。图 1 中的含硒蛋白组分以其荧光强度(代表硒含量)2 倍于对应空白确定。在此种条件下,可以认为所分离的 27 种大豆蛋白或多肽中,有 13 种为含硒蛋白组分,根据第 6 槽标准分子量蛋白所确定的分子量依次为: H58.3—60.3、52.5—53.7、M46.8—50.1、N29.5—30.9、O28.8、P25.1—25.7、S24.3、T19.7—20.9、U18.4—18.6、X16.8—17.9、Y16.1—16.2、Z15.2—15.8 和 AA14.3—14.8 kDa(英文字母为条带编号)。此结果和 Sathe<sup>[4]</sup>实验结果比较可看出: H、I 属于 Sathe 工作报道中的 7S 组分, M、N 属于 11S 组分中酸性组分, U 属于 11S 组分中的碱性组分, 其它属于 11S 或清蛋白组分。

### 3 小结

用 SDS-PAGE 及 HPLC-荧光检测技术研究恩施高硒地区中硒在蛋白组分中的形态分布,在分离得到的 27 条蛋白条带中检出了 13 条含硒条带,并初步估算了它们的分子量。

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waste gas, energy recovery could be made while the goal of pollution control being achieved.

**Key words:** catalyst, noble metal, organic waste gases.

**Comparison in Vehicular Exhaust Emissions between Hong Kong and Guangzhou Cities.** L. Y. Chen (Ph. D) and W. T. Hung (Civil and Structural Eng. Dept. Hong Kong Polytechnic, Hunghom, Hong Kong), Y. Qin (Institute of Environ. Sci., Zhongshan University, Guangzhou 510275); *Chin. J. Environ. Sci.*, **15**(5), 1994, pp. 56—60

The monitoring results of vehicular exhaust emissions in the urban areas of Hong Kong and Guangzhou were discussed. The monitoring exercise was carried out by using automatic gaseous analyser mounted on road side in Hong Kong for four years and for nine days in Guangzhou. The results show that the pollution level at Castle Peak Road of Hong Kong was comparatively lower than that in Guangzhou. The pollution level in Hong Kong satisfied the national air quality standard (Class 2) of China in both years 1988 and 1989. The pollutant concentration along Jiefang Middle Road was very high and exceeded the national air quality standard (Class 3). The traffic flow speed and composition were discovered to be the major reasons for the difference in pollutant concentrations in both cities.

**Key words:** vehicular exhaust emission, combined emission factor, traffic flow speed and composition.

**SDS-PAGE Separation and HPLC-FID Identification of Selenoproteins in Soybean from Enshi Area Having a Higher Level of Selenium in Soil.** Xie Shenmeng, Wang Zijian and Peng An (Research Center for Eco- Environmental Sciences, Chinese Academy of Sciences, Beijing 100085); *Chin. J. Environ. Sci.*, **15**(5), 1994, pp. 61—62

Selenoproteins in soybean from Enshi area where selenium in soil was at a higher level were separated by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then identified by using high performance liquid chromatography with a fluorescent indication detector (HPLC-FID). Of 27 protein or protein subunit bands identified, 13 were found to be selenium species. According to a standard protein kit, their molecular weights were estimated at 58.3—60.3, 52.5—53.7, 46.8—50.1, 29.5—30.9, 28.8, 25.1—25.7, 24.3, 19.7—20.9, 18.4—18.6, 16.8—17.9, 16.1—16.2, 15.2—15.8 and 14.3—14.8 KDa, respectively.

**Key words:** SDS-PAGE, HPLC-FID, selenoprotein, speciation.

**Acute Toxicity of Organotin Compounds to Benthos.** Chen Tian'yi et al. (Dept. of Environ. Sci., Nankai University, Tianjin 300071); *Chin. J. Environ. Sci.*, **15**(5), 1994, pp. 63—64

The acute toxicities of three organotin compounds, i. e., monobutyltin (MBT), dibutyltin (DBT) and

tributyltin (TBT), to benthos (Chironomid larvae and tubificids) were reported. The results show that (1) the toxic effects of these compounds in a decreasing order were TBT > DBT > MBT; (2) the median lethal concentration (24h LC<sub>50</sub>) of TBT to 4 species of benthos was 26.85 ppb for chironomid larvae (*Chironomus plumosus*), 241.55 ppb for chironomid larvae (*Chaetocladius sexpilosus*), 145.55 ppb for tubificids (*Branchiura sowerbyi*), and 355.63 ppb for tubificids (*Limnodrilus hoffmesteri*); (3) there was a strong negative correlation between the concentration of TBT and the median lethal time (LT<sub>50</sub>) for *B. sowerbyi*.

**Key words:** organotin compounds, benthos, acute toxicity.

**Effects of CO<sub>2</sub> on the Grain Compositions of Winter Wheat and Soybean.** Gao Suhua and Wang Chun'yi (Chinese Academy of Meteorological Sciences, Beijing 100081); *Chin. J. Environ. Sci.*, **15**(5), 1994, pp. 65—66

Winter wheat and soybean crops were treated with different CO<sub>2</sub> concentrations in top-open chambers. The matured grains harvested from the crops were analysed for their compositions by using visible ultraviolet spectrometer, protein analyzer, gas chromatograph, YG-2 fat extractor and automanual nitrometer. The results show that an increased CO<sub>2</sub> concentration can have a positive effect on the contents of both rough protein and rough fat in soybean grain; and as the CO<sub>2</sub> concentration increases, the soybean grain would have an increased level of unsaturated acids and a decreased level of saturated acids. The change in CO<sub>2</sub> concentration had a more complicated effect on the levels of rough protein and lysine in winter wheat grain. In terms of both indicators of rough protein and lysine, a doubled concentration of atmospheric CO<sub>2</sub> had a negative effect on the quality of grains of the present varieties of winter wheat.

**Key words:** top-open chamber, CO<sub>2</sub> concentration, grain composition, winter wheat, soybean.

**Study on the Fluorometric Determination of Beryllium Using Morin.** Zhao Zhenhua et al. (Beijing Municipal Research Institute of Environmental Protection, Beijing 100037); *Chin. J. Environ. Sci.*, **15**(5), 1994, pp. 67—70

The fluorometric spectra of beryllium-morin complex were characterized and the graphs of excitation spectrum, emission spectrum and synchronous fluorescence spectrum were given. The synchronous spectrum of beryllium-morin in an alkaline solution had an optimum specific λ of 100nm. A synchronous scanning spectrofluorometry was used to determine beryllium at a level of submicrogram, with a detectable limit of 5 ng/ml Be<sup>2+</sup>. The results obtained with this method were comparable with those obtained with an atomic absorption spectrophotometric method for air samples and water samples.