Psoralen Photochemistry and Nucleic Acid Structure

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Many new psoralen derivatives have been synthesized in an effort to enhance their water solubility and their binding to nucleic acids. Availability of the very soluble strongly binding compounds has improved our abilities to follow the optical changes associated with the photochemistry of psoralens with DNA. Changes in both absorbance and fluorescence are presented in this review. A kinetic model for the photochemistry concludes that the detailed kinetics is dominated by the equilibrium constant for intercalation of the psoralen in the DNA, the quantum yield for photoaddition to DNA once intercalated and the quantum yield for photodestruction of the drug in water. With these 3 parameters the kinetics of photochemistry is predictable. The values of these parameters for numerous derivatives of 8-methoxypsoralen and 4',5',8 trimethylpsoralen are presented. Application of this photochemistry to a study of nucleic acid secondary structure in chromatin, fd bacteriophage, and in ribosomes is reviewed.

At present there exist over 100 psoralen derivatives in the chemical literature of which approximately half are naturally occurring and the remainder synthetically prepared. Of this large number, however, only 2 derivatives are currently in widespread scientific and clinical use in the United States. These 2 compounds are known by their trade names as Trioxsalen (4',5',8 trimethylpsoralen or TMP, 1) and Methoxsalen (8-methoxypsoralen or 8-MOP, 2).

My laboratory has become involved in many fundamental aspects of psoralen chemistry, including probing the secondary structures of Escherichia coli 16 S ribosomal RNA in vitro and in the 30 S ribosomal subunit [1-3], of Drosophila melanogaster 5 S ribosomal RNA in vitro [4], and of the circular single-stranded DNA genome of fd bacteriophage in the virus [5]. In the course of our investigations it became clear that modification of the psoralen nucleus in various ways would enable different types of experiments to be carried out which are not possible to do with either TMP or 8-MOP. With this in mind, an organic synthesis project was initiated which culminated in the production of many new psoralens, several of which have superior photoreactivity with both DNA and RNA as compared to TMP and 8-MOP. The 5 new derivatives shown here are 4' adducts of TMP and their structures are shown (3-7). The complete characterization of methoxymethyltrioxsalen (MMT, 4), hydroxymethyltrioxsalen (HMT, 5) and aminomethyltrioxsalen (AMT, 7) with respect to their reactivity with nucleic acids, including a theoretical treatment, is described in a recent paper by Isaacs et al [6]. A related set of soluble 8-methoxypsoralen derivatives have been synthesized by Isaacs, Chun, and Hearst [7] and are listed as compounds 8-11. Additional advances in psoralen synthetic chemistry have been presented by Bender, Hearst, and Rapoport [8] and in 3 associated patents [9-11].

Briefly, the advantages of the new compounds MMT (4), HMT (5), AMT (7), MMX (9), and HMX (10) are higher aqueous solubility and, in the case of the aminomethyl compound (7), a positively charged side chain. These factors greatly enhance the binding of the compound to nucleic acids and thus allow more drug to be covalently bound to DNA or RNA in the photoaddition reaction for a given dose of drug and light. One example of the advantage gained here is found in viral inactivation, as discussed below.

The current position on the therapeutic potential of these new derivatives falls mainly into 2 areas which should not be interpreted as limiting. A method enabling selective control of nucleic acid replication, hence viral and cellular reproduction, obviously lends itself to a broad range of application.

ANTIVIRAL VACCINE PRODUCTION

Current methods for inactivating viruses for the purpose of vaccine production consist primarily of formaldehyde or heat denaturation of the intact virus. The disadvantage of such procedures is the concomitant denaturation or chemical modification of viral protein, changing the antigenic structures to which antibodies are formed.

With psoralens, which have very weak reactivity with protein, it has been possible to completely inactivate both DNA and RNA viruses with an efficiency several orders of magnitude greater than the denaturation methods. In the case of the RNA virus, Vesicular Stomatitis virus (Hearst and Thiry [12], Trioxsalen was found capable of reducing the number of survivors to 10% of the original viral titer for a given dose; an equivalent dose of the new derivative AMT was found to leave a fraction of 10^-9 surviving plaques after treatment. In both cases the required time of irradiation for inactivation was on the order of minutes. Even more effective AMT inactivation has been demonstrated by Hansson, Riggs, and Lenette [13] using Western Equine Encephalitis virus.

