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ENZYMATICALLY GENERATED ELECTRONICALLY EXCITED MOLECULES INDUCE TRANSFORMATION OF 4-THIOURIDINE TO URIDINE

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SUMMARY: Oxidation of 4-thiouridine-5'-monophosphate in t-RNA, from E. coli, by singlet oxygen generated via self-sensitization, photosensitization or by energy transfer from bioenergized systems yields uridine-5'-monophosphate. Studies with absorption, fluorescence and circular dichroism techniques showed similar interactions between singlet oxygen and the nucleotide in t-RNA generated by either optical or enzymatic systems. Protection by histidine and an enhancement of the photodegradation in the presence of D_2O corroborates the important role of singlet oxygen in these processes.

The stability of several thioketones towards oxygen in the presence or absence of light has been investigated by several groups of workers (1-6).

Recently, Rammath et al. (7), Tamagaki et al. (8) and Ramesh et al. (9,10) have carried out systematic investigations on the light-induced oxidation of a series of carefully chosen thioketones. A zwitterionic-diradical intermediate, arising out of the primary interaction of singlet oxygen with the thiocarbonyl group, was believed to be the common intermediate for the ketone and the sulfine, while a 1,2,3-dioxathietane would lead exclusively to the ketone (7).

Few reports on the photochemical oxidation of biologically interesting thioketones have been published (11-14).

In our studies on the energy transfer of bioenergized processes (enzymatically generated excited molecules) in t-RNA (15-17) we found an effi-

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Abbreviations: ^{4}Srd, 4-thiouridine; HRP, horseradish peroxidase; ^{3}IAl*, excited indole-3-aldehyde; IAA, indole-3-acetic acid; Cyd, cytidine; CD, circular dichroism.

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cient transfer from IA1 in its triplet state, generated by the peroxidase-catalyzed oxidation of IAA, to ^{4}Srd. As these processes are probably important in the growth control function, we decided to study the possible transformation of ^{4}Srd into uridine in t-RNA by these bioenergized processes and its relation to the role of t-RNA.

MATERIALS AND METHODS

HRP (Type VI), sodium uridine-5'-monophosphate, ammonium ^{4}Srd-5'-monophosphate, E. coli strain W t-RNA and acridine orange were from Sigma Chem. Co.; IAA, D_2O (99.9%), magnesium acetate, magnesium chloride, and sodium cacodylate were from Merck. 1,1,3,3-Tetraethoxypropane was from Aldrich Chem. Co. Malonaldehyde was prepared by the method of Grabowski et al. (18).

Oxygen consumption was measured with Yellow Springs Instr. Model 53 (YSI 53) Oxygen Monitor. Fluorescence spectra were obtained with a Perkin Elmer 44-B Spectrometer. The photon emission from enzymatic systems were measured with a Beckmann LS 100c Liquid Scintillation Counter. CD measurement were made on a Cary 50 Spectropolarimeter equipped with a Model 5001 CD attachment. Circular cells with an optical path of 1.0 cm were used and the specific elipticity (Ψ) was expressed in deg-cm^2 dmol^{-1}. Absorption spectra were measured on a Zeiss DMR-21 Spectrophotometer.

The standard reaction conditions for triplet IA1 generating systems were IAA (0.1 mM), HRP (0.25 µM) and EDTA (30 µM) in 0.05 M acetate buffer (pH 3.8). The t-RNA containing system was prepared as follows: To a final volume of 2.3 ml of 0.05 M acetate buffer (pH 3.8) was added 1.0 mg/ml of t-RNA, 0.2 mM IAA, 1.0 µM HRP, 1.6 mM Mg^{++} and 30 µM EDTA. The standard reaction conditions for the singlet oxygen generating system were malonaldehyde (10.5 mM), HRP (2.3 µM) and Mn^{++} (3.1 mM) in 0.2 M acetate buffer (pH 4.8).

Photooxidation of t-RNA (1.0 mg/ml) or ^{4}Srd-5'-monophosphate (2.6 mM) was carried out in the presence of 15.5 µM of acridine orange (previously purified by the method of Armstrong et al. (19) in 0.05 M Tris-HCl buffer (pH 7.6).

For all the spectral studies on t-RNA the following method was used: After photoxidative or enzymatic treatment, the t-RNA was precipitated by addition of 0.1 M NaCl and 2 volumes of cold ethanol, followed by centrifugation in a Sorval Superspeed RC-2B Centrifuge equipped with a SS-34 rotor at 10,000 x g for 15 min, followed by storage at freezer temperature.

