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An RNA–protein contact determined by 5-bromouridine substitution, photocrosslinking and sequencing

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ABSTRACT

An analogue of the replicase translational operator of bacteriophage R17, that contains a 5-bromouridine at position −5 (RNA 1), complexes with a dimer of the coat protein and photocrosslinks to the coat protein in high yield upon excitation at 308 nm with a xenon chloride excimer laser. Tryptic digestion of the crosslinked nucleoprotein complex followed by Edman degradation of the tryptic fragment bearing the RNA indicates crosslinking to tyrosine 85 of the coat protein. A control experiment with a Tyr 85 to Ser 85 variant coat protein showed binding but no photocrosslinking at saturating protein concentration. This is consistent with the observation from model compound studies of preferential photocrosslinking of BrU to the electron rich aromatic amino acids tryptophan, tyrosine, and histidine with 308 nm excitation.

INTRODUCTION

Substitution of thymidine in DNA for 5-bromodeoxyuridine (BrdU) has been utilized extensively to promote photocrosslinking of DNA to associated proteins (1–12). In only a few cases; however, has the crosslinked amino acid been identified (9–12). In the case of the *Oxytrica nova* telomer binding protein (11), BrdU was substituted for both thymidine and deoxyguanosine in the single stranded model for telomeric DNA, and crosslinks were observed to Trp and Tyr residues. 5-Bromouridine (BrU) has also been substituted for uridine in RNA and photocrosslinking to associated proteins observed (13,14), but the crosslinked amino acids have not been identified. The success of bromouracil–nucleic acid photocrosslinking to protein has been limited in part by the yield of the reaction, which is often less than 5%.

Earlier we reported a specific photocrosslink between the bacteriophage R17 coat protein and a small BrU-substituted RNA hairpin (RNA 1) which models the binding site (RNA 2) within the phage genome (Figure 1) (14). The nucleoprotein complex serves as a translational repressor of replicase synthesis (15) and as a nucleation site for encapsidation (16,17). *In vitro*, the coat protein binds as a dimer to the wild type hairpin and to hairpins of similar structure (18) with high affinity. RNA 1 (Figure 1) undergoes photocrosslinking to the coat protein in yields as high as 45% when irradiated with monochromatic emission at 308 nm from a xenon chloride excimer laser. The RNA contains a single BrU located at the −5 position which slightly improves affinity for coat protein. RNase digestion of the photocrosslinked hairpin followed by two-dimensional thin layer chromatography established that the crosslink was to the single BrU. The −5 position was established as the only reactive position by studying six different hairpins variously substituted with BrU.

Model studies with 5-bromouracil have established that photoreactivity is surprisingly dependent upon the wavelength of excitation (19). Monochromatic excitation at 308 nm yields the reactive triplet state via a low energy excited singlet state, presumably an n,π^* state. The triplet state reacts by initial single electron transfer from an oxidizable group in an amino acid residue such as an aromatic ring of Trp, Tyr, or His following by ion radical coupling and elimination of HBr to yield a crosslink (20). Excitation at 254 nm with a low pressure mercury lamp populates a higher energy singlet state, a π,π^* state, which undergoes C–Br bond homolysis to yield a uracilyl radical and a bromine atom in competition with intersystem crossing to the triplet state. Subsequent reaction of the uracilyl–bromine atom geminate radical pair may yield some crosslinking to nearby amino acid residues in an associate protein but also, because of the high reactivity of radicals such as these, likely yields other reactions. Hence, upon excitation of the 5-bromouracil chromophore with shorter wavelength ultraviolet light, two different reactions can occur, C–Br bond homolysis in an upper singlet state and single electron transfer from a neighboring group in the triplet state. These pathways are summarized in Figure 2. The identified crosslinks from excitation of BrU substituted nucleic acids to associated proteins all involve Trp, Tyr, or His (9,11,12) except for one which involves Ala (10). A low yield of crosslinking of Ala 238 of the yeast transcriptional activator GCN4 to an associated, 5-bromo-2′-deoxyuridine substituted, double stranded DNA was observed upon 254 nm excitation (10). This crosslinking to Ala probably occured via C–Br bond

