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SPECIFIC PHOTOREACTIONS BETWEEN PSORALENS AND YEAST-tRNA^{Phe}.

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The photoreactions between yeast-tRNA^{Phe} and two psoralens, 8-methoxypsoralen (8-MOP) and 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT) have been investigated. It is found that AMT reacts more efficiently and with more sites on the tRNA than does 8-MOP, and that the AMT-photoreaction is inhibited by ethidium bromide and enhanced by EDTA. The results indicate that psoralens photobind to specific sites on the yeast-tRNA^{Phe} molecule.

INTRODUCTION

Psoralens, a class of compounds belonging to the furocoumarins, have been used in ancient medicine for skin disease treatment and are presently employed in the light therapy of psoriasis (1). The compounds are also used as tools in the study of the structure and conformation of double stranded nucleic acids, since they induce interstrand cross-links upon irradiation (2). It was recently shown that 8-methoxypsoralen (8-MOP)[1] photobinds to transfer-RNA thereby decreasing its amino acid acceptor capacity (3). Since tRNAs constitute a very specialized class of nucleic acids having a well described tertiary structure, the cloverleaf which is folded into the L-shape (4), containing both single and double stranded regions, the photobinding of psoralens could be anticipated to be restricted to specific sites. In the hope that the identification of these sites could lead to a better understanding of both the structure of the tRNA and the interaction of psoralens

1. Abbrevations: AMT: 4'-aminomethyl-4,5',8-trimethylpsoralen
8-MOP: 8-methoxypsoralen.

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with nucleic acids, we investigated the photobinding of 8-MOP and AMT (4'-aminomethyl-4,5',8-trimethylpsoralen) to yeast-$tRNA^{Phe}$. The results indicate the presence of one major photoreaction site for 8-MOP and two or more sites for AMT.

MATERIALS AND METHODS

Yeast-$tRNA^{Phe}$ and nucleases S_1 (EC 3.1.4-) and T_1 (EC 3.1.27.3) were purchased from Boehringer Mannheim. [^{3}H]-8-MOP was prepared by heating 25 mg of 8-MOP (Fluka) dissolved in 3.3 ml dry dioxane containing 380 µl fuming H_2SO_4 and 200 µl tritiated water (25 Ci/ml, Amersham) at 60°C for 3 h. The mixture was cooled to room temperature, 10 ml of ice-water was added and the [^{3}H]-8-MOP was extracted into 2 x 5 ml ethyl acetate. The organic phase was reduced to 500 µl and run through a silicagel column eluted with toluene ethyl acteate; 1:1. The fractions containing [^{3}H]-8-MOP were pooled and evaporated. The product (∿ 1.5 mCi) had a specific activity of ∿ 40 mCi/mmole and was > 95% radiochemically pure by TLC-analysis. An analogous experiment done with D_2O showed that the exchange took place (mass-spectral and NMR analysis, J.B. Hansen and P.E. Nielsen, unpublished results) at the 5-position. [^{3}H]-AMT was a kind gift from Dr. John E. Hearst and the specific activity of the sample was ∿ 10 mCi/mmole.
Irradiations were performed with light from an Osram Sp 200 mercury lamp filtered through 5 mm acetone giving a light intensity of 2 x 10^{17} qunata/s·cm^2 (350-450nm). The stirred samples were kept in an ice bath during the experiments. Before nuclease digestions the photoreacted tRNA was precipitated and washed with ethanol to remove excess radioactivity.
Digestions with nuclease T_1 (3 units/µg tRNA) or nuclease S_1 (30 units/µg tRNA) were performed in 50 mM Na acetate, pH 6.5, 5 mM $MgCl_2$ (and 2mM $ZnSO_4$ in the case of S_1) for 20 min at 37°C. The tRNA fragments were analyzed on 10% (S_1) or 29% polyacrylamide slab-gels (5,6) which were scanned at 260 nm and subsequently sliced (2 mm) for scintillation counting (luma solve, lipo luma; Lumac).

RESULTS AND DISCUSSION

Both 8-MOP and AMT were found to react covalently with yeast-$tRNA^{Phe}$ in a light dependent reaction (Fig. 1). However, it was observed that AMT reacted more efficiently than 8-MOP both in terms of photochemical yield and number of psoralens bound per tRNA (Table 1). This behaviour of the compounds is in full accord with results obtained with other types of RNA and DNA (7).

