

半胱氨酸对DNA辐射保护修复机理的ESR研究

屠铁城 林念芸 李学鹏

(中国科学院上海原子核研究所)

摘要 一九七九年本实验室曾用半胱氨酸对甲苯磺酸盐代替它的盐酸盐首次证实其对dTMP与嘧啶碱基的辐射保护效应,并用程序升温法详细地研究证实了它对dTMP的修复机理。最近又进一步证实了它对辐照DNA自由基修复机理。

关键词 自由基修复机理, 巯基类保护剂, 脱氧核糖核酸。

前言

对于巯基类保护剂辐射保护效应的修复机理,英,美两国的几个实验室从六十年代初以来曾多次用ESR研究了半胱氨酸对于DNA及其组份的辐射保护效应机理,但大量的实验工作仅仅证实了半胱氨酸修复靶分子自由基的氢原子转移机理^[1],而对于另一个重要巯基保护剂-半胱氨酸与DNA或其组份的二元体系,直至七十年代初即使用较先进的ESR,也未曾直接证实自由基修复机理。

先前本实验室曾用半胱氨酸对甲苯磺酸盐代替其盐酸盐首次用ESR证实它对dTMP与嘧啶碱基的辐射保护效应^[2],随后又用低温(77K)辐照、测谱及程序升温等方法详细研究并证实了它对dTMP自由基的修复机理^[3]。

实验

1. 试剂

半胱氨酸对甲苯磺酸盐是实验室合成的生化试剂^[2],小牛胸腺DNA的纯化如前文所述^[4]。

2. 样品的制备和辐照

将DNA和半胱氨酸对甲苯磺酸盐称量后分别溶于三次重蒸馏水中,然后按DNA中的核苷酸与半胱氨酸的克分子比1:1配成二元体系水溶液并进行冷冻干燥,干燥后的样品除氧封管及辐照条件均与文献^[4]相同。

3. ESR波谱的测定

被辐照样品的ESR波谱的测定均与文献^[4]相同。

结果及讨论

如图1所示,77K辐照并测定的DNA的ESR波谱为一不对称的单峰,略为显示胸腺嘧啶5位自由基的痕迹;升至室温退火后,则明显地显示出该自由基的特征(见图2)。

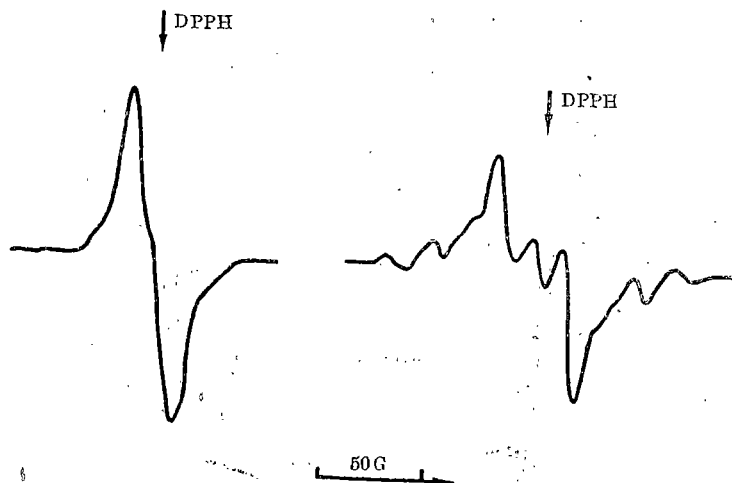


图 1 DNA 77K 辐照, 77K 测定的 ESR 波谱

Fig. 1 ESR Spectrum from DNA irradiated and observed at 77K

图 2 DNA 77K 辐照, 升至室温退火后测定的 ESR 波谱

Fig. 2 ESR Spectrum from DNA irradiated at 77K, recorded after annealing at room temperature.

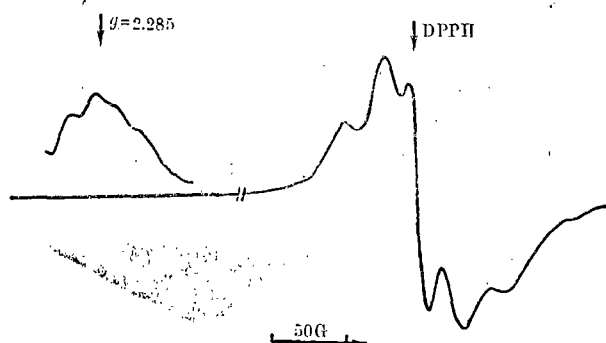


图 3 半胱氨酸对甲苯磺酸盐 77K 辐照, 77K 测定的 ESR 波谱

Fig. 3 ESR Spectrum from cysteine paramethyl benzenesulfonate irradiated and observed at 77K

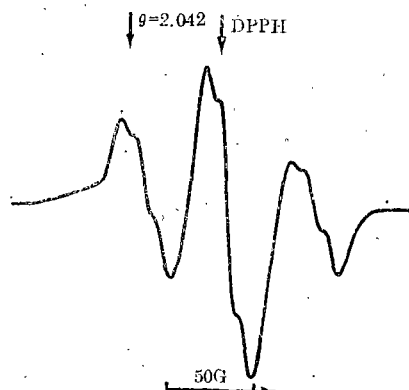


图 4 半胱氨酸对甲苯磺酸盐 77K 辐照升至室温退火后测定的 ESR 波谱

Fig. 4 ESR Spectrum from cysteine paramethyl benzenesulfonate irradiated at 77K, recorded after annealing at room temperature

