ther work on these and related derivatives, including attempts to isolate the metal–sulfinate species free of Lewis acid, are presently in progress.

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Photosensitized Inactivation of Alanine Transfer RNA

Sir:

Several recent reports have indicated that photochemical dimerization of pyrimidines can be achieved by ketone-sensitized energy transfer without concomitant photohydration.[1-4] Application of this technique to DNA by Lamola and Yamane has provided the first example of photosensitized thymine–thymine dimer formation in a nucleic acid.[5] Because of the potential usefulness of sensitized pyrimidine photochemistry as a structure–action probe, we have examined this procedure with tRNA. We have found that the aminoacyl acceptor activities of $tRNA^{Ala}_{Iab}$, $tRNA^{Ala}_{II}$, and $tRNA^{Tyr}$ from yeast are inactivated rapidly by acetone-sensitized photochemistry. This communication describes our initial studies on acetone-sensitized photomodification of $tRNA^{Ala}_{Iab}$. The results suggest that inactivation is caused by pyrimidine dimers formed by energy transfer from acetone to tRNA.

Commercial bakers yeast tRNA was fractionated as described previously, and $tRNA^{Ala}_{Iab}$ of 94% purity was obtained.[6] Acetone-sensitized photolysis was carried out at 310 ± 10 nm with a Bausch and Lomb high-intensity, grating monochromator and a super-pressure mercury lamp. The monochromator was fitted with a quartz, plano-convex, collecting lens (1.5-in. i.d., 5-cm F.L. Model A-11-651-20, Oriel Optics Corp., Stamford, Conn.) and Corning 0-54 and 7-54 glass filters (Corning Glass Works, Corning, N. Y.), giving a window (97% transmission) between 304 and 410 nm. Under these conditions acetone was the only absorbing species in the reaction mixture. Irradiations were carried out in stoppered 1-cm light-path quartz cuvettes. The solutions were deoxygenated with N_2 and stirred mechanically during the irradiation. The acetone concentration in the reaction mixture was calculated from the absorbance at 300 nm and the molar extinction coefficient, ϵ_{300} 2.01 l./(mol cm). Actinometry performed with ferrioxalate[7] gave $I_0 = 0.13$ μE/min.

In a typical experiment, a solution (3.2 ml) containing $tRNA^{Ala}_{Iab}$ (1.3×10^{-3} *M*), $MgCl_2$ (10^{-2} *M*), and acetone (0.085 *M*) was irradiated as described above. Half the alanine acceptor activity[6] was lost after about 90 min. Loss of activity did not occur in the absence of acetone or with acetone in the dark. The rate of inactivation was dependent upon the acetone concentration, but for technical reasons the kinetic order is still uncertain. We wish to defer discussion of this important point until we are able to gather more data. It is clear, however, that the inactivation is sufficiently rapid at low acetone concentrations to be useful in structure–action studies.

These results show that the inactivation of tRNA is dependent upon acetone photochemistry. Two different processes might be involved: (1) energy transfer from excited acetone to tRNA followed by photochemistry of the excited tRNA to form pyrimidine dimers or possibly dihydropyrimidines[8] or (2) addition of excited acetone to tRNA to form oxetanes with uracil or cytosine.[2]

The role of photoaddition in the inactivation process was examined with acetone-2-^{14}C (specific activity 0.46 Ci/mol) under the conditions described above. After irradiation for 2.5 hr the acetone was removed from the reaction mixture by repeated evaporation *in vacuo* in the presence of added unlabeled acetone. Nonvolatile radioactive impurities were separated from the tRNA on benzoylated DEAE-cellulose[9] as described previously.[6] A large, radioactive, nonultraviolet absorbing peak was eluted with 85 ml of solution I.[6] A nonradioactive, ultraviolet absorbing peak was eluted between 79 and 120 ml of solution I. The exact nature of the material in these peaks is unknown, but they are not tRNA. After 120 ml of solution I had passed through the column, solution II[6] was started, and tRNA was eluted between 110 and 340 ml of this solution.

The pooled tRNA peak accounted for 91% of the material used in the reaction. It had only 15% of its initial alanine acceptor activity. Its specific radioactivity was 0.023 Ci/mol of tRNA, which corresponds to 0.05 mol of acetone adduct/mol of tRNA. Therefore, acetone addition is not a significant cause of inactivation under these conditions, and most, if not all, the inactivation is caused by energy transfer.

Although the evidence presented here is indirect, the most reasonable interpretation of the data in terms of current knowledge is that dimerization of adjacent pyrimidines causes inactivation of the tRNA. This conclusion is consistent with earlier observations which show that formation of a single dimer is sufficient, though not necessary, for the inactivation of $tRNA^{Ala}_{Iab}$ by *direct irradiation* at 254 nm.[10]

These findings have important implications for structure–action studies on tRNA. Irradiation at 254 nm produces pyrimidine dimers and photohydrates.[10] This leads to very complex photochemistry because

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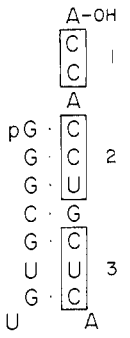

Figure 1. Aminoacyl acceptor stem of $tRNA^{Ala}_{Iab}$. Dimer targets in this area of the molecule are shown in the boxes.