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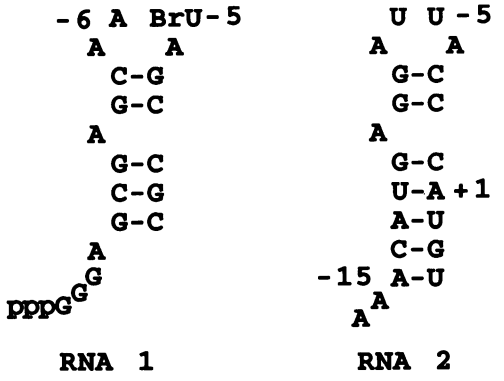

Figure 1. Sequences and secondary structures of RNA 1 and a 21mer (RNA 2) which has a sequence identical with the R17 replicase translational operator. The minimal coat protein binding site includes positions −15 to +2 (Gott, J. M., Pan, T., LeCuyer, K. A. and Uhlenbeck, O. C. (1993) *Biochemistry*, 32, 13399).

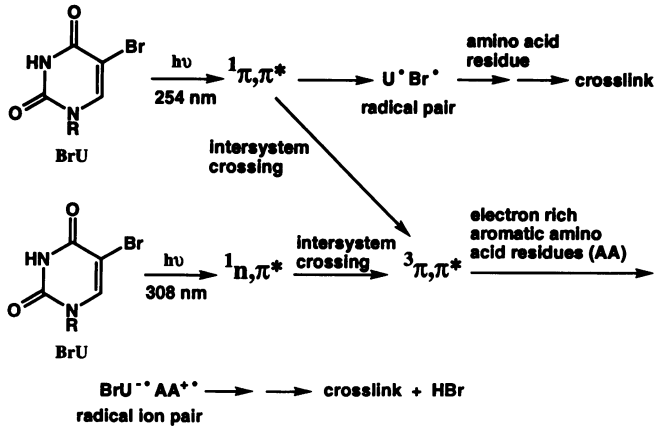

Figure 2. Scheme showing the wavelength dependent mechanisms for photocrosslinking of 5-bromouracil substituted nucleic acids to associated proteins.

homolysis in the π,π^* singlet state because no oxidizable groups were present for reaction in the triplet state, and the yield was low because of favorable competing reactions of the geminate radical pair which do not yield a nucleoprotein crosslink. An advantage to photocrosslinking of nucleoprotein complexes with 308 nm light rather than 254 nm light, when an aromatic amino acid residue is near a 5-bromouracil group, is that other photoreactive chromophores in the complex are not excited or minimally excited relative to excitation of the bromouracil chromophore. Hence, less degradation of both protein and nucleic acid are observed.

We now report the identification of the photocrosslink between the −5 position of RNA 1 and Tyr 85 of the R17 coat protein. We further report that a Tyr 85 to Ser 85 variant coat protein binds but does not photocrosslink to RNA 1.

EXPERIMENTAL

Preparation of RNA 1 and wild-type coat protein

RNA 1 was prepared by *in* vitro transcription from synthetic DNA templates by T7 RNA polymerase (22). Transcription reactions contained 40 nM Tris−HCl (pH 8.1 at 37°C), 1 mM spermidine, 5 mM dithiothreitol (DTT), 50 μg/ml bovine serum albumin (BSA), 0.1% (v/v) Triton X-100, 80 mg/ml polyethylene glycol (m_r 8000), 0.1 mg/ml T7 RNA polymerase, 3 mM of each of the NTPs, 25 mM $MgCl_2$, and 1 μM template. Non-radioactive RNA was treated with calf intestinal alkaline phosphatase (USB) and 5′ end labeled using T4 polynucleotide kinase (USB). Nucleotides, including the 5-BrUTP, were obtained from Sigma. R17 wild type coat protein was obtained and purified as described by Gott and co-workers (14) using the overexpression system of Studier and Moffat (23).