如图 3 所示, 77K 辐照并测定的半胱氨酸 ESR 波谱显示氨基酸阴离子自由基的特征^[3], 升至室温退火后转化为室温下较稳定的次级巯基自由基 ($\text{RCH}_2\text{SS}\cdot$ 或 $\text{RCH}_2\text{S}\cdot\text{-SH}_2\text{CR}$) 的特征(见图 4)^[3]。

DNA-半胱氨酸二元体系 (DNA 中的核苷酸与半胱氨酸的克分子比为 1:1) 77K 辐照并测定的 ESR 波谱显示类似于 DNA 的波谱(见图 5)而不同于图 3 所示半胱氨酸阴离子自由基的特征, 升至室温退火后也转化为类似于次级巯基自由基的 ESR 波谱特征(见图 6), 并不见 DNA 在室温下呈现以胸腺嘧啶 5 位自由基为特征的 ESR 波谱的痕迹。

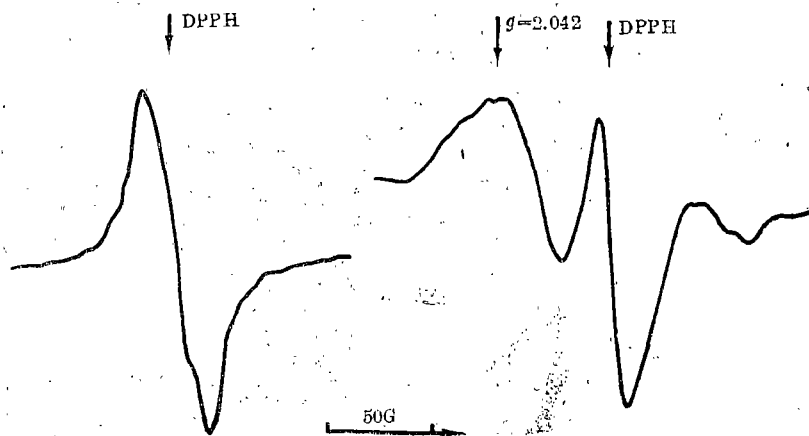


图5 DNA-半胱氨酸对甲苯磺酸盐分子混合物(核苷酸与半胱氨酸的克分子比为1:1) 77K 辐照测定的ESR波谱

Fig. 5 ESR Spectrum from DNA-cysteine paramethyl benzenesulfonate with molar ratio of nucleotide to cysteine (1:1) irradiated and observed at 77K

↓ DPPH

$g=2.042$

↓ DPPH

图6 DNA-半胱氨酸对甲苯磺酸盐分子混合物(核苷酸与半胱氨酸的克分子比为1:1) 77 K 辐照, 升至室温退火后测定的ESR波谱

Fig. 6 ESR Spectrum from DNA-cysteine paramethyl benzenesulfonate with molar ratio of nucleotide to cysteine (1:1) irradiated at 77K observed after annealing at room temperature

综上所述用半胱氨酸对甲苯磺酸盐代替其盐酸盐在发现并证实电荷转移机理的同时^[3]还较完整地证实了半胱氨酸对DNA, dTMP 基于氢原子转移的修复机理, 这是对证实 Wall 和 Magat^[5], Alexander 和 Charlesby^[6]五十年代初提出的最初建议所取得的一个新进展。

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ESR STUDY OF DNA RADIOPROTECTIVE REPAIR MECHANISM BY CYSTEINE

Tu Tiecheng Lin Nianyun Li Xuepeng

(Institute of Nuclear Research, Academia Sinica, P. O. Box 8204, Shanghai, China)

ABSTRACT Since the preliminary proposal of "free radical repair" via H-transfer process was suggested, a series of ESR studies, such as by Ormerod and Alexander, Pulman and Milvy et al.^[1] have been performed to clarify the repair mechanism exerted by the sulfhydryl compounds such as octyl mercaptan, cysteine, but only cysteamine was studied in detail in binary system containing DNA or dTMP and cysteamine. As for cysteine it was failed to be confirmed in binary system containing cysteine-HCl and DNA or dTMP.

In our previous work cysteine paramethyl benzenesulfonate was used to instead of its hydrochloride, thus ESR spectrum from dTMP-cysteine paramethyl benzenesulfonate irradiated and recorded at 77K revealed predominately characteristic from dTMP, but after a long range variable temperature from 189 to 323K accompanying the decay of ESR spectrum from dTMP the signal from cysteine grew gradually and a stable state assigned to secondary organosulfur radical $\text{RCH}_2\text{SS}\cdot$ or $\text{RCHS}\cdot\text{-SH}_2\text{CR}$ attained at higher temperature (323K) or long time (~ 20 hrs) annealing at room temperature.

Recently, the ESR spectrum from DNA-cysteine paramethyl benzenesulfonate irradiated and recorded at 77K revealed the characteristic similar to that from cysteine alone. After annealing at room temperature ESR pattern also changed into that from cysteine alone, no trace signal from 5-thymyl radical can be found.

KEY WORDS Free radical repair mechanism; Sulfhydryl protectors; DNA.