技术与方法

蛋白质二级结构的真空紫外圆二色性研究

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摘要　利用同步辐射真空紫外圆二色谱仪和特制的样品池，测定溶液中蛋白质的真空紫外圆二色谱，测定波长低至175nm，并应用一种新的计算法分析计算了蛋白质5种二级结构的含量，所得结果与用X射线衍射法测定的结果一致．讨论了获得好的真空紫外圆二色谱的几个重要因素．结果表明，真空紫外圆二色法是目前测定溶液中蛋白质二级结构的较好方法之一．

关键词　真空紫外圆二色谱，同步辐射，蛋白质二级结构

用真空紫外圆二色（VUV-CD）谱研究蛋白质的二级结构比通常的圆二色（CD）谱更灵敏和可靠，但由于仪器和技术上的困难，以往人们在这一领域的工作做得不多．因为用VUV-CD法研究蛋白质的二级结构，测定波长应扩展到178nm，但目前商品圆二色谱仪都测不到这一波长[1]．通常的圆二色谱仪用氙灯做光源，氙灯200nm以下波段的光很弱，噪声大，要测准200nm以下波段的圆二色谱是很困难的．

同步辐射光源提供了很强的紫外连续光谱，用同步辐射光源代替氙灯做圆二色谱仪的光源，使得真空紫外圆二色谱的测定既灵敏又准确．本文应用同步辐射真空紫外圆二色谱仪，测定蛋白质的VUV-CD谱，并应用了新的计算法计算了蛋白质5种二级结构[2—4]（α-螺旋，平行β-折迭，反向平行β-折迭，β转角和无规卷曲）的含量，得到了比较满意的结果．

1　仪器和方法

蛋白质的VUV-CD谱用美国Brookhaven国家实验室同步辐射光源（NSLS）的真空紫外圆二色谱仪测定[5,6]．测定的波长范围由260nm至175nm．由于大气中的氧气在200nm以下对光线有很强的吸收，所以实验应在真空中进行，或整个仪器光路都充以氮气．另外，在190nm以下溶剂对光线的吸收很大，为了得到较高的信噪比，以获得好的圆二色谱，应采用尽量短的光程，使溶剂对光线的吸收尽可能少．为此，我们改进了样品池的设计[7]，图1为特制样品池的结构图，该样品池便于注入样品，便于拆开清洗及更换样品，其密封性能也有改善．样品池的光程由垫圈厚度决定，可在1—300μm之间选择．

测定VUV-CD谱时，样品的浓度和样品池的光程长度应适当选择．实验表明，样品的光密度在0.8—0.9时信噪比最好，所获得的圆二色谱也最好．样品光密度的可用范围通常为0.4—1，如果不在此范围，应重新配制样品或选用其它合适的光程长度．在往特制样品池注入样品时应小心，避免在样品溶液中产生气泡，否则在圆二色谱测定中会引起较大误差．

选用细胞色素c和胰凝乳蛋白酶为样品，其浓度均为5mg/ml，溶剂为0.025mol/L HBS（Hepes-buffered saline），pH7，美国Sigma公司产品，之所以选用这两种蛋白质，主要是为了便于进行对照研究，因为曾有人用CD法和

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X 射线法对其进行过测定[8].