Cloning, purification and binding of the serine 85 variant

The parent plasmid (pKCONA) encoded an R17 coat protein variant containing two mutations (V75E;A81G) which prevent the coat protein from assembling into viral capsids. Characterization of this non-aggregating protein which has the same RNA binding properties as the wild type protein will be reported elsewhere (24). A self-complementary mutagenic oligonucleotide containing the Tyr to Ser substitution at the 85 position was cloned into the *BglII*−*Eco*RI fragment of this plasmid. The sequence of the Ser 85 oligonucleotide was as follows: 5′-TGC AGA TCT TCC TTA AAT ATG GAA CTA ACC ATT CCA ATT TTC GCT ACG AAT TCG T-3′. All enzymatic reactions were carried out according to the manufacturer's instructions. The plasmid was prepared by digesting with *BglII* (NEB) and then with *Eco*RI (NEB). The DNA was phenol/chloroform extracted and ethanol precipitated after each digest. The plasmid was then treated with calf intestinal alkaline phosphatase (Boehringer Mannheim). Purification on a low melting temperature agarose gel was followed by extraction with Gene Clean (BIO 101). The insert was annealed by heating to 75°C and cooling to ambient temperature. To extend, dNTPs were added to 250 μM along with 5 units of Klenow DNA polymerase (NEB). Phenol/chloroform extractions and ethanol precipitations were performed after each step. The inserts were then digested with *BglII* and *Eco*RI (NEB). Vector and insert were incubated with T4 DNA Ligase (NEB) overnight at 16°C. Ligation mixtures were transformed into JM83 cells and individual colonies selected for sequencing. Sequencing was performed using the USB Sequenase protocol. Clones with the correct sequence were transformed into BL21(DE3) pLYSs cells. The Ser85 protein was purified using FPLC techniques (24).

Protein excess filter binding of the Ser variant to RNA 1 was performed as described by Gott and coworkers (14). The protein concentration was corrected for the percent active protein as determined by an RNA excess titration. Data were corrected for 100% retention on the filters (actual retention was 25%).

Preparation and purification of crosslinked nucleoprotein complex

A 10 ml solution, 300 nM in 5′ end labeled-RNA and 500 nM in R17 coat protein, was incubated on ice in the presence of TMK (100 mM Tris−HCl (pH 8.5 at 4°C), 10 mM $Mg(OAc)_2$, 80 mM KCl), 80 ng BSA, and 5 mM DTT for 10−90 min. A Lambda Physik EMG-101 excimer laser was used to achieve monochromatic 308 nm light. The laser cavity was charged with 60 mbar of xenon, 80 mbar of 5% hydrogen chloride in helium, and filled to 2500 mbar with helium. The laser beam output was measured at 69 ± 5 mJ/pulse at 10 Hz with a Scientech 360-001 disk calorimeter power meter. Approximately 15% of the beam was passed through a 7 mm-diameter circular beam mask into

Ser[37]-Arg-Ser-Gln-Ala-Tyr-Lys-Val-Thr-Cys-Ser-Val-Arg-Gln-Ser-Ser-Ala-Gln-Asn-Arg-Lys-Tyr-Thr-Ile-Lys-Val-Glu-Val-Pro-Lys-Val-Ala-Thr-Gln-Thr-Val-Gly-Gly-Val-Glu-Leu-Pro-Val-Ala-Ala-Trp-Arg-**Ser-Tyr[85]-Leu-Asn-Met-Glu-Leu-Thr-Ile-Pro-Ile-Phe-Ala-Thr-Asn-Ser-Asp-Cys[101]-Glu-Leu-Ile-Val-Lys-Ala-Met-Gln-Gly-Leu-Leu-Lys**-Asp-Gly-Asn-Pro-Ile-Pro-Ser-Ala-Ile-Ala-Ala-Asn-Ser-Gly-Ile-Tyr[129]

