

# ESR STUDIES OF RADIOPROTECTION MECHANISM ON DNA BY SINAPIC ACID AND ITS DERIVATIVE

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**ABSTRACT** Connecting with the character of ESR spectra at 77 K and calculated LUMOs, HOMO the linear relationship between spin transfer efficiency and molar ratios of nucleotide in DNA to sinapic acid or sinapine provides quantitative evidence of long range electron transfer within mixed aggregates.

**KEYWORDS** ESR, Radioprotection mechanism, Sinapic acid, Longrange charge transfer

**CLC** O644.2

## 1 Introduction

A series of mixed aggregates composed of TMP and member of hydroxycinnamic acid derivatives have been studied by ESR and MO calculations.

ESR spectra from solid binary system including TMP-caffeic acid, TMP-ferulic acid, TMP-sinapic acid or sinapine (all with molar ratio 10:1) irradiated and observed at 77K reveal the character ( $\Delta H_{pp}$  and g-factor) from radical anion of carboxylic acid. On subsequent annealing at room temperature the ESR patterns changed to that from more stable conformations of protectors but no trace signal from 5-thymyl radical can be found. Frontier orbital energies of hydroxycinnamic acid derivatives calculated by CNDO/2 method are summarized in Tab.1.

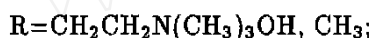
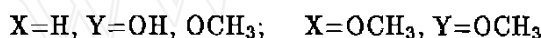
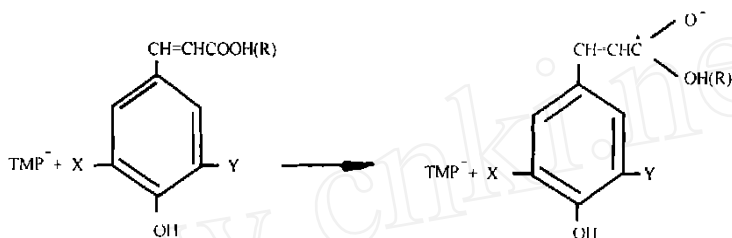
**Tab.1** Frontier orbital energies of hydroxycinnamic acid derivatives

Compound	$\epsilon_{HOMO}/eV$	$\epsilon_{LUMO}/eV$
Caffeic acid	-11.74	1.79
Ferulic acid	-11.35	1.89
Sinapic acid	-11.27	1.81
Thymine	-11.75	2.60

From Tab.1 it is obvious that the EAs ( $-\epsilon_{LUMO}$ ) of all the members of hydroxycinnamic acid derivatives are higher than that of thymine but IPs ( $-\epsilon_{HOMO}$ ) decrease following the increment of numbers of methoxy substituents. Accordingly, sinapic acid may be the more effective charge transfer protector and better than caffeic acid and ferulic acid. Ref.[1].

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Both the ESR spectra from mixed aggregates at 77K and the  $\epsilon_{LUMOS}$  appeared in Tab.1 demonstrate that long range electron transfer from anion radical of TMP to protector leads to the effective protection on TMP:



## 2 Mixed aggregates of DNA-sinapic acid

In order to explore the long range electron transfer within mixed aggregates composed of DNA and sinapic acid a series of binary mixtures with molar ratios of nucleotides in DNA to sinapic acid varied from 4:1 to 50:1 have been studied by ESR. The g-factor of a series of mixed aggregates irradiated and observed at 77 K reveal predominately the character from sinapic acid. After annealing at 261 K for 5 min, the ESR patterns changed from that at 77 K to the composite features composed of major central peak from sinapic acid and the side signal from 5-thymyl radical with different fraction dependent on the content of DNA.

Simulation and resolution of the composite spectra from a series of mixed aggregates are used to explore the radioprotection mechanism in detail.

Suppose the composite spectrum can be resolved as the linear combination of single spectrum from protector (P) and DNA with fraction factor  $W_P$ ,  $W_{DNA}$  respectively, a series of  $W_P$  and  $W_{DNA}$  from different mixed aggregates have been tabulated in Tab.2, based on the consistency of simulation and resolution of composite patterns, as shown in Fig.1 conducted on HP-9835B computer connected with a Varian E-112 ESR spectrometer.

Tab.2  $W_P$ ,  $W_{DNA}$  from simulation of spectra from DNA-sinapic acid after annealing at 261K

Molar ratio of nucleotide to protector	$W_{DNA}$	$W_P$
4:1	0.49	1
10:1	0.62	1
25:1	0.91	1
50:1	1.09	1
90:1	1.29	1

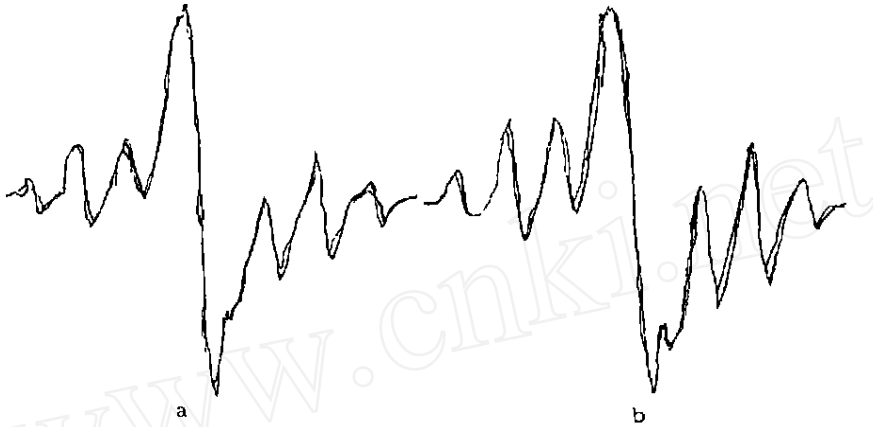


Fig.1 Simulation and resolution of composite spectra from DNA-sinapic acid with different molar ratios of nucleotide to protector  
(a) 10:1, (b) 5.0:1