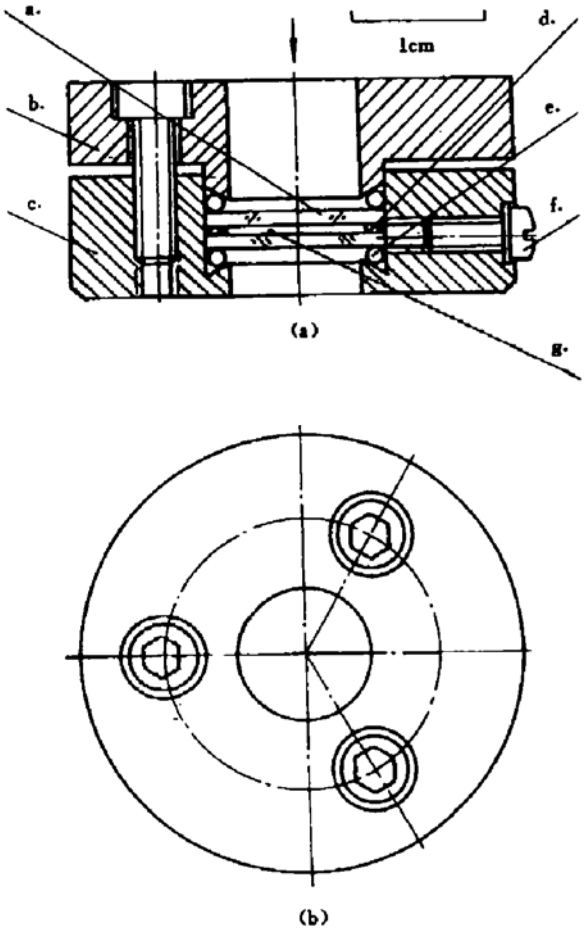

图 1 特制样品池的结构图

(a) 主视图 (b) 俯视图

a. 为石英窗（为了测定更短波长的 VUV-CD 谱，可改用氟化钙窗），*b*. 为上盖，*c*. 为样品池座，*d*. 为垫圈，*e*. 为密封圈，*f*. 为密封螺丝，*g*. 为样品.

2 结果和讨论

用真空紫外圆二色谱仪测定细胞色素 c 和胰凝乳蛋白酶的 VUV-CD 谱，其 VUV-CD 谱如图 2 所示. 根据所测定的 VUV-CD 谱，按照 Compton[2]计算法，分别算出这两种蛋白质的 5 种二级结构的含量. 表 1 为 CD 法计算结果与 X 射线衍射法计算结果之比较. 由表 1 可见，两种方法计算的结果相近. VUV-CD 法分别测定到 178nm 和 184nm.

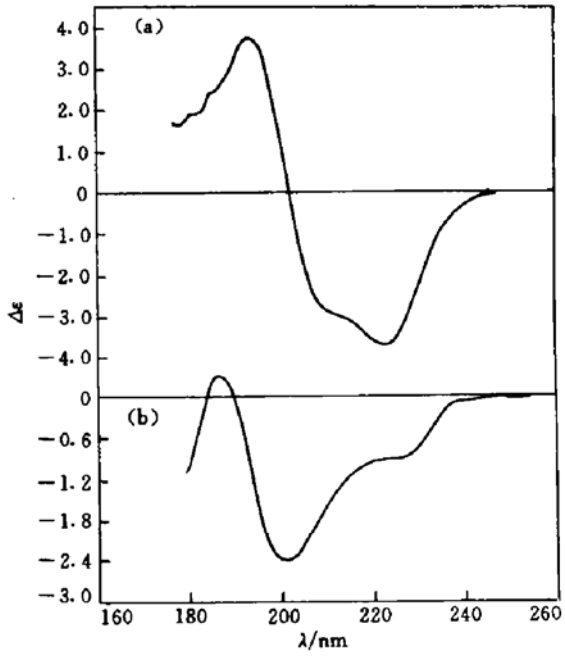

图 2 两种蛋白质的同步辐射真空紫外圆二色谱

(a) 为细胞色素 c；(b) 为胰凝乳蛋白酶.

表 1 用 VUV-CD 法与用 X 射线衍射法计算两种蛋白质二级结构结果之比较

（含量/%）

	α-螺旋	反向平行 β-折迭	平行 β-折迭	β-转角	无规卷曲
细胞色素 c					
测定到 178nm	41	1.4	2	15	40
测定到 184nm	48	2.8	2	15	31
X 射线法计算结果	38	0	0	17	45
胰凝乳蛋白酶					
测定到 178nm	13	25	0	23	38
测定到 184nm	13	21	1	24	41
X 射线法计算结果	10	34	0	20	36

通过 X 射线衍射技术可以得到蛋白质二级结构的比较全面和准确的信息，但它需要合格的晶体，分析周期长，工作量大，并且由于样品是在结晶状态下测定，不易说明大分子在生理状态下结构与功能的关系[9]. 而 CD 法在溶液状态下测定，较接近其生理状态.

蛋白质 5 种二级结构在紫外和真空紫外区

有其独特的圆二色谱，尤其在 200nm 以下，蛋白质 5 种二级结构的圆二色谱很不相同，这正是真空紫外圆二色谱对分析蛋白质二级结构比长波长的圆二色谱更灵敏和可靠的原因所在.

应用相同的方法，我们还测定了血纤维蛋白溶酶原，尿激酶等多种蛋白质的 VUV-CD 谱[10,11]，也获得了满意的结果.