Residue	pmoles	Residue	pmoles
Ser	133	Phe	43
Tyr	---	Ala	47
Leu	120	Thr	31
Asn	90	Asn	31
Met	103	Ser	10
Glu	88	Asp	23
Leu	99	Cys	---
Thr	98	Glu	12
Ile	94	Leu	8
Pro	58	Ile	7
Ile	53		

Figure 3. Amino acid sequence of the R17 coat protein in the region of the crosslink and the abundance of amino acid residues in the tryptic fragment crosslinked to RNA 1 as determined by Edman degradation. The order of amino acids detected by the sequinator, appearing in vertical columns, established the tryptic peptide crosslinked to RNA 1 which is shown in bold, underlined type in the partial protein sequence. The absence of Tyr in the Edman report at the second position indicates that Tyr 85 in the coat protein has been modified by the photocrosslinking. Cys 101 is also absent from the report because it was not alkylated prior to degradation (see text for further discussion).

a 1 cm path length quartz cuvette in a cell holder thermostatted at 4°C. The 10 ml reaction mixture was prepared just prior to the irradiations. The mixture was irradiated in 2 ml fractions in a quartz cuvette. After 5 min of irradiation the protein concentration was increased to 1 μM and the mixture incubated for 3 min and irradiated for an additional 5 min. Nine 10 ml reaction mixtures were prepared, irradiated and combined. The extent of crosslinking was measured using denaturing polyacrylamide gel electrophoresis (PAGE) with quantitation on a Molecular Dynamics Phosphor Imager. Samples were heated to 95°C for 3–5 min in 7 M urea/TBE buffer/10 mM dithiothreitol (DTT)/0.01% bromophenol blue/0.01% xylene cyanol prior to loading on a 20% denaturing polyacrylamide gel. The 90 ml sample contained 5.9 nmol of crosslinked material, 21 nmol of free RNA, 97 nmol of free coat protein and 7.2 mg of BSA. The total volume was reduced to 20 ml by evaporation, and the reaction mixture was ethanol precipitated over night at −20°C. The pellet was resuspended in 2 ml of 0.5 M urea/50 mM Tris−HCl (pH 8.3)/0.2% SDS for 48 h at 4°C with shaking. SDS was added to the sample to aid in the resuspension of the pellet. Once the pellet was resuspended, the SDS was precipitated by bringing the solution to 40 mM KCl and removed with a 0.22 μm cellulose acetate centrifuge filter unit.

Tryptic digestion and purification of the fragment crosslinked to RNA 1

The trypsin conditions were optimized using 25% of the crosslinked material. The remaining 75% of the crosslinked RNA−protein complex containing also free RNA, coat protein, and BSA was brought to 6 ml in 1 M urea/20 mM $CaCl_2$/6 mM DTT/1.6 mg (251 u/mgP) Worthington Trypsin-TPCK (1:5 w/w). The reaction proceeded at 36°C for 2 h at which time 1.6 mg more trypsin was added. At 4 h the reaction was stopped by quick freezing. The conditions selected resulted in maximal tryptic digestion with minimum RNA digestion. The majority of crosslinked products migrated on a 20% polyacrylamide denaturing gel as a single band, slightly above the free RNA. The trypsin reaction mixture was diluted to reduce the molar concentration of salt and run through a 240 μl DEAE ion exchange centrifuge column. The column was washed with 100 mM NaCl to remove the majority of the free peptide. The column bound material, containing the RNA and crosslinked tryptic fragment, was washed off the column with 600 mM NaCl, and ethanol-precipitated to remove the salt. The pellet was resuspended in 25 μl of 1 × TBE containing 7M urea, 10 mM DTT, 0.1% bromophenol blue, and 0.1% xylene cyanol, and heated to 85°C for 4 min. The digested protein−RNA crosslinked material was then isolated by 20% denaturing polyacrylamide gel electrophoresis and electrolytic blotting from the gel onto a PVDF protein transfer membrane (0.2 micron) from Bio-RAD. An autoradiogram was made of the membrane and used as a template to excise the digested protein RNA crosslinked material from the membrane. The experimental protocol was designed to minimize exposure to surfaces to avoid product loss. Although the R17 coat protein was retained on most surfaces, the fragment did not bear the same property. The immobilized peptide was sent for sequencing by automated Edman degradation directly off the membrane. This was performed on an Applied Biosystems 470A sequencer using manufactures methods and protocols (Clive Slaughter, Howard Hughes Medical Institute, University of Texas, Southwestern).