For evaluation of spin transfer efficiency  $E_{ST}$  can be used as an index

$$E_{ST} = W_p \frac{g_{P_o}}{g_{P_b}}$$

Where the  $g_{P_o}$  and  $g_{P_b}$  are the weight of single protector and weight of protector within mixed aggregates in samples within ESR cavities respectively, A series of  $E_{ST}$  have been plotted against molar ratios ( $R$ ) of nucleotides in DNA to protector varied from 4:1 to 90:1. A linear relationship between  $E_{ST}$  and  $R$  as shown in Fig.2. demonstrates that the spin transfer efficiency increase linearly with the decrease of sinapic acid content and provides quantitative evidence of radiation induced long range electron transfer from nucleobase of DNA to electron affinic protector.

On subsequent annealing at room temperature for 5 min. a new series of  $W_{DNA}$   $W_P$  were derived from the simulation of composite spectra with different molar ratios of nucleotide to protector. Then the spin concentration of DNA and sinapic acid radical have were calculated by computer respectively. The increase of spin concentration of sinapic acid radical, accompanying the decrease of spin concentration of DNA radical, in the same mixed aggregate following the annealing to room temperature indicates that H-transfer repair of DNA radical by sinapic acid could also play an important role at room temperature, because the two methoxy substituents on benzene ring of sinapic acid increase the antioxidation activity of phenolic hydrogen supported either by redox potential determinations of phenolic antioxidants Ref.[2] or  $\epsilon_{HOMOS}$  appeared in Tab.1.

### 3 Mixed aggregates of DNA-sinapine

Quite similar to DNA-sinapic acid the g-factors of a series of mixed aggregates with molar ratios of nucleotides in DNA to sinapine varied from 4:1 to 25:1 irradiated and

observed at 77K reveal predominately the character from sinapine.

After annealing at 253K for 5min, a series of  $W_P$ ,  $W_{DNA}$  from mixed aggregates have been derived from the simulation of the composite patterns. A series of  $E_{ST}$  have also been plotted against molar ratio of nucleotide in DNA to protector. An excellent linear relationship between  $E_{ST}$  and  $R$  as shown in Fig.3. also demonstrates that the spin transfer efficiency increases linearly with the decrease of sinapine content.

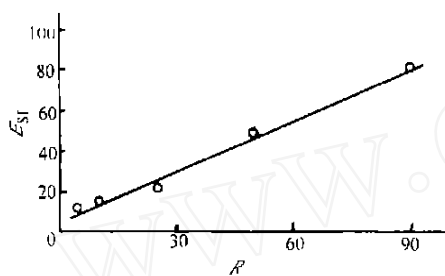


Fig.2 Relationship between  $E_{ST}$  and  $R$   
(DNA-sinapic acid)

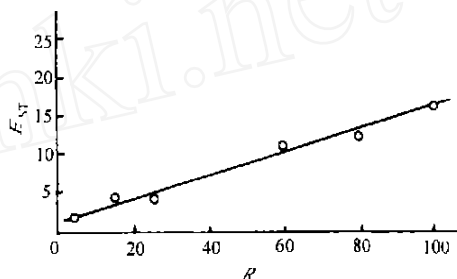


Fig.3 Relationship between  $E_{ST}$  and  $R$   
(DNA-sinapine)

On subsequent annealing at room temperature the characteristic pattern from 5-thymyl radical decayed rapidly and disappeared less than 30 min, for most of mixed aggregates (with molar ratios varied from 4:1 to 80:1), while the life time of DNA radical is longer than 30 min and decay slowly after annealing for 30 min.

Accordingly, the rapid decay of DNA radical from mixed aggregates also reveal the repair of DNA radical by phenolic hydrogen on sinapine.

#### 4 Discussion

Based on our series researches<sup>[1,3,4]</sup> and the kinetic studies by Miller<sup>[5-7]</sup> the major character of radiation induced electron transfer is its strong dependence on exothermicity and for larger exothermicity the rate of electron transfer can exceed diffusion controll limit<sup>[5-7]</sup>. Accordingly, in the case of electron transfer within mixed aggregates of DNA hydroxycinnamic acid derivatives the dependence on the difference of electron affinity ( $\Delta EA$ ) or ionization potential ( $\Delta IP$ ) predominates,

Another character is that both the electron donor and acceptor induced by radiation to their excited states are always beneficial for long distance charge and excitation transfer. Furthermore in the solid binary mixtures composed of DNA and aromatic hydroxycinnamic acid derivatives the two components can form stacked arrays through good overlap of electron cloud of DNA bases and the benzene ring of hydroxycinnamic acid derivatives. Thus within the above mixed aggregates electron transfer occur substantially over long distance.

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## 芥子酸及其衍生物辐射保护 DNA 的电子自旋共振研究

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**摘要** 将低温获取的电子自旋共振波谱与 DNA 组分及羟基肉桂酸衍生物的前线轨道能量相关联,依据自旋自 DNA 向芥子酸及其衍生物的转移效率与核苷酸和后者摩尔比的线性关系揭示混合堆集中电荷长程传递的证据。

**关键词** 电子自旋共振,辐射保护机理,芥子酸,长程电荷传递

**中图分类号** O644.2