A PROTEIN-NUCLEIC ACID CROSSLINK IN 30S RIBOSOMES

R.A. Kenner*

Département de Biologie moléculaire
Université de Genève
Geneva, Switzerland

Received February 26,1973

Summary

The data indicate that probably a single 30s ribosomal protein has been crosslinked to the 3'-ribosyl terminus of 16s RNA in intact 30s ribosomes. The crosslink was made by oxidizing the 3'-ribosyl moiety of 16s RNA to a dialdehyde with $NaIO_4$. One of the aldehyde groups generated in the oxidation reacted with an α- or ε-amino group of an adjacent protein to form a Schiff's base. The Schiff's base was reduced with $NaBH_4$ to a stable, specific covalent crosslink between the protein and the 16s RNA molecules. The crosslinked protein was tentatively identified as S1 according to the nomenclature of Wittmann *et al.* (1971), Mol. Gen. Genetics *111*, 327.

In order to understand the events which take place on ribosomes during protein synthesis, it has become evident that precise information on the positions of individual proteins, relative to each other and to ribosomal RNA, will be required. The use of crosslinking agents, binding together neighboring proteins or RNA and proteins, appears to offer useful means toward this end (1). The next step in ordering or mapping proteins on RNA in ribosomes is to study protein-16s RNA interactions by protein-RNA fragment binding (2-4) or chemical crosslinking experiments. In this report a crosslink between the 3'-terminus of 16s RNA and probably a single ribosomal protein in intact 30s ribosomes is described. This crosslink, by virtue of its location at one terminus of the RNA, not only gives information on protein-RNA interaction but also yields a starting point on which orientation of proteins on RNA can be made.

Experimental

Salt-washed ribosomal subunits from a ribonuclease I deficient strain

* Present address: Department of Oceanography, University of Washington, Seattle, Washington 98195.

of *E. coli* (MRE 600) (5) were prepared as previously described (6). Subunits were dialyzed just before use against 50 mM NaCl--20 mM $MgCl_2$--1 mM Tris-HCl, pH 8.0 to remove all K^+ which would precipitate periodate.

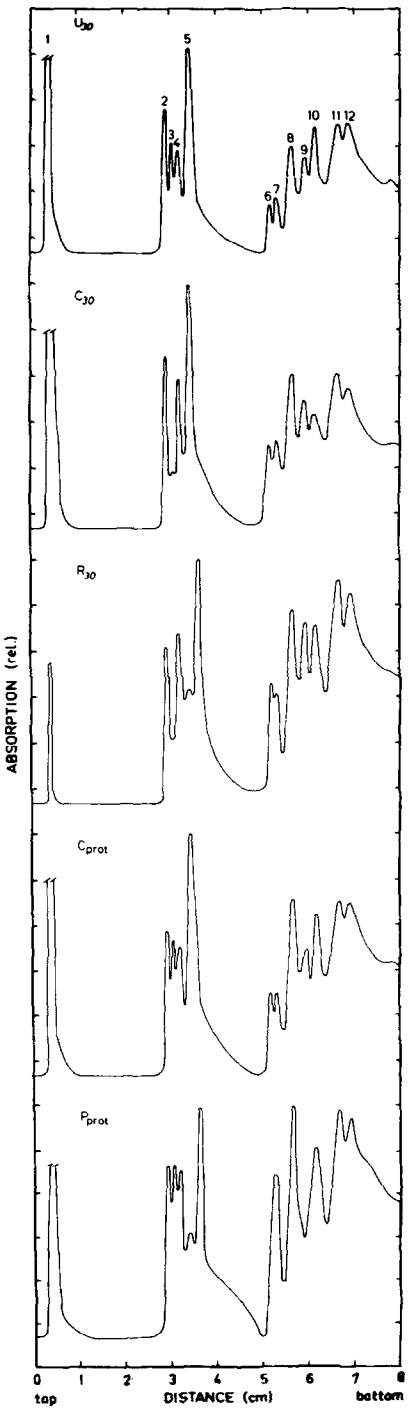
Intact ribosomal subunits (8-10 mg/ml) were oxidized with 0.09 M $NaIO_4$ buffered at pH 6.5 with 50 mM sodium phosphate--20 mM $MgCl_2$ using essentially the conditions described for oxidizing the 3'-ribosyl moiety of polyadenylic acid (7). The oxidation was carried out with stirring in dark at 22° for usually ten min, and oxidation was terminated by addition of 1 M KCl to a final concentration of 0.2 M. The reaction mixture was placed at 0° for ten min before centrifuging out precipitated KIO_4. After an additional forty-five min at 0°, sufficient 1 M Tris-HCl, pH 8.0, was added to the oxidized material to raise the pH to 8.0. After thirty min at 22°, the solution was again chilled to 0°, made 20 mM in $NaBH_4$ and left for fifteen min. Modified ribosomal subunits were dialyzed at 3° against several changes of 50 mM NaCl--10 mM $MgCl_2$--10 mM Tris-HCl, pH 8.0 to remove borates and traces of periodate. The modified ribosomal subunits were dissociated and analyzed by sodium dodecyl sulfate (SDS[1]) (0.1%) stacking disc gel electrophoresis. SDS disc gels were made essentially as described by Laemmli (8). Refer to the legend of Fig. 1 for details.

