研究简报

牛肝 tRNA^{Ile}的序列分析和二级结构

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摘要 用随机降解法和Donis-Keller酶分析法，测定了牛肝tRNA^{Ile}序列. 牛肝tRNA^{Ile}长77个碱基；G5·G69不配对为其显著特征. 依据tRNA螺旋区和环区自由能大小及Holley模型，确定了tRNA^{Ile}的二级结构.

关键词 tRNA^{Ile}，序列分析，二级结构

由于精胺对哺乳动物异亮氨酸tRNA (tRNA^{Ile}) 氨基酰化反应的特异刺激作用[1,2]，我们测定了牛肝tRNA^{Ile}的序列，以探讨哺乳动物tRNA^{Ile}序列和二级结构的特点. 1981年，Shinriki等测定了小鼠L_{1210}细胞tRNA^{Ile}序列[3]. 1990年，本文作者发表了大鼠肝tRNA^{Ile}序列及二级结构[2]. 对tRNA序列的分析方法，主要有化学降解法和限制性碱基特异酶降解法. 由于tRNA中含有大量稀有碱基，它们呈现出对化学法及酶法降解具有不同的抗性，致使对tRNA序列的分析较对其它RNA及DNA的序列分析困难得多.

1 材料和方法

牛肝tRNA^{Ile}的分离和纯化按我们以前的报道方法[2]，从新鲜牛肝中制备.

tRNA^{Ile}序列的测定方法 a. 随机降解法：取1.0 μg纯化的tRNA^{Ile} (10μl)，在80℃水浴6min，tRNA被降解为含5′-OH的核苷酸或寡核苷酸片段. 用[γ-^{32}P]ATP (1.11×10^5—2.22×10^{5}GBq，370MBq/ml，NEN) 和T4多核苷酸激酶 (Takara) 标记这些片段的5′末端[4]. 然后以12%聚丙烯酰胺-7mol/L尿素凝胶电泳分离，再从凝胶中抽提出每条带 (77条)，并用多核苷酸酶T2 (Sigma) 降解为5′-^{32}P核苷二磷酸，最后经PEI-纤维素板 (Merck) 薄层层析分析. b. 通过Donis-Keller酶法分析[5]，将tRNA 3′末端用[5′-^{32}P] pCp (NEN) 和T4 RNA连接酶 (Takara) 标记[6]，然后用RNase T1，RNase U2，RNase PhyM和RNase BC (均为Sigma产品) 进行特异性降解. c. tRNA^{Ile}的核苷酸组成分析[7]. 1.0 A_{260}单位的tRNA^{Ile}用RNase混合液 (50mmol/L Tris-HCl pH8.0，20μg RNase A，4μg磷酸二酯酶，0.32μg磷酸单酯酶)，在37℃水解过夜，然后用高效液相色谱法分析. 上述的随机降解法和Donis-Keller酶法可分别直接从PEI薄层层析板上和电泳凝胶上读出序列，高效液相色谱法仅用于确定tRNA^{Ile}中的核苷酸组成和数目. 三种方法并用可互相验证，以达到结果的准确性.

2 结果和讨论

测定结果表明，牛肝tRNA^{Ile}含77个碱基，其中含10种15个稀有碱基. 与大鼠和小鼠肝tRNA^{Ile}有96.1% (74/77) 的同源性 (大鼠和小

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鼠肝 $tRNA^{Ile}$ 序列相同)，与 *E. Coli* $tRNA^{Ile}$ 仅有 61.0%同源性. 哺乳动物 $tRNA^{Ile}$ 含有较多且集中的 G-C 对. 氨基酸臂 G5 · G69 不配对为哺乳动物 $tRNA^{Ile}$ 的显著特征. 根据 tRNA 螺旋区和环区自由能的大小及 tRNA 分子二级结构(三叶草结构)的普遍模型[8]，牛肝 $tRNA^{Ile}$ 的序列及二级结构见图 1.

