MOLECULAR RULERS WITH 5 OR 11 PROLINES INTRAMOLECULARLY CROSSLINK TO G45 OF YEAST PHENYLALANINE tRNA

Thomas D. YAGER, Michael A. REUBEN[†], Parviz R. AINPOUR[+] and Eric WICKSTROM*

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, [†]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, [+]Howmedica, Inc., Groton, CT 06340 and **Recombinant DNA Division, Southern Medical and Pharmaceutical Corp., 3500 E. Fletcher Ave., Tampa, FL 33612, USA*

Received 10 June 1981; revision received 17 August 1981

1. Introduction

To probe the solution structures of biological macromolecules, we have developed a series of rigid, variable-length oligoproline crosslinking reagents, which we call molecular rulers [1]. We have reported the synthesis of oligoprolines having lengths of 5,11 and 15 residues [2], their modification at the N-terminus by chlorambucil (CAB), their attachment through the C-terminus to phenylalanyl-tRNA^{Phe} (yeast), and the intramolecular crosslinking of tRNA which is effected by the attached reagents [1]. We recently identified the sites in tRNA^{Phe} (yeast) which are crosslinked by molecular rulers containing zero and fifteen proline residues [3]. CAB-Phe, which is 15 Å long, crosslinked to G71 and A73, while CAB-$(Pro)_{15}$–Phe, which is 62 Å long, crosslinked to G20 and Y37.

These results agreed reasonably well with the crystal structure of yeast tRNA^{Phe} [4,5], assuming that the rulers were rigid, and that the tRNA in solution has some flexibility in the anticodon loop, and considerable flexibility in the aminoacyl end. Thus it appeared valid to use molecular rulers as structural probes.

We report here further measurements on yeast tRNA^{Phe}, which were obtained with rulers containing 5 and 11 prolines. In both cases, the only prominent site of crosslinking was G45.

* To whom reprint requests should be addressed

Fifth paper in the series, 'Molecular Rulers for Measuring RNA Structure'; paper 4 is [3]

2. Materials and methods

CAB-$(Pro)_n$–[^{3}H]Phe-tRNA^{Phe} (yeast) was synthesized as described [1] in 80% Me_2SO (n = 5) or in 65% Me_2SO and 15% $CHCl_3$ (n = 11), the particular conditions chosen to minimize intramolecular crosslinking during the reaction [1], and to maximize yield [6]. Intramolecular crosslinking in 1.0 mM $Mg(OAc)_2$, 10 mM NH_4OAc (pH 6.0) was carried out for 4 h at 37°C [1]. The sites of crosslinking were determined by rapid gel sequencing [3,7,8]. Labeling of the 3′-OH of crosslinked tRNA molecules with cytidine-3′,5′-[5-^{32}P]bisphosphate (New England Nuclear) [9] was allowed to proceed for 48 h to allow deacylation to occur in the labeling reaction mixture. This eliminated the need for a separate deacylation step. The labelling reaction was catalyzed by T4 RNA ligase; preparations of this enzyme from P-L Biochemicals and New England Biolabs were contaminated by an RNA-exonuclease activity, which we suspect removed the last few unpaired nucleotides of the tRNA 3′-terminus. In sequencing reactions, the alkylation of purine bases was achieved with chlorambucil [1], instead of with dimethyl sulfate. After the aniline-catalyzed chain-scissions, the aniline was removed by EtOH precipitation instead of lyophilization [7].

3. Results

In fig.1, lane 4 shows the rapid gel sequencing pattern of intramolecularly crosslinked CAB-$(Pro)_5$–[^{3}H]-Phe-tRNA^{Phe}. This pattern was obtained after 4 h of crosslinking. As a control for any alkylation which