In order to determine the specificity of the reactions yeast-$tRNA^{Phe}$, which had been photoreacted with [^{3}H]-8-MOP or [^{3}H]-AMT, was digested with nuclease S_1 or RNase T_1. The nucleotide frag-

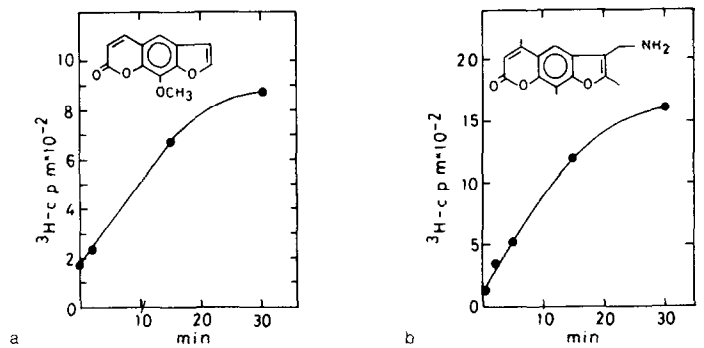


Figure 1. Time course of photoreactions of a) [^{3}H]-8-MOP and b) [^{3}H]-AMT with yeast-tRNA^{Phe}. 50 µg of tRNA were irradiated in 25 mM Na acetate, pH 6.5, 5 mM $MgCl_2$ in the presence of a) 2.5 x 10^6 cpm or b) 10^5 cpm tritiated psoralens. Aliquots were withdrawn at the times indicated. The samples were run on a polyacrylamide gel, from which the tRNA bands were cut out and counted.

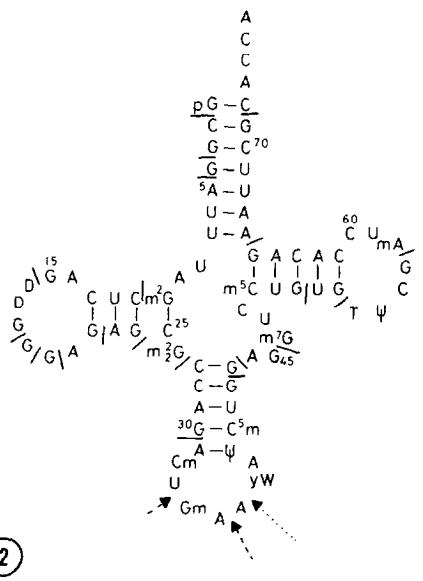
ments from these digestions, which have been well characterized (Fig 2, 6,8,9), were analyzed on polyacrylamide slab gels. The results indicate (Figs. 3,4 and 5) that 8-MOP binds to one major specific site in the 3'-half of the molecule, since the radioactivity is found associated with the A_{35}-A_{76} fragment of the S_1-digest (Fig 3b) and apparently only with one oligonucleotide of the T_1-digest (Fig 4). The identity of this nucleotide has not been determined yet since the influence of the psoralen on the migration of the nucleotide in the gel is not known, but the position in the gel indicates an octanuleotide[2] thereby suggesting the major 8-MOP photobinding site to be between nucleotide A_{58} and G_{65} of the yeast-tRNA^{Phe}. The problem is being pursued. In contrast to 8-MOP binding, more sites on the tRNA molecule are available for AMT photoreaction as indicated by the experiments with nuclease S_1 (Fig 3a) and RNase T_1 (Fig 5).

The qualitative difference between the binding sites for the two psoralens was further demonstrated by the pattern of inhibition of the photoreactions by ethidium bromide. The experiments

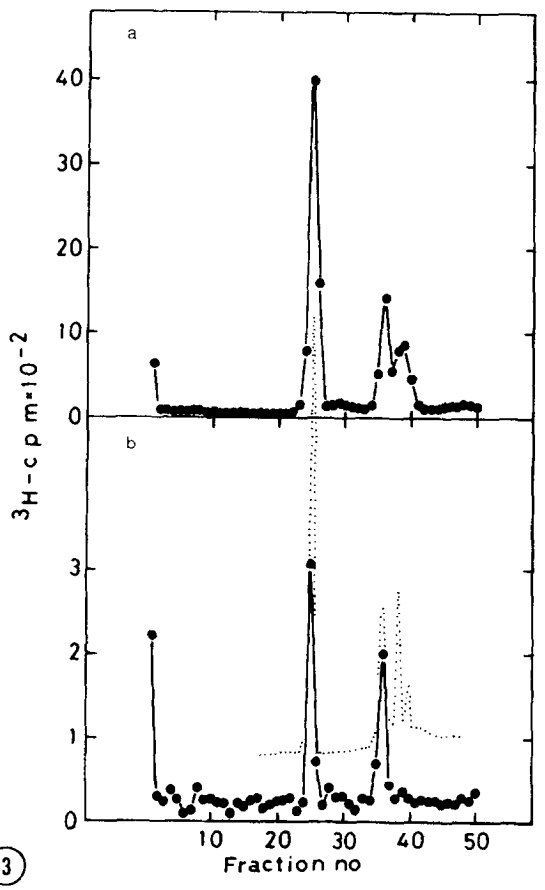
2. An analysis on DEAE cellulose was consistent with this conclusion.