In theory, a psoralen inactivated virus should produce a superior vaccine, as the protein antigenic component of the virus is, in all probability, unmodified by the inactivation procedure.

A final point on viral inactivation deserves comment. Recent attention has been given to the oncogene theory. If this theory is correct, an inactivated virus may contain an oncogene which could be excised and reinserted into the host genome, giving rise to transformation. With psoralen inactivation, however, it is possible to chemically alter 1 out of every 4 base pairs, clearly eliminating the possibility of the repair or recombination of viral information.

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Abbreviations:
AMT: aminomethyltrioxsalen
HMT: hydroxymethyltrioxsalen
MMT: methoxymethyltrioxsalen
8-MOP: 8-methoxypsoralen
TMP: 4',5',8-trimethylpsoralen
TUMOR CHEMOTHERAPY

One possible approach to tumor chemotherapy employing psoralens and light combines oral or local administration of the drug followed by local irradiation of solid tumor mass via needle guided light pipes. Such an approach is essentially the same in principle as the photochemotherapy of psoriasis, but it does not limit itself to an external surface. If found to be workable, the procedure would provide a means to affect the controlled killing of neoplastic cells without the trauma of surgery or high energy ionizing radiation.

OPTICAL PROPERTIES OF THE PSORALENS AND THEIR REACTION PRODUCTS WITH DNA

The absorption spectrum of AMT is shown in Fig 1. Typically, the photochemistry is activated at wavelengths between 340 and 380 nm, well away from the absorption bands of nucleic acids alone, which absorb below 300 nm. The absorption spectrum of the psoralens is similar to that of the corresponding coumarins, suggesting that conjugation in the furan ring is rather weak. In fact, benzo furan does not absorb significantly above 320 nm. For this reason one would predict that the furan ring monoadduct to pyrimidine would still absorb between 320 to 380 while the pyrrole monoadduct should not. This also suggests that the precursor to crosslink is the furan ring monoadduct and that only this monoadduct will be fluorescent when excited at 365 nm [14-16]. These conclusions have recently been verified in our laboratory.

Figure 2 shows the excitation (left) and emission spectra (right) of AMT alone, where the excitation spectrum was recorded at the fluorescence wavelength of 450 nm while the emission spectrum was recorded utilizing exciting radiation at 330 nm [17]. The fluorescence peaks at 457 nm independent of the wavelength of the exciting light between 240 nm and 380 nm. There are 3 obvious bands in the excitation spectrum corresponding to the 3 absorption bands seen in Fig 1. These bands lead to fluorescence with an efficiency which is the reverse of their absorption efficiencies, the shortest wavelength band leading to the least fluorescence.

In the presence of poly d(A-T) (Fig 3), there is a 23% depression of fluorescence intensity of AMT at 457 nm. Under the conditions of this experiment, essentially all the AMT was intercalated into the alternating poly d(A-T) before photoreaction because of its strong binding constant to DNA.

As this reaction mixture is irradiated at 365 nm, Fig 3 shows that the 457 nm fluorescent peak is depressed and a new peak at 380 nm appears, corresponding to the buildup of furan monoadduct. Further irradiation leads to the loss of the 380 nm fluorescence, leaving a nonfluorescent crosslinked product [17].
In a similar experiment using the helix of homopolymers poly d(A-T) [(poly dA-poly dT)], Johnston [17] has shown the buildup of the 380 nm fluorescent peak still occurs with the formation of furan monoadduct, but the loss of this fluorescence is slower and leads to a thus far undefined photoadduct product which is fluorescent at 460 nm.

KINETICS OF PHOTOADDITION TO DNA

Isaacs et al. [6] have presented a tentative kinetic model for psoralen addition to DNA involving 3 steps: (a) drug intercalation, (b) photoadDITION, (c) photobreakdown of drug free in solution,

\[ P + S \xrightarrow{k_1} PS \]
\[ PS + h_r \xrightarrow{k_2} A \]
\[ P + h_r \xrightarrow{k_3} B \]

where P is the psoralen, S is an intercalation site, PS is an intercalated drug, A is photoaduct, and B is breakdown product. The adduct A may absorb a second photon to form crosslink, but it is still considered adduct A, for the experiment only measures \(^7\)H psoralen covalently added to the DNA.