Results

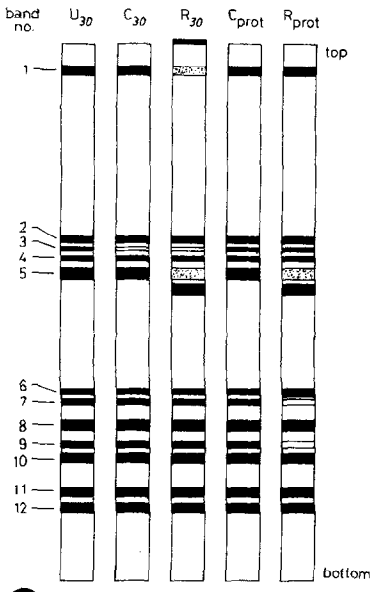
Protein which may crosslink to the oxidized 3'-terminus of the RNA molecule can be evaluated by use of SDS disc gels. In these gels, 16s RNA molecules migrate through the upper stacking gel and remain at the interface between the lower and upper gels. The presence of RNA at the interface is evident from the very strong UV absorption prior to staining for protein. Thus proteins which crosslink with RNA should stain at the interface and should be revealed as missing or depleted bands in the lower gel.

A time-course of $NaIO_4$ oxidation of 30s ribosomal subunits was made

[1] Abbreviations: SDS, sodium dodecyl sulfate; poly U, polyuridylic acid.



1



2

Figure 1. Scans of Polyacrylamide Gels of Proteins from modified 30s Ribosomes. The lower gels were 15% acrylamide and the upper gels were 3% acrylamide. The best results were obtained by electropphoresing the tracking dye through the upper gel at 1 mA/tube and through the lower gel at 2 mA/tube. Samples of ribosomal subunits or protein with approximately 2 ug of protein per band were applied to each gel after dissociation by heating to 90° for three min in 0.2 ml of 10% glycerol--5% 2-mercaptoethanol--3% SDS--0.063 MTris. Polyacrylamide gels were analyzed for protein by staining with Coomassie Brilliant Blue and scanning the gels in a Gilford 2400 spectrophotometer. U, C and R correspond to untreated, borohydride-treated and periodate-borohydride-treated ribosomes, respectively. The subscript "30" refers to treatment of intact 30s subunits and the subscript "prot" to treatment of proteins of dissociated 30s subunits. Due to technical problems of scanning gels at interfaces, absorption corresponding to RNA-protein complex is not shown in the Fig.

Figure 2. Summary and Comparison of Polyacrylamide Gels of Proteins from Modified 30s Ribosomes. The details are the same as those given in Fig. 1. The band numbers correspond to those given above each protein peak in Fig. 1. Blank or stippled bands indicate missing or significantly reduced bands, respectively.

using times between three and one hundred min of oxidation. The oxidation was probably complete in three min as judged by presence and position of protein-staining bands in SDS gels. A standard time of ten min oxidation was chosen for all remaining experiments.

SDS gels of oxidized and reduced 30s ribosomes reveal that certain protein bands are either missing, displaced or remain at the interface between upper and lower gels along with RNA (Fig. 1). Bands 1 and 3 of oxidized and reduced 30s ribosomes (scan R_{30}, Fig. 1) are essentially missing from the scan while band 5 is slightly displaced toward the lower molecular weight proteins as compared to untreated ribosomes (U_{30}). Although not shown in scan R_{30} (see legend to Fig. 1), *protein-stained material remains at the interface* along with the RNA. A control experiment (scan C_{30}, Fig. 1) in which robosomes were treated with all the chemicals used for R_{30} except $NaIO_4$ shows that band 3 is missing but band 1 and 5 are unaffected and *no protein material stains at the interface.*

Another type of control experiment was made on HOAc-extracted protein mixtures derived from dissociated 30s ribosomes (9). The only difference in treatment of protein mixtures and intact ribosomes is that 6 M urea was added to keep the proteins in solution. The results are shown in Fig. 1. Proteins which were only $NaBH_4$-treated and not oxidized are shown as scan C_{prot}. The gel is identical to the one for proteins from untreated, control ribosomes (U_{30}). The gel of proteins both oxidized and reduced, R_{prot}, shows that as with intact ribosomes band 5 is displaced; but, in addition, bands 7 and 9 are missing.

For convenience, the results in Fig. 1 are summarized and compared in

Oxidized and reduced 30s ribosomes (R_{30}) bind only about 10% as much poly U as untreated 30s ribosomes (U_{30}) (Table 1). Treatment of 30s subunits with only the $NaBH_4$ reduction step results in only a small effect on poly U binding. These $NaBH_4$-treated ribosomes (C_{30}) bind 75% as much poly U as untreated ribosomes (U_{30}) (Table 1).