Table I. Efficiency of psoralen photobinding to yeast-tRNA^{Phe}. The numbers were calculated from the results shown in Figure 1 (30 min).

	psoralen bound per tRNA	yield of photoreaction (rel.to added psoralen)
8-MOP	0.07	0.15%
AMT	0.3	7.7%



(2)



(3)

Figure 2. Structure of yeast-tRNA^{Phe} showing T_1 (——), primary S_1 (---) and secondary S_1 (···) cleavage sites.

Figure 3. S_1-digestion of tRNA reacted with [^{3}H]-psoralens. 30 µg of tRNA were irradiated for 15 min in the presence of a) 5 x 10^5 cpm of [^{3}H]-AMT or b) 10^6 cpm of [^{3}H]-8-MOP. The tRNA was precipitated with ethanol and subsequently digested with S_1. The products were analyzed on a polyacrylamide gel. The dotted line is the UV trace (260 nm) of the gel, showing intact tRNA, and the fragments of lengths 41-42 (fraction 36), 35 (fraction 38) and 33 (fraction 39) nucleotides.

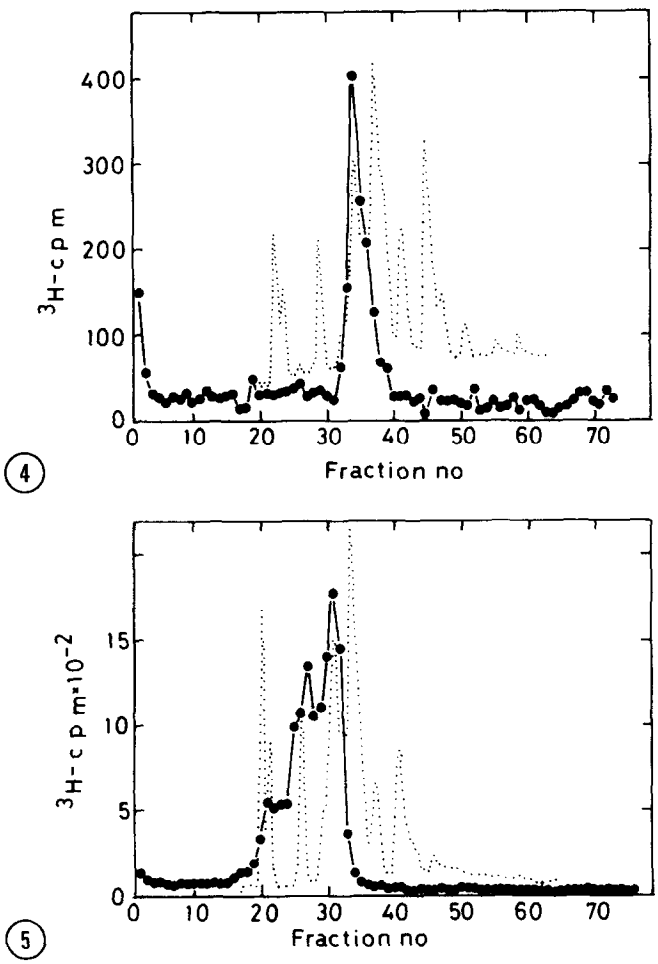


Figure 4. T_1-digestion of tRNA reacted with [^{3}H]-8-MOP. The tRNA was treated as described in the legend to Fig 3 except that nuclease T_1 was used instead of S_1. The fragments (dotted line) are of lengths 20,19 (intermediates) 12 (fraction 29, identified on the basis of the fluorescence from the yW base), 8 (fraction 36), 6, 5 and 4 nucleotides.