We have characterized the psoralen derivatives that we handled in terms of 4 parameters relating to this scheme. First, the dissociation constant associated with the equilibrium represented by Equation (1).

\[ K_D = \frac{[P][S]}{[PS]} = \frac{k_{-1}}{k_1} \quad (1) \]

Typically, this constant is measured by equilibrium dialysis utilizing radiolabeled psoralen. Dissociation constants, extinction coefficients, and solubilities can be found in Table I [7]. The solubility of the compound in water at room temperature as shown in Table I allows for the calculation of the maximum amount of intercalation prior to photoreaction shown as [PS]/[S] for the saturated solution.

\( \phi_r \) is the quantum yield for monoaddition assuming the extinction coefficient of the psoralen at 365 nm intercalated in the DNA is not different from the free solution extinction coefficient, an approximation which has been experimentally verified for AMT. \( \phi_b \) is the quantum yield for photobreakdown by a first order reaction in psoralen concentration. We find at the very dilute conditions of this chemistry that photodimerization of free drug is not an important process.

From Table II [7], we see a range of quantum yields for addition to DNA of 8% down to 0.06%, the highest value being associated with Trioxsalen, the lowest with the pyridyl derivative of 8-MOP. The quantum yields for drug breakdown in solution range from 4.7% down to 0.08%. Typically, the quantum yield for addition is 1 to 5 times larger than the quantum yield for photobreakdown. Only 8-methoxypsoralen and 5 hydroxymethyl 8-methoxypsoralen are variants from this pattern and appear to resist photobreakdown about 10 times better than the other compounds in comparison to addition. Figure 4 shows the kinetics of addition of these compounds to DNA and one can see the qualitatively different behavior of 8-MOP derivatives in that the addition of compound to DNA continues for times about 10 times longer than for the remaining compounds. These long times are associated with the pumping of more and more 8-MOP into the DNA, a process which is less likely in the other derivatives because of photobreakdown. At present the relationships between these quantum yields and the structures of the compounds are not explicable but with a sufficiently large sampling such understanding should be realizable.

Johnston et al. [18-20] have studied the kinetics of crosslink formation in DNA with AMT using laser flash photolysis. There are 3 major conclusions in these references. First, a single laser pulse of 10 ns duration at modest energy densities forms only monoadducts. Second, the delay required between the 1st and 2nd of such laser pulses for efficient crosslink formation is 1 \( \mu s \), suggesting the need for a DNA conformational change in the course of crosslink formation. Finally, at higher energy densities a small number of single pulse crosslinks do form. While the mechanism for formation of these single pulse crosslinks is uncertain, one possibility is that a small fraction of intercalated molecules are at sites which are already conformationally modified at the time of the pulse, so 2 photons can be absorbed over a very short time, both leading to reaction. A second attractive possibility is that a small number of excited molecules intersystem cross to a triplet biradical which is capable of reaction at both ends.

\[ \text{Fluorescence intensity} \]

\[ \lambda_{em} = 450 \text{ nm} \]
\[ \lambda_{ex} = 330 \text{ nm} \]

\[ 256 \quad 336 \quad 457 \quad 505 \quad 540 \quad 580 \]

\[ \text{Corrected excitation (left) and emission (right) spectra of} \]
\[ \text{aminomethyltrirosalan (AMT) alone at a concentration of 27.8 \mu M and} \]
\[ \text{of AMT (25.9 \mu M) with polyd (A-T) (0.15 mM phosphate) in the Tris/} \]
\[ \text{EDTA buffer.} \]
Fig. 3. Fluorescence emission spectra in the course of the photoreaction AMT with poly d(A-T). Note that fluorescence is depressed when AMT intercalates in the poly d(A-T). The numbers associated with each curve in the figure on the left represent the time in seconds of photoreaction at constant intensity of 365 nm light. The buildup of the fluorescence peak at 380 nm is associated with monoaddition of AMT to thymine residues in the polymer. Loss of the same peak is associated with crosslink formation and is shown in the figure on the right where the numbers associated with each curve represent minutes of photoreaction.