The photoproducts of ^{4}Srd-5'-monophosphate either by photochemical or enzymatic treatment were analyzed by TLC, F-254 Silica gel (Merck).

Amino acyl-t-RNA synthetase preparation free of t-RNA and aminoacetylation of t-RNA were carried out as before (17).

RESULTS AND DISCUSSION

As reported earlier (16,17), t-RNA dramatically enhances the emission from the IAA/HRP/O_2 system, as a result of energy transfer from ^{3}IA1* to the ^{4}Srd group in t-RNA^{Phe}. If Mg^{++} ions are present, maximum emission is more intense and delayed with respect to time. ^{4}Srd-5'-monophosphate at the concentration used (39 µM) had only a modest sensitizing effect, (although it was ~10 times higher than to the ^{4}Srd concentration in t-RNA) noticeable only after extensive oxygen depletion. This might indicate that a more

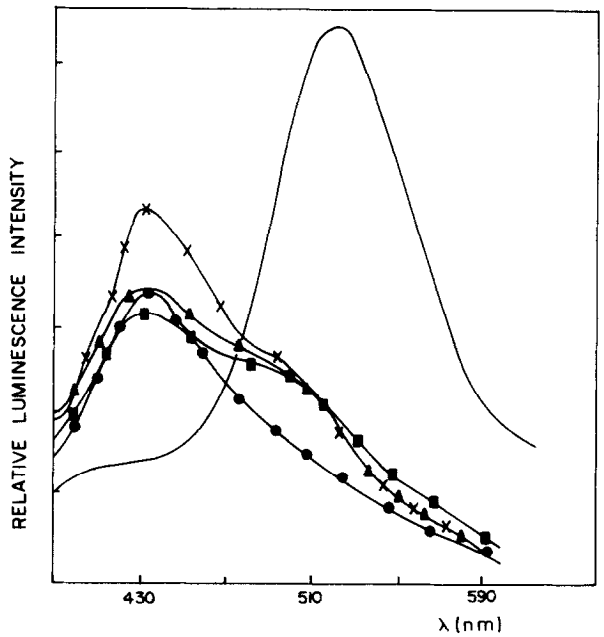


FIG. 1. Emission spectra of 1.0 mg/ml t-RNA (λ Exc. 335 nm) in 0.05 M cacodylate buffer pH 7.0, 0.1 M NaCl and 5 mM $MgCl_2$ (——). t-RNA after IAA/HRP/O_2 treatment (-▲-); t-RNA after IAA/HRP/Mg^{++}/O_2 treatment (-■-); t-RNA after IAA/HRP/O_2/D_2O buffer treatment (-●-), and t-RNA after IAA/HRP/Mg^{++}/O_2 D_2O buffer treatment (-X-).

efficient energy transfer from enzyme-generated excited IAl to the macromolecule or ^{4}Srd is somewhat protected from deactivating oxygen collisions.

Fig. 1 shows the emission spectra of t-RNA (λ exc. 335 nm) in 0.05 M cacodylate buffer (pH 7.0) and 0.1 M NaCl both in the presence and absence of 5 mM Mg^{++} ions from photochemical or enzymatic systems. In deuterated buffer and absence of Mg^{++} ions the disappearance of ^{4}Srd is enhanced when compared to undeuterated buffer. The presence of Mg^{++} ions supresses the D_2O effect producing essentialy equivalent yield of ^{4}Srd, an in addition the concentration of the product centered at 440 nm was increased. Surprisingly, the same type of D_2O effect was observed in the photoirradiation of t-RNA at λ 335 nm for 8 hrs at the conditions where the |8-13|^{4}Srd-Cyd photoadduct is formed (20). Also, in this case, D_2O exerts an effect on the production of a photoproduct at 440 nm in the absence of Mg^{++} ions.

The photochemical and enzymatic treatment was studied following the CD spectra at ^{4}Srd region. Fig. 2 shows enzymatic treatment of t-RNA for 15