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

Fig.1. Autoradiogram of yeast Phe-tRNA^{Phe}, crosslinked by a $(Pro)_5$ ruler and then analyzed by rapid gel sequencing [7]. CAB-$(Pro)_5$–[^{3}H]Phe-tRNA^{Phe} (yeast) was synthesized and allowed to crosslink intramolecularly for 4 h as in [1]. To control for crosslinking during the initial attachment of the ruler to the tRNA, a second sample was synthesized and allowed to crosslink for 0 h. The crosslinked tRNA samples, and also native tRNA, were 3′-OH end-labelled with cytidine-3′,5′-[5-^{32}P]bisphosphate [9], and then sequenced [7]; in sequencing, CAB substituted for dimethyl sulfate as a purine-alkylating reagent, to allow detection of the sites of crosslinking. The 4 h crosslinking and 0 h crosslinking patterns effected by the CAB-$(Pro)_5$ ruler are shown in lanes 4 and 0, respectively. Lanes T1 (T1 digestion, for G), H (hydrazine/$NaBH_4$ treatment, for U and C), and A (alkaline hydrolysis, for all bases) were obtained from native tRNA, and are shown to help identify sites of crosslinking.

may have occurred during the initial attachment of the ruler to the tRNA [1,3], a sample which had been modified, but had not undergone crosslinking in aqueous solution (a '0 h' sample) was analyzed in lane 0. In [1] ~10% of the modified tRNA appeared to have been crosslinked during modification. Lanes T1 (T1 digestion, for G), H (hydrazine/$NaBH_4$ treatment, for U and C), and A (alkaline hydrolysis, for all bases) are present to help identify the sites of chlorambucil alkylation.

The pattern of reactivity in lane 0 closely resembles that for bimolecular chlorambucil alkylation (fig.2 in [3]). At G45, we found considerable reaction in the 4 h sample above that seen in the 0 h sample. No other nucleotide showed significantly darker bands in the 4 h sample, compared with the 0 h sample on this autoradiogram, or on another from a second gel of this sample (not shown).

Fig.2 shows a similar autoradiogram for tRNA^{Phe} crosslinked with the $(Pro)_{11}$ molecular ruler. The right-hand alkaline hydrolysis lane is delayed by 1/2–1 band, due to a late start at the top of the gel. We again found considerable reactivity at G45. The greater exposure of this autoradiogram, compared with that in fig.1, resulted in the G45 band in the 0 h sample being much more noticeable. The same results were seen when a second preparation of crosslinked tRNA^{Phe} was analyzed on another gel (not shown).

On the autoradiogram shown in fig.2, the G bands at the top of the gel appear darker in the 4 h sample than in the 0 h sample. However, this situation was