**Table I.** Dissociation constants, extinction coefficients and solubilities

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Abbrev.</th>
<th>Molec. Weight</th>
<th>Extinction coefficient $\epsilon_{250\text{nm}}$ (l/mole cm$^{-1}$)</th>
<th>Solubilities (µg/ml)</th>
<th>K$_D$ (mol/l)</th>
<th>[PS]/[S] Saturated solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,5,8-Trimethylpsoralen (Trioxsalen)</td>
<td>TMP</td>
<td>228</td>
<td>$1.5 \times 10^4$</td>
<td>0.6</td>
<td>$2.6 \times 10^{-4}$</td>
<td>$1.3 \times 10^{-4}$ .05</td>
</tr>
<tr>
<td>4'Aminomethyltrioxsalen</td>
<td>AMT</td>
<td>257</td>
<td>$2.5 \times 10^4$</td>
<td>$&gt;10^4$</td>
<td>$3.4 \times 10^{-2}$</td>
<td>$6.6 \times 10^{-6}$ 5000</td>
</tr>
<tr>
<td>4'Hydroxymethyltrioxsalen</td>
<td>HMT</td>
<td>258</td>
<td>$2.1 \times 10^4$</td>
<td>41</td>
<td>$1.6 \times 10^{-4}$</td>
<td>$2.9 \times 10^{-4}$ .55</td>
</tr>
<tr>
<td>4'Methoxymethyltrioxsalen</td>
<td>MMT</td>
<td>272</td>
<td>$2.5 \times 10^4$</td>
<td>10</td>
<td>$3.7 \times 10^{-4}$</td>
<td>$5.4 \times 10^{-4}$ .39</td>
</tr>
<tr>
<td>N(4-methylenetrioxsalen)-pyridinium chloride</td>
<td>PMT</td>
<td>356</td>
<td>$2.5 \times 10^4$</td>
<td>$&gt;10^4$</td>
<td>$2.8 \times 10^{-2}$</td>
<td>$2.6 \times 10^{-5}$ 1080</td>
</tr>
<tr>
<td>Psoralen</td>
<td>PSOR</td>
<td>186</td>
<td>$2.1 \times 10^4$</td>
<td>38</td>
<td>$1.8 \times 10^{-4}$</td>
<td>$1.3 \times 10^{-4}$ .14</td>
</tr>
<tr>
<td>(8-Methoxypsoralen)</td>
<td>8-MOP</td>
<td>216</td>
<td>$2.1 \times 10^4$</td>
<td>35</td>
<td>$1.9 \times 10^{-4}$</td>
<td>$4.0 \times 10^{-3}$ .05</td>
</tr>
<tr>
<td>5-Hydroxymethylxanthotoxin</td>
<td>HMX</td>
<td>246</td>
<td>$1.8 \times 10^4$</td>
<td>46</td>
<td>$1.8 \times 10^{-4}$</td>
<td>$5.0 \times 10^{-3}$ .04</td>
</tr>
<tr>
<td>5-Methoxymethylxanthotoxin</td>
<td>MMX</td>
<td>280</td>
<td>$1.7 \times 10^4$</td>
<td>55</td>
<td>$2.1 \times 10^{-4}$</td>
<td>$1.0 \times 10^{-2}$ .02</td>
</tr>
<tr>
<td>N-(5-methylene-8-methoxy-psoralen)-pyridinium chloride</td>
<td>PMX</td>
<td>344</td>
<td>$2.3 \times 10^4$</td>
<td>$&gt;10^4$</td>
<td>$2.9 \times 10^{-2}$</td>
<td>$5.2 \times 10^{-5}$ 560</td>
</tr>
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</table>