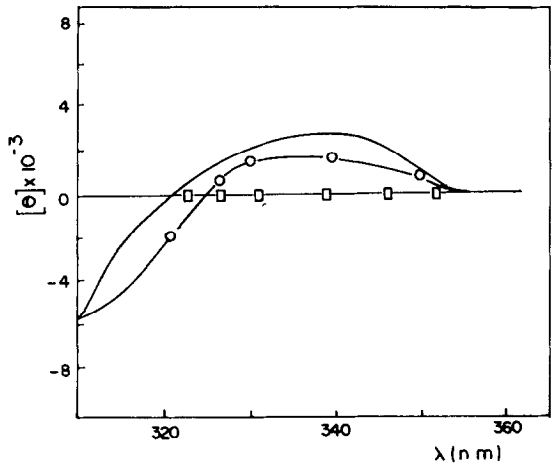


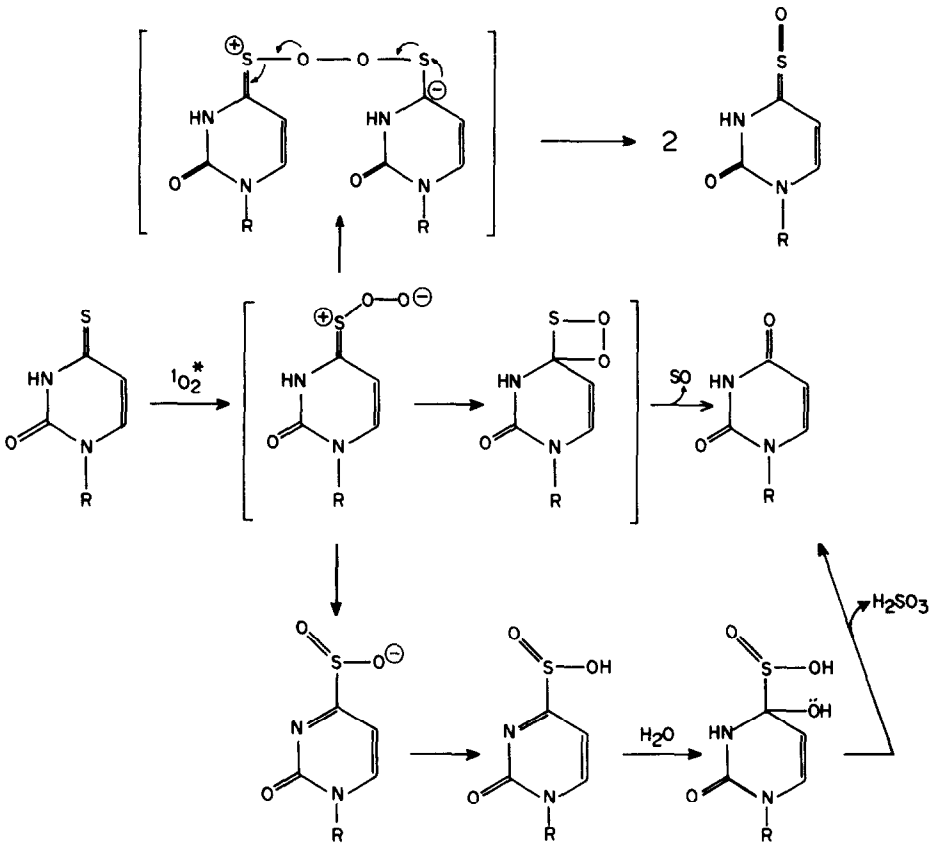
FIG. 2. CD spectra of 1.0 mg/ml t-RNA in 0.05 M acetate buffer pH 3.8 with and without 1.4 mM mg^{++} ions (——). t-RNA after IAA/HRP/O_2 treatment, (-○-) in aqueous buffer and (-□-) in deuterated buffer.

min. It is immediately evident that D_2O exerts a strong effect on ^{4}Srd. We found out through CD spectra that since D_2O does not change the t-RNA conformation under the reaction conditions then presumably, the D_2O effect is due to a higher photodegradation of ^{4}Srd. Then, not only |8-13|^{4}Srd-Cyd photoadduct in these conditions is formed (21), but a concommitant photodegradation of ^{4}Srd occurred.

In order to assess the importance of singlet oxygen interaction with ^{4}Srd, we used the malonaldehyde/peroxidase/O_2 system (22) and the photosensitization by acridine orange, which generates singlet oxygen enzymatically and photochemically respectively. The absorption spectrum of t-RNA after treatment with malonaldehyde/HRP/O_2 system, in the presence of D_2O showed a large enhancement of consumption of ^{4}Srd as compared with that in water. The CD spectra in this case showed a comparative effect of bioenergized and photochemical singlet oxygen generation on t-RNA. These facts are consistent with the singlet oxygen degradation of ^{4}Srd, in both processes, due to an increment of the effect in the presence of D_2O and protection by histidine (not shown).