Figure 5. T_1-digestion of tRNA reacted with [^{3}H]-AMT. Experiment analogous to the one described in Fig 4.

showed a drastic decrease in AMT binding in the presence of ethidium bromide, while 8-MOP photobinding was only moderately affected (Fig 6). These results indicate that one or more of the AMT binding sites are close to or overlapping the ethidium bromide site (10,11,12), while the 8-MOP site(s) seems little related to the latter. A recent study has also shown that AMT is able to induce a conformational change to yeast-tRNA^{Phe}, not very different

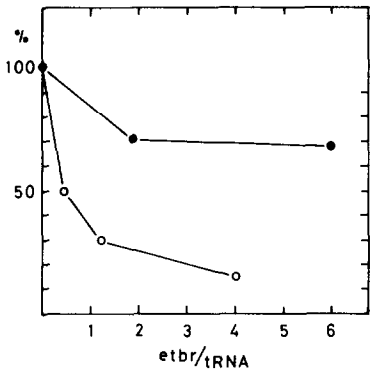


Figure 6. Effect of ethidium bromide on the photoreactions. Yeast-$tRNA^{Phe}$ was photoreacted with psoralens as in Fig 1 but in the presence of ethidium bromide. At the higher levels of ethidium bromide the results have been divided by the transmission of the solution at 350 nm (40-90%) thereby taken the inner filter effect into account. 8-MOP: ●-●-●, AMT: o-o-o.

than one induced by ethidium bromide, while 8-MOP did not exhibit such an effect (6).

The difference in behaviour between 8-MOP and AMT may be due to the presence of the amino group in the latter and the interaction of this with the phosphate backbone of the tRNA as the results in Table II suggest. In the presence of EDTA, for example, the AMT photoreaction was significantly enhanced while the 8-MOP reaction was inhibited. This may be due to either a general "opening" of the tRNA tertiary structure (6,9,13) or to an increased availability of phosphates in the absence of Mg^{2+} ions. It has been shown that more sites are available for ethidium bromide binding in the absence of Mg^{2+} (14). Conversely, spermine, which

Table II. Influence of EDTA and spermine on psoralen photobinding. Yeast-$tRNA^{Phe}$ was photoreacted with the psoralens as described in Figure 1 (15 min) except for the addition of EDTA or spermine, respectively.

	8-MOP		AMT	
Addition	cpm	%	cpm	%
none (control)	1000	100	1400	100
EDTA (10 mM)	680	68	2800	200
spermine (5 mM)	-	-	400	29

bind to the phosphate backbone (15), had an inhibitory effect on the AMT binding (Table II).

We did not intend to saturate the tRNA with psoralens and this may explain the discrepancy between the results presented here and those of Ou and Song (3) who reported that four 8-MOP molecules could be photoreacted per tRNA.

The present results have several implications. First of all they show that the photoreactions of yeast-tRNA^{Phe} with psoralens are not random but involve specific sites, and these sites are not identical for the two psoralens investigated in this study. Furthermore, the influence of both ethidium bromide, EDTA and spermine on the photoreactions infer that it may be possible - when the sites have been characterized more thoroughly - to use psoralens as probes for tRNA conformation e.g. during protein synthesis.

ACKNOWLEDGEMENTS

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REFERENCES

1. Anderson, T.F. and Voorhees, J.J. (1980) Ann. Rev. Pharmacol. Toxicol 20, 235-257.
2. Song, P.-S. and Tapley, K.J.Jr. (1979) Photochem. Photobiol 29, 1177-1197.
3. Ou, C.-H. and Song, P.-S. (1978) Biochemistry 17, 1054-1059.
4. Rich, A. (1977) Acc. Chem. Res. 10, 388-396.
5. Donis-Keller, H., Maxam, A. and Gilbert, W. (1977) Nucl. Acids Res. 4, 2527-2538.
6. Nielsen, P.E. (1981) Biochim. Biophys. Acta 655, 89-95.
7. Isaacs, S.T., Shen, C.-K.J., Hearst, J.E. and Rapoport, H. (1977) Biochemistry 16, 1058-1064.
8. RajBhandary, U.L., Stuart, A. and Chang, S.H. (1968) J. Biol. Chem. 243, 584-591.
9. Wrede, P. Wurst, R., Vournakis, J. and Rich, A. (1979) J. Biol. Chem. 254, 9608-9616.
10. Jones, C.R. and Kearns, D.R. (1975) Biochemistry 14, 2660-2665.
11. Wells, B.D. and Cantor, C.R. (1977) Nucl. Acids. Res. 4, 1667-1680.
12. Liebman, M., Rubin, J. and Sundralingham, M. (1977) Proc. Natl. Acad. Sci USA 74, 4821-4825.
13. Rich, A. and RajBhandary, U.L. (1976) Ann. Rev. Biochem. 45, 805-860.
14. Urbanke, C., Römer, R. and Mass, G. (1973) Eur. J. Biochem. 33, 511-516.
15. Quigley, G.J., Teeter, M.M. and Rich, A. (1978) Proc. Natl. Acad. Sci USA 75, 64-68.