Irradiation at 308 nm of wild-type and Tyr 85 to Ser variant protein with RNA 1

RNA 1 was renatured by heating to 85°C in water for 3 min and cooling to 4°C. The RNA was mixed with 100 μl of 5×TMK buffer [100 mM Tris−HCl (pH 8.5 at 4°C), 10 mM $Mg(OAc)_2$, 80 mM KCl, 40 μg BSA, 5 mM DTT] and R17 wild type coat protein to make a solution 10 nM in RNA and 100 nM in coat protein. The 500 μl solution was allowed to equilibrate on ice for 20 min prior to irradiation. The laser beam intensity at the front window of the cell was measured at 4.5 mJ/pulse at 10 Hz. The solution was irradiated at 4°C in a 1.5 ml polymethacrylate cuvette with a path length of 1 cm. Solutions of RNA 1/R17 Ser 85 variant coat protein complex were prepared in an analogous manner to solutions of RNA 1/R17 wild type coat protein complex except that the R17 Ser 85 variant coat protein concentration was 2.1 μM. The percent crosslinking was quantitated from a phosphor imager scan of a 15% denaturing gel.

RESULTS AND DISCUSSION

Determination of the amino acid involved in the crosslink

To determine the site of the specific crosslink on the protein, the reaction scale was substantially increased over previous crosslinking experiments (14) to obtain enough material (100 pmol) such that a crosslinked tryptic fragment could be sequenced by Edman degradation. Because the coat protein forms aggregates at high protein concentration, the volume rather than the concentrations of reactants was increased. The ideal concentrations were determined to be 500 nM protein and 300 nM end labeled RNA **1** equilibrated in a volume of 2.5 ml. Irradiation was carried out for 5 min with 308 nm light. The

protein concentration was increased after 5 min to replace photo damaged protein, and irradiation was carried out for an additional 5 min. The final yield of crosslinked nucleoprotein complex from irradiation of 90 ml of reaction mixture was 6 nmol (22% based upon RNA).

The isolation of the crosslinked nucleoprotein complex was complicated by the large amount of BSA in the reaction mixture. Though gel purification provided excellent separation of the crosslinked material from the BSA, it resulted in a large loss at the elution step. Consequently, the mixture of BSA, free coat protein, free RNA and crosslinked nucleoprotein was subjected to tryptic digestion. The amount of enzyme was increased to allow for the additional protein. On a 15% SDS/polyacrylamide gel, the digested complex migrated slightly above the free RNA, suggesting that the peptide was quite small and that the digestion was successful. Following the digestion, the crosslinked fragment was isolated from the other peptide fragments via ion exchange chromatography, gel purification, and electrolytic blotting from the gel onto a PVDF protein transfer membrane. Calculations based on the number of c.p.m. that remained indicated that approximately 100 to 500 pmol of material were present.

The results of Edman degradation of the tryptic fragment and the peptide sequence consistent with the data are shown in Figure 3. The sequence report shows blanks for a tyrosine corresponding to the 85 position of the coat protein and a cysteine corresponding to the 101 position. The microsequencing utilizes an HPLC profile of the derivatized amino acids. An amino acid that has a modification, such as the covalently attached RNA in this experiment, is represented as a blank because it elutes at a different position than the other 20 amino acids that are recognized. The data strongly support photocrosslinking to Tyr 85 because of the blank in the data for the second amino acid residue in the peptide which should have been a Tyr and because of the intensity of the signals observed for the amino acid residues on either side of the blank.