**Table II.** Kinetic constants for photochemistry—quantum yields for DNA addition and breakdown

<table>
<thead>
<tr>
<th></th>
<th>$\phi_2$</th>
<th>$\phi_3$</th>
<th>$\phi_3/\phi_2$</th>
<th>Equilibrium bound per 1000 b.p.</th>
<th>Plateau bound per 1000 b.p.</th>
<th>Plateau of photodaddition equilibrium bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP</td>
<td>$8.4 \times 10^{-2}$</td>
<td>$4.7 \times 10^{-2}$</td>
<td>1.8</td>
<td>15</td>
<td>28</td>
<td>1.9</td>
</tr>
<tr>
<td>AMT</td>
<td>$2.2 \times 10^{-2}$</td>
<td>$2.2 \times 10^{-2}$</td>
<td>1.0</td>
<td>61</td>
<td>67</td>
<td>1.1</td>
</tr>
<tr>
<td>HMT</td>
<td>$1.6 \times 10^{-2}$</td>
<td>$4.9 \times 10^{-3}$</td>
<td>3.2</td>
<td>14</td>
<td>38</td>
<td>2.7</td>
</tr>
<tr>
<td>MMT</td>
<td>$4.7 \times 10^{-3}$</td>
<td>$1.6 \times 10^{-3}$</td>
<td>2.9</td>
<td>13</td>
<td>23</td>
<td>1.8</td>
</tr>
<tr>
<td>PMT</td>
<td>$1.8 \times 10^{-3}$</td>
<td>$3.1 \times 10^{-4}$</td>
<td>5.8</td>
<td>49</td>
<td>55</td>
<td>1.1</td>
</tr>
<tr>
<td>PSOR</td>
<td>$3.0 \times 10^{-3}$</td>
<td>$8.3 \times 10^{-3}$</td>
<td>3.6</td>
<td>1.2</td>
<td>9</td>
<td>7.5</td>
</tr>
<tr>
<td>8-MOP</td>
<td>$1.3 \times 10^{-2}$</td>
<td>$5.5 \times 10^{-4}$</td>
<td>24</td>
<td>3.7</td>
<td>(42)</td>
<td>(11.4)</td>
</tr>
<tr>
<td>HMX</td>
<td>$1.4 \times 10^{-3}$</td>
<td>$1.4 \times 10^{-4}$</td>
<td>10</td>
<td>1.0</td>
<td>(4)</td>
<td>(4.0)</td>
</tr>
<tr>
<td>MMX</td>
<td>$1.2 \times 10^{-3}$</td>
<td>$8.9 \times 10^{-4}$</td>
<td>1.3</td>
<td>0.5</td>
<td>(2)</td>
<td>(4.0)</td>
</tr>
<tr>
<td>PMX</td>
<td>$6.2 \times 10^{-4}$</td>
<td>$8.3 \times 10^{-4}$</td>
<td>0.74</td>
<td>39</td>
<td>(67)</td>
<td>(1.7)</td>
</tr>
</tbody>
</table>
A Specific DNA Orientation in the Filamentous Bacteriophage fd as Probed by Psoralen Crosslinking and Electron Microscopy

Shen and Hearst [5] have stabilized the molecular structure of single-stranded fd DNA inside its filamentous virion by the photochemical crosslinking with psoralens in solutions of different concentrations of NaCl, short (100–300 pair) hairpins can be stabilized on denatured Simian virus 40 (SV40) DNA for electron microscopy visualization. Six major hairpins were mapped. This study was extended by Shen & Hearst [26] by crosslinking single-stranded SV40 linear molecules, EcoRI-SV40 and Bgl I-SV40, in the presence of magnesium ions. After the reaction, in addition to the hairpins detected before, 7 DNA hairpins, and hairpin loops with sizes from 300 to nearly 2,000 nucleotides, are observed. These loops are located at specific regions on the SV40 genome. At least 3 of these loops are found in regions thought to be involved in splicing of the early and late SV40 transcripts. These loop structures are most likely to arise from base pairing between distant regions of the single-stranded DNA. RNA transcribed from the double-stranded DNA template is expected to behave in a similar way, possibly providing recognition sites for processing (splicing) enzymes.

Base-Pairing Between Distant Regions of the Escherichia coli 16 S Ribosomal RNA in Solution

Wollenzien et al [3] have introduced intramolecular crosslinks into Escherichia coli 16 S ribosomal RNA in aqueous solution by irradiation in the presence of hydroxymethyltrimethylpsoralen. When the crosslinked RNA is denatured and examined in the electron microscope, the most striking features are a variety of large open loops. In addition, because the crosslinked molecules are shortened compared to noncrosslinked molecules, there are likely to be small hairpins not resolved by the present technique. The sizes and positions of 11 loop classes have been determined and oriented on the molecule. The frequency of occurrence of the different classes of loops depends on the crosslinking conditions. When the crosslinking is done in solutions containing Mg2+, at least 4 of the loop classes appear with greater frequency than they do in 3.5 mM-NaCl. The loops presumably arise because complementary sequences separated by long intervening regions are being crosslinked. These base-pairing interactions between residues distant in the primary structure appear to be prominent features of the secondary structure of rRNA in solution.