用真空紫外圆二色法研究蛋白质二级结构的含量，首先应有好的真空紫外圆二色谱仪，测定波长应延至 184nm，最好测到 178 或 175nm. 但此波段内的圆二色谱测定，在技术上有一些难度，为了测得好的真空紫外圆二色谱，应注意以下几点：a. 仪器内部应抽真空或在所有光路中充以氮气，前面提到的特制样品池也可在充氮气条件下测定. b. 由于许多溶剂在真空紫外区的光吸收很大，信噪比不高，对实验很不利，所以应选择光吸收尽量小的溶剂. c. 为了提高信噪比，当样品的 CD 谱不是很尖锐时，可适当增大狭缝宽度（谱带宽度），增大时间常数和降低扫描速度，这样可以获得较光滑的 CD 谱，而又不影响实验结果. d. 通常的圆二色谱仪，在 200nm 以下波段噪声很大，仪器不能很好工作. 用同步辐射光源或特种型号的氘灯做光源的圆二色谱仪可以测得好的 VUV-CD 谱. 另外，仪器的各个光学部件，如偏振器，光弹性调制器，光栅，反射镜等应保持清洁，使其具有良好的透过率和反射率. 光源的位置和仪器光轴也应调整好，这样才可能在 200nm 以下波段获得高信噪比的 CD 信号.

由于圆二色法用于溶液测定，其测定方法快速简便，对构象变化灵敏，所以它是目前研究蛋白质二级结构的主要手段之一. 对于研究核酸的构象，尤其是在不同条件下核酸构象的变化来说，圆二色法也是一种很有用的工具[12]. 我们用同步辐射真空紫外圆二色谱仪对核酸和多糖的结构进行了测定与研究，同样也得到了满意的结果[13,14].

随着北京正负电子对撞机及其同步辐射实验室和合肥国家同步辐射实验室的建立，人们期待着应用我国自己的同步辐射真空紫外圆二色谱仪开展更多生物大分子溶液构象的研究工作.

致谢 本文之 VUV-CD 谱在美国能源部支持的 The National Synchrotron Light Source 测定. 作者对 John Sutherland 教授和 John Trunk 先生的技术帮助表示感谢.

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tation. CpG dinucleotides of p53 and Rb gene are easily methylated. Hypermethylation of the tumor-suppressor genes, with consequent gene inactivation and the loss of the suppression of the cellular proliferation, has been postulated as one of the potential mechanism for oncogenesis.

Key words tumor-suppressor gene, retinoblastoma, p53, methylation of DNA

Roles of Corpus Callosum in Early Visual Information Processing. Diao Yuncheng. (*Laboratory of Visual Information Processing, Institute of Biophysics, Academia Shinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 50

Results of a series of studies by this research group on the functional roles of the callosal connections in primary visual cortices were reviewed. Based on these, a topographical projection model of the vertical retinal bilaterally projecting strip was proposed, which not only agrees well with experimental results but also explains the necessity of the corpus callosum: signals conveyed by these fibers compansate information loss in the cortex due to the existence of retinal bilaterally projecting strip.

Key words visual cortex, corpus callosum, retinal bilateral projection

Recent Progress and Prospect in the Studies of Cell Biophysics. Zhang Jinzhu. (*Institute of Biophysics, Academia Sinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 55

Recent studies of cell biophysics have been introduced about cell ultrastructure effects of physical factors on living cells, cellular motilities, ion channels and cellular signalling. Some progress in methodology for studying living cells are also reviewed. It has been emphasized that the main purpose of cell biophysics is to understand the nature of the living cell in order to explain why and how the cell is alive. Accordingly, some questions and problems on principles and methodology have been discussed in this article as well.

Key words cell, biophysics, progress, prospect

The Protecting Effect of Mg^{2+} on the Changes of Cardiac Mitochondrial F_1F_0 Induced by Adriamycin. Lin Zhihuan, Li Shengguang, Cao Maosun, Chen Yunjun, Feng Chaoyang, Deng Junpeng. (*Institute of Biophysics, Academia Sinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China), 1994; **21** (1): 61

Adriamycin (ADM) is a widely used anticancer drug, but the chronic cardiotoxicity severely limits the use of it in the treatment of neoplastic disease. The experimental results obtained from F_1F_0-ATPase activity assay, ^{31}P-NMR spectra measurement, fluorescent probe NBD-PE detection, packing and fluidity of membrane lipids and intrinsic fluorescence measurements can be summarized as follows: ADM induces the phase transition of mitochondrial membrane lipids at first, as a consequence affecting on the lipid packing and fluidity of the lipid molecules and then influencing the conformation of the F_1F_0-ATPase and finally resulting in the decreasing of the enzymatic activity. And Mg^{2+} can protect all the effects induced by ADM thus reducing the harmful effect of ADM.

Key words cardiac mitochondrial F_1F_0-ATPase complex, Mg^{2+}, Adriamycin, non-bilayer-lipid, conformation

Vacuum Ultraviolet Circular Dichroism Studies

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-