The cysteine of the tryptic fragment was not methylated and thus was not recognized in the Edman analysis independent of crosslinking. Consequently, the data do not rule out Cys 101 as another possible site of crosslinking. Earlier model studies with cysteine and cystine derivatives indicated that cysteine reacts with 5-bromouracil excited at 308 nm to give uracil and cystine and that cystine reacts to give the analogue of a photocrosslink, 5-(S-cysteinyl)uracil (24). Although the model studies do not predict photocrosslinking to Cys 101, they are not necessarily an accurate measure of potential reactivity within a nucleoprotein complex where only a single Cys may reside; the balanced equation for the model reaction shows two cysteines. In spite of this ambiguity, photocrosslinking to Cys 101 is unlikely because the X-ray structure of the coat protein dimer (20) shows that Cys 101 is located in the α-helix on the outside of the virus and not in the β sheets facing inward. Further evidence against photocrosslinking to Cys 101 comes from studies with a variant coat protein (*vide infra*).

Synthesis and 308 nm irradiation of RNA 1 bound to a variant of R17 coat protein with a Tyr 85 to Ser 85 mutation

The specificity of the photocrosslinking reaction was tested with a variant of the coat protein in which the tyrosine at position 85 was replaced with a serine. This mutation was selected because model studies of the photocrosslinking reaction predict no photocrosslinking to a serine residue. Further, the hydroxyl group of the serine residue might preserve at least some of the hydrogen