As a model of this process we selected the ^{4}Srd-5'-monophosphate to compare the photosensitization of acridine orange and the bioenergized process by enzymatic system. The Rf values of the components after enzymatically

and photochemically sensitized reaction were studied. From this data and by spectral analysis is observed that ^{4}Srd-5'-monophosphate (Rf = 0.0) was transformed to uridine-5'-monophosphate (Rf = 0.16) either by an optical or bioenergized process. On the basis of the paper describing the photooxidation of thioketones (7), the following mechanism can be rationalized (SCHEME 1):



(Scheme 1)

Studies of the effect of iodine oxidation of thiopyrimidine nucleotides in E. coli t-RNA (23) did not yield conclusive information about the functional role of these minor components, and unfortunately no information is available about the amino acyl accepting capacity of t-RNA affected by mild and selective oxidation conditions used by Pleis et al. (12).

The t-RNA obtained after interaction with the IAA/HRP/O_2 system was tested for aminoacid accepting capacity and it was shown that this capacity is slightly reduced in comparison to the unmodified t-RNA.

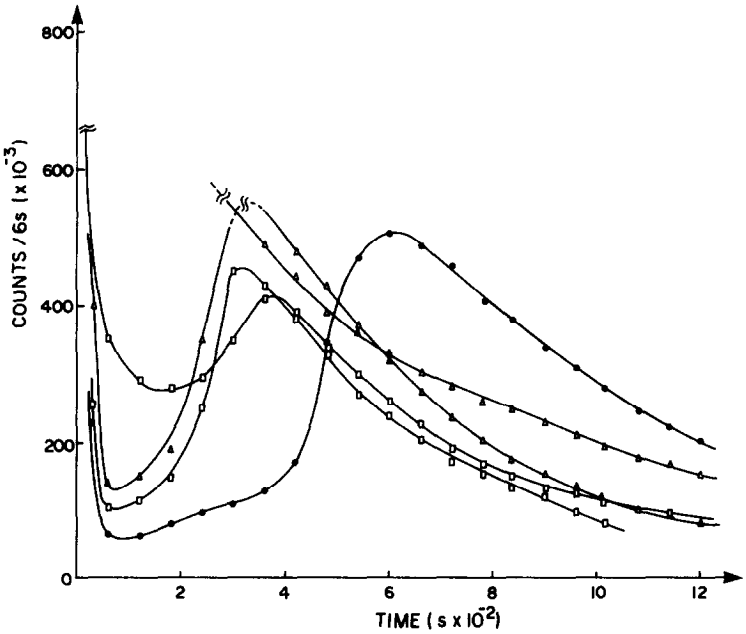


FIG. 3. Temporal behavior of the t-RNA (0.86 mg/ml) sensitized emission from the IAA/HRP/O_2 system: Control (-●-, Intensity x 1); t-RNA treated with malonaldehyde/HRP/Mn^{++}/O_2 system (-Δ-, Intensity x 2); t-RNA treated with Acridine orange/hν (-□-, Intensity x 2); t-RNA treated with IAA/HRP/O_2 system (-▲-, Intensity x 2); and t-RNA treated with direct irradiation at 335 nm for 6 hrs (-■-, Intensity x 2).

The t-RNA modified by singlet oxygen either by malonaldeyde or IAA systems or photosensitization with acridine orange or by direct irradiation at 335 nm, was tested in the energy transfer efficiency of $^3IAl^*$ to ^4Srd. Fig. 3 shows that the emission at 6 min at the reaction conditions disappears completely in all the systems. Previously it was shown that at this time, this corresponds to ^4Srd emission centered at 540 nm (16). The enhancement at 3 min probably correspond to an energy transfer to a photoproduct which fluoresces at 440 nm as observed by the fluorescence spectra after different treatment (Fig. 1). This observation indicates a photodegradation of ^4Srd. Presumably, this decrease is also due to the formation of the $|8\text{-}13|^4Srd$-Cyd photoadduct. Another possibility is that the 440 nm emission corresponds to the excited uridine after cleavage from 1,2,3-dioxathietane intermediate in a similar way as observed by Suzuki et al. (24).

Supression of these oxidation processes in the presence of histidine (10 mM, results not shown), a singlet oxygen quencher, the D_2O effect and the product distribution suggest the involvement of singlet oxygen. D_2O decreases the photon emission from the energy transfer of $^3IAl^*$ to ^4Srd in t-RNA (not shown), and this is presumably due to an increase of the lifetime of singlet oxygen and leads to a higher degradation of ^4Srd in its ground state, diminishing the actual concentration of ^4Srd available for energy transfer.

This method of t-RNA transformation led to a rapid ^4Srd modification without affecting the aminoacid accepting capacity.

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