Crosslinking Studies on the Organization of the 16 S Ribosomal RNA within the 30 S E coli Ribosomal Subunit

Thammana et al [1] have examined the structure of 16 S RNA in the 30 S subunit. The location and frequency of RNA crosslinks induced by photoreaction of hydroxymethyltrimethylpsoralen with 30 S E coli ribosomal subunits have been determined by electron microscopy. At least 7 distinct crosslinks between regions distant in the 16 S rRNA primary structure are seen in the inactive conformation of the 30 S particle. All correspond to crosslinked features seen when the free 16 S rRNA is treated with hydroxymethyltrimethylpsoralen. The most often observed crosslink occurs between residues near one end of the molecule and residues about 600 nucleotides away to generate a loop of 570 bases. The size and orientation of this feature indicate it corresponds to the crosslinked feature located at the 3' end of free 16 S RNA.

When active 30 S particles are crosslinked in 5 mM Mg2+, 6 of the 7 features seen in the inactive 30 S particle can still be detected. However, the frequency of several of the features, and particularly the 570-base loop feature, is dramatically decreased. This suggests that the long-range contacts that lead to these crosslinks are either absent or inaccessible in the active conformation. Crosslinking results in some loss of functional activities of the 30 S particle. This is consistent with the notion that the presence of the crosslink that generates the 570-base

APPLICATIONS OF PSORALEN PHOTOCHEMISTRY TO THE SOLUTION OF SIGNIFICANT STRUCTURAL PROBLEMS

Chromatin Structure

The nucleosome structure of chromatin imparts a specificity to the photochemical reaction of the psoralens with DNA. The photoaddition occurs primarily in the interbead regions of this structure. This has been demonstrated by denaturation electron microscopy on high molecular weight DNA isolated from Dro sophila melanogaster nuclei after photoreaction of the nuclei with Trioxsalen, and also by denaturation electron microscopy on short pieces of DNA obtained by micrococcal nuclease digestion of these same nuclei (Wieselhahn et al [21, 22], Hyde & Hearst [23]). Tritium labeled Trioxsalen is removed preferentially from these nuclei by micrococcal cleavage, demonstrating that the sites of nuclease attack in nuclei and the sites of photoaddition of Trioxsalen to chromatin are in close proximity on the DNA.

Secondary Structure in Single-Stranded fd DNA

The photochemical crosslinking of DNA by 4,5',8-trimethylpsoralen (Trioxsalen) has been used by Shen and Hearst [24] to freeze the secondary structures of single-stranded DNA molecules of bacteriophage fd at different ionic strengths. These secondary structures (hairpins or looped hairpins) have been visualized in the electron microscope. Most of the single stranded circular fd DNA molecules show only one hairpin after irradiation at 15° in 20 mM NaCl in the presence of Trioxsalen. As the ionic strength is increased, more hairpins appear on the DNA molecules. To map these secondary structures, double-stranded supercoiled fd DNA (RFI) was cleaved with the restriction enzyme Hind II, which makes only one cut on each RFI molecule. After denaturation and crosslinking of the linear single-stranded fd DNA (a mixture of viral and complementary strands), all the hairpins have been mapped on the DNA molecule with respect to this Hind II site. The results show that these hairpins occur at specific sites. The most stable hairpin has been assigned to the position where the initiation site for the conversion of single-stranded fd DNA to the double-stranded covalently closed form has been mapped.

Fig 4. Kinetics of photoaddition of derivatives of Trioxsalen, derivatives of 8-MOP and psoralen to calf thymus DNA. Abbreviations are as follows: AMT, aminomethyltrioxsalen; xanthotoxin or 8-methoxy psoralen; HMT, hydroxymethyltrioxsalen; TMP, Trioxsalen; MMT, methoxymethyltrioxsalen; and PSOR, psoralen.
loop traps the subunit in an inactive form, which cannot associate with 50 S particles.

The arrangement of the interacting regions crosslinked by hydroxymethyltrimethylspsoralen suggests that the RNA may be organized into 3 general domains. A striking feature of the crosslinking pattern is that 3 of the 7 products involve regions near the 3' end of the 16 S rRNA. These serve to tie together large sections of rRNA. Thus structural changes at the 3' end could, in principle, be felt through the entire 30 S particle.

I must acknowledge the many scientists with whom I have collaborated in these last 5 yr which I have devoted to psoralen chemistry. In particular, I acknowledge the assistance and friendship of Jean-Pierre Bachellerie, Stephen Isaacs, Henry Rapoport and Gary Wiese. TheREFERENCES

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