Table 1

BINDING OF POLYURIDYLIC ACID TO MODIFIED RIBOSOMES

Ribosomes	cpm	% of unmodified ribosomes
U_{30}	1250	100
C_{30}	940	75
R_{30}	100	8
U_{70}	1425	100
C_{70}	1475	103
R_{70}	1250	91

The Millipore filter method of Moore (10) was used to determine the capacity of modified ribosomes to bind tritiated poly U. U, C and R correspond to untreated, borohydride-treated and periodate-borohydride-treated, respectively. The type of ribosome is indicated by a subscript 30 or 70 for 30s or 70s ribosomes.

Ribosomal 70s particles were also $NaIO_4$-oxidized and $NaBH_4$-reduced, but it was not possible to analyze the effects of the treatments on SDS gels as was done with the smaller 30s subunit. There is, however, no significant effect of either oxidation and reduction or reduction alone on poly U binding by 70s ribosomes (Table 1).

Discussion

$NaIO_4$ treatment of intact 30s ribosomes oxidizes the 3'-ribosyl group to a dialdehyde. One or both of the aldehyde groups are capable of reacting with a nearby α- or ε-amino group to form a Schiff's base. The resulting protein-nucleic acid crosslink is stabilized by $NaBH_4$ reduction *after* the crosslink has already been made; thus any secondary modification caused by $NaBH_4$ is of no consequence to the crosslink experiment.

Tentatively, a single protein appears to be covalently attached to the 3'-terminus of oxidized 16s RNA in intact 30s ribosomes. The crosslinked protein, illustrated by its absence from its usual position and concomitant appearance of a protein-staining band associated with RNA at the top of SDS gels (Fig. 1 & 2), is the largest of the 30s ribosomal proteins. It corresponds to protein P1 of Nomura *et al.* (11) and S1 of Wittmann *et al.* (12).

Ribosomal 30s subunits do not appear to undergo gross structural changes

on forming a crosslink of nucleic acid with protein, but there is at least one function which is modified. Modified 30s subunits do not bind poly U (Table 1). Loss of ability to bind poly U following chemical modification of protein S1 by Rose Bengal photooxidation was also observed by Noller *et al.* (13). Moreover, addition of S1 to 30s subunits deficient in that protein stimulates poly U binding (14). This protein may be involved in poly U binding (13), but the data do not allow a distinction to be made between a direct and an indirect involvement of the protein in that function.

As with most chemical modifications, the treatments reported here are not without some side reactions. Periodate treatment modifies only one protein (in addition to oxidizing RNA) in intact 30s ribosomes, but the oxidation affects at least three protein bands in dissociated ribosomes. Thus it appears that the intact structure of the 30s ribosome protects certain protein adjuncts (sugars?) from periodate oxidation.

With due consideration of the pitfalls of chemically modifying biological material, $NaIO_4$ oxidation and $NaBH_4$ reduction of various ribonucleoprotein complexes should prove a valuable tool as a first approach in illucidating the structures of intact ribonucleoprotein complexes in solution.

Acknowledgment

The author is grateful to Dr. Alfred Tissières for his advice and generous encouragement during the course of this investigation. Special thanks are due Dr. Josef Eisinger of Bell Laboratories for his involvement in the conception of this study and for his help and encouragement during the early stages. This work was supported by grants to Dr. Alfred Tissières from the Fonds National Suisse de la Recherche Scientifique.

References

1. Bickle, T.A., Hershey, J.W.B., and Traut, R.R. (1972), Proc. Nat. Acad. Sci. U.S. *69*, 1327.
2. Zimmermann, R.A., Muto, A., Fellner, P., Ehresmann, C., and Branlant, C. (1972), Proc. Nat. Acad. Sci. U.S. *69*, 1282.
3. Schendel, P., Maeba, P., and Craven, G.R. (1972), Proc. Nat. Acad. Sci. U.S. *69*, 544.
4. Brimacombe, R., Morgan, J.M., and Cox, R.A. (1971), Eur. J. Biochem. *23*, 52.
5. Cammack, K.A., and Wade, H.E. (1965), Biochem. J. *96*, 671.
6. Kenner, R.A., and Aboderin, A.A. (1971), Biochem. *10*, 4433.
7. Millar, D.B.S., and Steiner, R.F. (1965), Biochim. Biophys. Acta *102*, 571.

8. Laemmli, U.K. (1970), Nature *227*, 680.
9. Hardy, S.J.S., Kurland, C.G., Voynow, P., and Mora, G. (1969), Biochem. *8*, 2897.
10. Moore, P.B. (1966), J. Mol. Biol. *18*, 8.
11. Nomura, M., Mizushima, S., Ozaki, M., Traub, P., and Lowry, C.V. (1969), Cold Spr. Harb. Symp. Quant. Biol. *34*, 49.
12. Wittmann, H.G., Stoeffler, G., Hundennach, I., Kurland, C.G., Randall-Hazelbauer, L., Birge, E.A., Nomura, M., Kaltschmidt, E., Mizushima, S., Traut, R.R., and Bickle, T.A. (1971), Mol. Gen. Genetics *111*, 327.
13. Noller, H.F., Chang, C., Thomas, G., and Aldridge, J. (1971), J. Mol. Biol. *61*, 669.
14. Van Duin, J., and Kurland, C.G. (1970), Mol. Gen. Genetics *109*, 169.