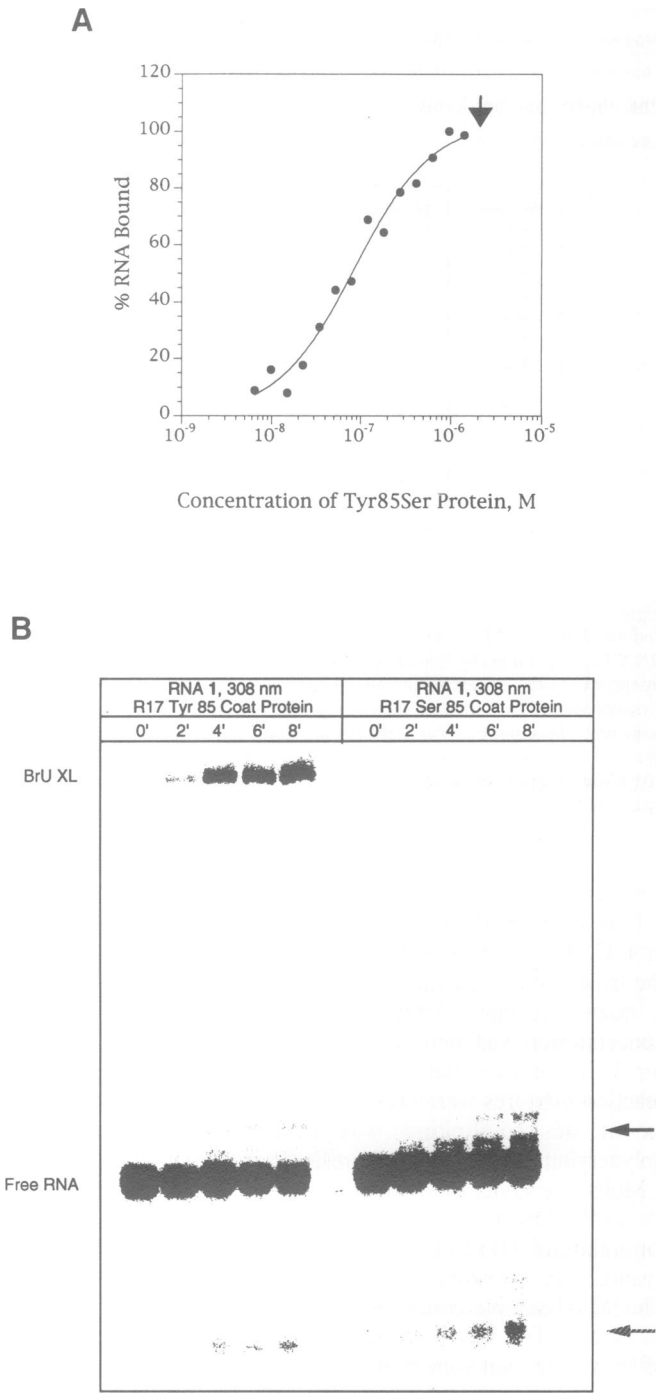

Figure 4. (**A**) Protein excess binding curve for Tyr 85 to Ser 85 variant coat protein using ^{32}P-labeled RNA 1 in TMK buffer at 4°C. The points fit a bimolecular equilibrium with a K_d of 86 nM, compared to a K_d of 250 nM for the non-bromouracil substituted RNA. The black arrow shows the concentration at which photocrosslinking was performed. (**B**) A phosphor imager scan of a 15% polyacrylamide denaturing gel showing the time course (0, 2, 4, 6 and 8 min) of irradiation at 308 nm of RNA 1 — R17 coat protein complex (lanes 1–5) and RNA 1 — Tyr 85 to Ser 85 variant coat protein complex (lanes 6–10). The free RNA and crosslinked nucleoprotein complex bands are labeled on the left side. The solid arrow on the right indicates bands possibly resulting from intramolecular RNA–RNA photocrosslinking, and the hashed arrow indicates bands possibly resulting from RNA photocutting.

bonding in the nucleoprotein complex anticipated from the hydroxyl group of the tyrosine.

For convenience, this variant of the coat protein contained two mutations, Val75Glu and Ala81Gly, in addition to the desired Tyr85Ser. These changes prevent the coat protein from assembling into viral capsids but do not affect RNA binding (24). The protein was obtained by cloning a mutagenic oligonucleotide containing the Y85S substitution into the *Bgl*II−*Eco*RI fragment of the parent plasmid bearing the V75E and A81G substitutions. The clones were transformed into BL21(DE3) pLYSs cells designed to overexpress coat protein gene. A nitrocellulose filter binding curve for the variant coat protein binding to RNA 1 is shown in Figure 4A. Specific binding of the variant protein to RNA 1 is indicated (K_d = 86 nM); however, the variant binds with lower affinity than the wild type protein [K_d = 3.5 nM (14)].

Nucleoprotein complexes of RNA 1 with the variant R17 coat protein and with wild type protein as a control were prepared. Sufficient concentrations of both proteins were used to insure complete binding to the RNA. Both complexes were irradiated at 308 nm and time points were taken at 0, 2, 4, 6 and 8 min. The time course of the reaction is shown in Figure 4B. The percent crosslinking was quantitated with a phosphor imager and found to be 27% for the control and 0% for the variant protein, both after 8 min of irradiation. The absence of photocrosslinking to the variant protein with Ser at position 85, maintaining Cys at position 101, further supports photocrosslinking to Tyr 85 and no photocrosslinking to Cys 101 in the wild-type protein.

The gel in Figure 4B also shows some weak bands slightly above the free RNA, indicated with a solid arrow in the figure, which increase with irradiation time. These bands are especially evident with the RNA bound to the variant protein. The structure of the materials giving rise to these bands has not been identified; however, possibilities are RNAs bearing intramolecular crosslinks. Intramolecularly crosslinked RNAs might migrate with lower mobility than free RNA on a denaturing gel. With excitation at 308 nm, the 5-bromouracil chromophore reacts primarily by abstracting a single electron from an oxidizable group nearby; covalent bond formation results from the subsequent reaction of the resulting radical ion pair (19, 20 and 25). In the absence of an oxidizable tyrosine near the bromouracil, the excited bromouracil may abstract an electron from an adenine or guanine group in proximity with subsequent formation of an intramolecular RNA−RNA crosslink. The gel also shows an irradiation dependent weak band significantly below the free RNA band, indicated with a hashed arrow in the figure. This band is also stronger with the variant protein and probably represents photocutting of the RNA. A model reaction in the literature for intramolecular nucleic acid photocrosslinking is the photocoupling of 5-bromo-2′-deoxyuridine with 2′-deoxyadenosine in a frozen aqueous medium (26). The frozen medium likely forced the 5-bromo-2′-deoxyuridine and 2′-deoxyadenosine molecules into the required close proximity for photocoupling. The 5-bromouracil group of RNA 1 may be in close proximity to an adenine group such as the one at position −7. An analogy for the proposed RNA nucleic acid photocutting is the report of single strand photocleavage of a duplex DNA specifically between a deoxyadenosine 5′ to a 5-bromo-2′-deoxyuridine (BrdU) (27). The adenosine at position −6 of RNA 1 is 5′ to the 5-bromouridine. Both of these reactions from the literature have been proposed to occur via an initial electron transfer from an adenine group to a 5-bromouracil group (26,27).