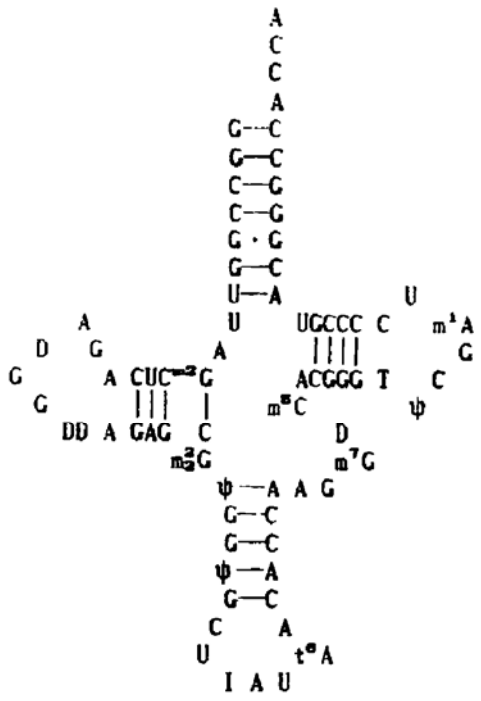

图 1　牛肝 $tRNA^{Ile}$ 的序列及二级结构

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离体缺血再灌注鼠心肌钙离子的变化*

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摘要　用液体闪烁计数法测定离体再灌注鼠心肌肌质网 (SR) 和线粒体 (Mit) 内 $^{45}Ca^{2+}$ 放射性强度 (cpm)，比较能量制剂 ATP-$MgCl_2$，活性氧自由基清除剂 SOD 和钙阻滞剂 Verapamil 对离体缺血再灌注鼠心肌细胞 SR 和 Mit 钙离子浓度的影响. 结果表明，SR 内 ATP-$MgCl_2$，SOD 和 Verapamil 组 $^{45}Ca^{2+}$ 的 cpm 均高于对照组 ($P<0.01$ 或 $P<0.05$)，而 Mit 内均低于对照组 ($P<0.01$). 此三种药均能提高离体大鼠心肌细胞内 SR $^{45}Ca^{2+}$ 和降低 Mit $^{45}Ca^{2+}$ 积聚，从而保护了心肌细胞，防止缺血再灌注损伤.

关键词　缺血-再灌注，心肌肌质网，线粒体，钙离子

近年来的研究结果[1]表明，细胞内钙离子的积聚及氧自由基的产生是心肌缺血再灌注损伤的重要因素. 肌质网 (sarcoplasmic reticulum vesicle，SR) 是心肌细胞储存和释放钙的重要部位，SR 摄钙可因其膜上 Ca^{2+}-ATPase 受抑制而减弱，导致细胞内钙过荷，且缺血再灌注的心肌细胞常伴有线粒体 (mitochondria，Mit)

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on Secondary Structure of Proteins. Lin Bohai. (*Institute of Biophysics, Academia Sinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China). 1994: **21** (1): 67

Vacuum ultraviolet circular dichroism (VUV-CD) spectra of proteins in solution have been measured using synchrotron radiation vacuum ultraviolet circular dichroism spectrometer and a special cell. The measurement wavelength is down to 175nm. A new calculation method has been applied for calculating the content of five kinds of secondary structures of proteins. Their results are coincident with that from X-ray diffraction method. In order to get good VUV-CD spectra, several important factors have been discussed. The experiments show that so far, VUV-CD analysis is one of the favorable method for secondary structure studies of proteins.

Key words vacuum ultraviolet circular dichroism, synchrotron radiation, secondary structure of proteins

The First Sixteen N-terminal Amino Acids of Firefly Luciferase Involve in Catalytic Activity. Lu Jianrong, Yang Jian, Jin Zhenhua. (*Institute of Developmental Biology, Academia Sinica, Beijing* 100080). *Prog. Biochem. Biophys.* (China). 1994: **21** (1): 70

The full-length intronless firefly luciferase gene and its deletion mutant which lacked 48 nucleotides from the initiation codon (ATG) were inserted into the high expression secretion vector, pIN-Ⅲ-ompA3, and introduced into *E. coli* cells in which high level and no luciferase activity were detected, respectively. This result shows that the first sixteen N-terminal amino acids of firefly luciferase involve in catalytic activity.

Key words firefly luciferase, enzymatic activity

Studies of the Immobilized Extracellular Catechol 1, 2-Dioxygenase. Li Li, Li Qin. (*Institute of Microbiology, Academia Sinica, Beijing* 100080). *Prog. Biochem. Biophys.* (China). 1994; **21** (1): 71

The extracellular catechol 1, 2-dioxygenase was immobilized. The apparent activity of the immobilized extracellular catechol 1, 2-dioxygenase was high, and range of use was extended. The pH-activity profile was altered by immobilization, and optimal pH from 6.0—9.0 was observed. Immobilization was shown to increase the thermal stability of the enzyme. The pureness and concentration of biotransformation of catechol to *cis, cis*-muconic acid were high. The product was easily separated from enzyme. The immobilized method of extracellular catechol 1, 2-dioxygenase was novel and simple. The results presented show that the immobilization of extracellular catechol 1, 2-dioxygenase offers an attractive means for the production of *cis, cis*-muconic acid.