$tRNA^{Ala}_{Iab}$ has 37 individual targets that can react when 254-nm light is absorbed. Although it has been possible to show that formation of a dimer in target 2 or 3 (Figure 1) is sufficient to inactivate the acceptor activity of $tRNA^{Ala}_{Iab}$, it has not been possible to isolate a dimer from either of these target regions.[10] One of the major difficulties in locating the actual photoproduct responsible for inactivation arises from the fact that dimers reverse during continued exposure to 254-nm light and photohydrates form in these areas. Thus, even though formation of a dimer is the initial inactivating event, photohydrates represent the major photoproducts at the end of the irradiation. Since we find no evidence of photohydrate formation by acetone-sensitized inactivation of $tRNA^{Ala}_{Iab}$ at 310 nm, the photochemistry should be much less complex than that produced by irradiation at 254 nm because there are only six areas that can form dimers in $tRNA^{Ala}_{Iab}$ (three of these are shown in Figure 1), and the dimers, once formed, should be stable. Therefore, it may be possible to isolate the actual photoproduct responsible for inactivation by first locating the inactivation target using the approach already developed for this purpose[10] and then isolating the photoproducts from the target oligonucleotide.

Further studies on the position of the inactivation targets, the nature of the photoproducts, and the use of acetone-sensitized photochemistry as a structure–action probe in tRNA are in progress.

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Photochemical Preparation of a Stable Oxetene

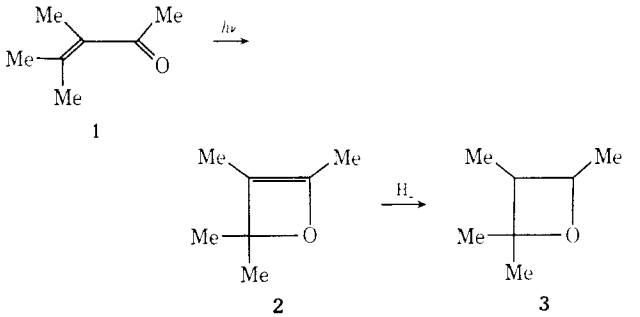
Sir:

We wish to report a significant result encountered in connection with our studies on oxabicyclobutanes. Irradiation[1] of 3,4-dimethylpent-3-en-2-one (**1**) in hexane solution through a Pyrex filter gave no observable reaction after 35 hr. Yang[2] and his coworkers have previously observed the same photochemical stability of compound **1** and other α,β-unsaturated ketones. The stability may result from the possible existence of a lowest energy $\pi-\pi^*$ triplet state[2,3] which is not prone toward hydrogen abstraction or other reactions familiar to $n-\pi^*$ triplet states.

When a 0.16 *M* solution of **1** in pentane was irradiated with Vycor-filtered ultraviolet light, however, the absorption maximum of the α,β-unsaturated ketone at 238 mμ rapidly disappeared with the simultaneous increase in end absorption at 215 mμ. The nmr spectrum of the crude product is greatly simplified if an unknown white solid is removed which precipitates when the pentane solution is cooled to −78°, followed by the evaporation of the solvent at 0°. The spectrum then shows mainly the presence of a small amount of starting material in addition to strong absorptions at δ 1.72 (narrow multiplet, $J < 1$ Hz), 1.58 (narrow multiplet, $J < 1$ Hz), and 1.38 (singlet) in a ratio 1:1:2. The low-field multiplet is partially obscured by a similar narrowly split absorption of the starting ketone at δ 1.75. The infrared spectrum shows an unresolved medium absorption centered at 1700 cm^{-1} accompanied with a shoulder at 1685 cm^{-1} attributed to the starting material.

Heating this partially purified photoproduct in carbon tetrachloride or pentane leads to a reappearance of starting material absorptions in the nmr, ir, and uv spectral regions. Silicone vpc chromatography showed only one major peak corresponding to starting material. Silica gel chromatography of the colorless product gave a red band on the column which would not elute with hexane. Our attempts at vacuum distillation gave only starting material. We estimate the product is formed in *ca.* 50% isolable yield and approximate the thermal half-life of the material in refluxing pentane to be 12 hr.

The structure of the suspected photochemically generated oxetene **2** was proved by hydrogenation over 5% palladium on calcium carbonate to give one major reduced material (vpc analysis) in *ca.* 60% yield based on vpc integration. The vpc collected product showed the following: nmr (δ, CCl_4) 4.47–4.94 (1 H, narrowly split pentet), 2.62 (1 H, pentet, $J = 6.5$ Hz), 1.38 (3 H, s), 1.18 (3 H, s), 1.17 (3 H, d, $J = 6.5$ Hz), 1.00 (3 H, d, $J = 6.5$ Hz);[4] ir (CCl_4) 958 cm^{-1}. Thus absorptions



(1) All radiations were performed with purified solvents in the usual immersion-well apparatus with a Hanovia 450-W medium-pressure lamp. In some cases, base-washed equipment was used, although to our knowledge it appears unnecessary.

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