Protection by *lac* repressor protein of singly BrdU substituted operator DNAs from photo cutting was observed by Matthews and co-workers (9) for most substitutions, even those which did not lead to photocrosslinking to protein. However, one substitution led to photocrosslinking to a Tyr residue without protection from DNA photo cutting. Simple comparison of photochemical results of the *lac* system with the experiments reported here is difficult because the *lac* experiments were performed with 254 nm light. Hence, both the bond homolysis and electron transfer mechanisms of the 5-bromouracil chromophore were operating.

Discussion of photocrosslinking experiments and R17 structure

Earlier experiments with R17 RNA−protein complex and with model photochemical reactions (14, 19, and 20) pointed to a tyrosine or tryptophan residue as the site of crosslinking. A histidine residue would also have been a possibility, except the coat protein contains no histidines. In the crystal structure of the near identical MS2 phage coat protein, the majority of Trp and Tyr residues are located within the protein interior (21); however, two tyrosines are accessible to the RNA. Tyrosine 85 is located on the βG sheet, on the RNA interaction side of the protein. This is a region of the protein that has been shown to be near electron density attributed to the RNA in the crystal structure (21). The other accessible tyrosine is Tyr 129 located at the carboxy terminus of the protein in a region that may be flexible and could have been in contact with the −5 position of the RNA.

The Edman degradation data, together with the result of the crosslinking experiment with Tyr85Ser variant protein, now indicate that photocrosslinking of RNA 1 occurs to Tyr 85 of one of the coat proteins of the dimer. Although the coat protein complexes as a dimer, the dimer has a C_2 axis of symmetry, and consequently, the two Tyr 85s are indistinguishable even to the chiral RNA. The sum of experiments reported here further suggest that in the assembled R17 bacteriophage, the −5 position of the translational operator of the replicase gene is in proximity to Tyr 85 of a coat protein. The earlier model studies of 5-bromouracil photocrosslinking established covalent bond formation between the 5-position of the uracil ring and the position ortho to the hydroxyl group of Tyr (20). Consequently, we suspect that even these atomic positions are close in the nucleoprotein complex and in the assembled phage. The observation that the Tyr85Ser variant binds to RNA 1, but less well than the wild-type protein, further suggests that interactions between the Tyr phenolic hydroxyl and the uridine at position −5 relative to the start codon for the replicase translational operator exists in the assembled phage. Interestingly, position 85 of a number of other bacteriophage coat proteins including MS2, f2, fr, JP 34, GA, and QB is occupied by a tyrosine residue (29), and the nucleoprotein contact described here for R17 may occur in an identical or analogous fashion in these other phages as well. Preliminary experiments indicate that GA coat protein also forms a high yield photocrosslink with RNA 1.

The lack of photocrosslinking of RNA 1 to the Tyr85Ser variant protein is also of interest with respect to the development of the general BrU photocrosslinking technique for establishing point contacts in nucleoprotein complexes. This experiment demonstrates that even with close proximity of a Ser residue to a 308 nm excited 5-bromouracil chromophore in a nucleoprotein complex, no photocrosslinking occurs. This is consistent with small molecule, model reactions which would predict that at best

the primary alcohol group of a serine residue would serve to reduce a 308 nm excited bromouracil group to a uracil group (19,28).

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