Key words extracellular catechol 1, 2-dioxygenase, immobilized enzyme

The Nucleotide Sequence and Cloverleaf Structure of Bovine Liver $tRNA^{Ile}$. Peng Zhaohui, K. IGARASHI, K. KUSAMA-EGUCHI. (*The First Military Medical University, Guangzhou* 510515). *Prog. Biochem. Biophys.* (China). 1994: **21** (1): 72

The nucleotide sequence of bovine liver $tRNA^{Ile}$ was determined by both the partial hydrolyzation in water and the Donis-keller method. The $tRNA^{Ile}$ was consisted of 77 nucleotides and relatively rich in GC base pairs. The acceptor stem of the $tRNA^{Ile}$ was characteristic of G5 • G69 mismatch. Forthermore, the clover-

leaf structure of $tRNA^{Ile}$ was showed according to the Holley mode as well as the amount of free energy in the each of stems versus loops.

Key words bovine liver tRNA, nucleotide sequence, cloverleaf structrue

The Change of Ca^{2+} in SR and Mit of Isolated Ischemia-Reperfusion Rat Heart. Che Chengri, Li Xiangshan, Jin Jihuan, Wang Mingyong, Li Suxiang, Li Dan. (*Department of Thoracic and Cardiovascular Surgery, Affiliated Hospital, Yian Bian Medical College, Yanji* 133000). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 73

The strength of radiation of $^{45}Ca^{2+}$ in sarcoplasmic reticulum vesicle (SR) and mitochondria (Mit) was measured by liguid scintillation counting method. Comparing the effects of ATP - $MgCl_2$, SOD and verapamil on the $^{45}Ca^{2+}$ concentration in SR and Mit which prepared from isolated ischemia reperfused rat hearts. The result showed that the $^{45}Ca^{2+}$ cpm of SR of the three groups were higher than that of control, while the $^{45}Ca^{2+}$ cpm of Mit of the three groups were lower than that of control. These results suggested that the three kinds of reagent could protect reperfusion injury of heart cell through enhance $^{45}Ca^{2+}$ storage in SR and inhibitting the $^{45}Ca^{2+}$ accumulation in Mit.

Key words ischemia-reperfusion, sarcoplasmic reticulum vesicle, mitochondria, calcium

Novel Properties of Bilayer Membrane Formed by Diazafluorenone Schiff Base Amphiphiles. Tai Zihou, Qian Xiangping, Zou Juan, Zhang Gencheng. (*State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing* 210008). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 76

A new kind of diazafluorenone Schiff base amphiphiles were synthesized. Bilayer membrane formed with these compounds possess good stability and oscillation properties. When 0.1 mol/L $AgNO_3$ presented in bathing solution and an electrical field was applied on this system, a maximum value of the current, 4.0 μA, was obtained. A possible application in the development is indicated.

Key words diazafluorenone, Schiff base, amphiphile, bimolecular membrane

The Purification and Characterization of Bacteriophage T7 Lysozyme of Recombinant Strains. Hua Ling, Li Dianjun, Xu Yongrui, Niu Zeling, Cui Daoshan. (*Institute of Biophysics, Academia Sinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 79

The culture solution of recombinant strains of lysozyme was treated by ultrasonic wave, purified by DE52 chromatography and CM52 chromatography, a polyacrylamide gel eletrophoresis pure T7 lysozyme of Mr 17000 was obtained, the optimal react pH is 8.0, 21% of total enzyme activity lost at 37°C in 5 min.

Key words T7 lysozyme, purification, characterization

Measurement of Creatine Kinase MM Isoforms by Chromatofocusing. Kong Qingyin, Yang Zhenhua, Yang Shude, Tang Zhiyi. (*Dept. of Laboratory Medicine, Beijing Hospital, Beijing* 100730). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 83

A rapid and sensitive method of isoforms of CK (EC 2.7.3.2) -MM in human serum by chromatofocusing was reported. The assay system involved Mono P (HR5/20) column, fast protein liquid chromatography (FPLC)