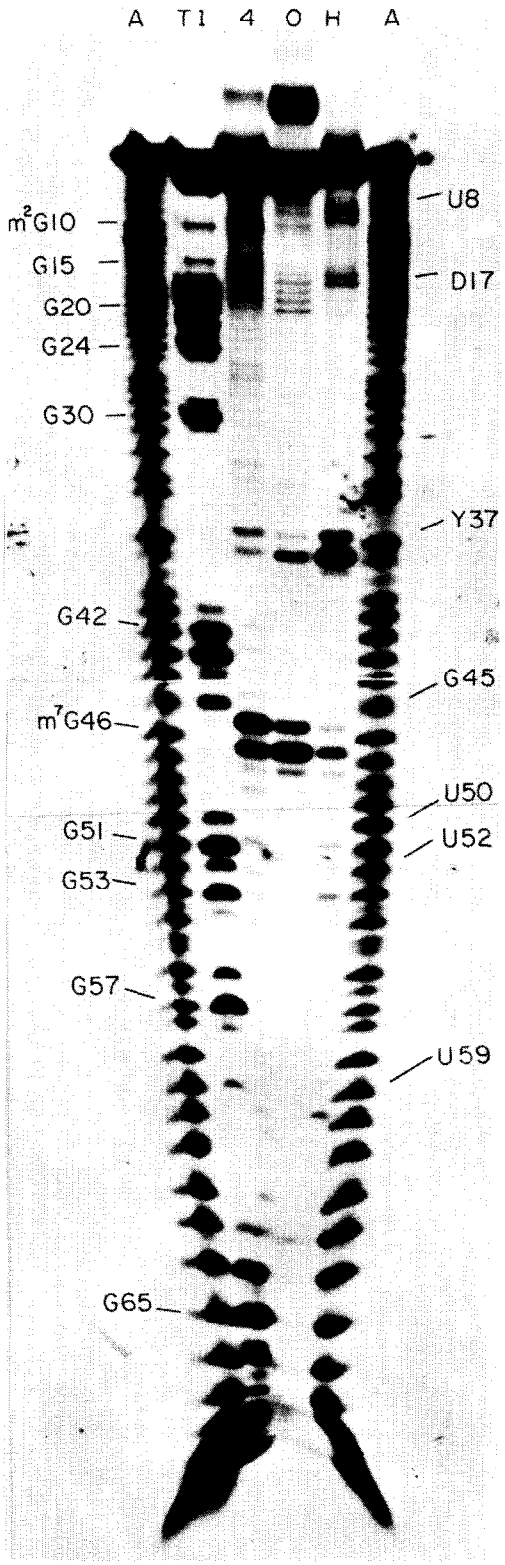

Fig.2. Autoradiogram of yeast Phe-tRNA^{Phe}, crosslinked by a $(Pro)_{11}$ ruler and then analyzed by rapid gel sequencing [7] as in fig.1.

reversed on the autoradiogram of the second preparation. Similarly, the A36 band is darker and the Y37 band is lighter in the 4 h sample, compared with the 0 h sample. Again, the situation was reversed on the autoradiogram of the second preparation. We do not know which step in the chemical sequencing causes this variability. However, the inconsistency of these bands, compared with the consistency of the G45 result, leads us to doubt that they indicate crosslinking.

4. Discussion

From the structure of poly(L-proline) II [10] and model building studies, we expect CAB-$(Pro)_5$ to be 31 Å long, and CAB-$(Pro)_{11}$ to be 49 Å long. An examination of atomic coordinates in the crystal structure of yeast tRNA^{Phe} (G. Quigley, personal communication) indicates that the N-7 of G45 is 58.6 Å distant from the 3′-OH. Thus a great deal of flexibility must exist to explain the crosslinking by both rulers at G45. From our data, we cannot determine whether the flexibility lies in the ruler or in the tRNA. It is consistent with solution studies of oligoprolines [11,12] and of tRNA [13,14] to ascribe flexibility to the tRNA, particularly at the aminoacyl terminus.

One might expect more crosslinking with the $(Pro)_{11}$ ruler than with the $(Pro)_5$ ruler, since the former comes closest in length to the 3′-OH-G45 distance in the crystalline tRNA. However, the yield of tRNA^{Phe} crosslinked with the $(Pro)_5$ ruler was 7%, while that for the $(Pro)_{11}$ ruler was only 3% [1]. One interpretation is that, in the solution structure of tRNA^{Phe}, G45 is more accessible to the $(Pro)_5$ ruler than to the $(Pro)_{11}$ ruler. Alternatively, due to the larger shell of space swept by the chlorambucil end of the $(Pro)_{11}$ ruler, the frequency of its interaction with the tRNA may be decreased enough to account for the lower yield.

Study of a space-filling model of tRNA which we constructed suggests an explanation for the crosslinking results above, and for those reported [3] for

rulers with zero and fifteen prolines. It is possible that, for tRNA in solution, the angle between the aminoacyl stem and the anticodon stem varies between the value of roughly 110° observed in the crystal [15] to ⩽90°. Such an extent of flexing, combined with freedom of motion of the 3′-ACCA terminus, may allow the 31 Å $(Pro)_5$ ruler and the 49 Å $(Pro)_{11}$ ruler to reach G45, even though the latter is 58.6 Å distant in the crystal structure. Similarly, such flexibility in solution may allow the 62 Å long $(Pro)_{15}$ ruler to reach Y37, which is 80.7 Å distant in the crystal structure [3]. The same 62 Å long ruler also reached G20, which is 60.1 Å distant in the crystal structure; however, this crosslinking occurred along the length of the short leg of the tRNA, whereas all the other crosslinking events observed occurred between the two legs.

Acknowledgements

We thank Dr Gary Quigley for communicating to us the crystal structure distance between G45 and the 3′-OH. This work was supported by grants from the US National Science Foundation (PCM 79-23471) and the US National Institutes of Health (GM 24128) to E. W.

References

[1] Reuben, M. A., Ainpour, P. R., Hester, H. L., Neveln, V. L. and Wickstrom, E. (1981) Biochim. Biophys. Acta 654, 11–25.
[2] Ainpour, P. R. and Wickstrom, E. (1980) Int. J. Pept. Prot. Res. 15, 225–235.
[3] Wickstrom, E., Behlen, L. S., Reuben, M. A. and Ainpour, P. R. (1981) Proc. Natl. Acad. Sci. USA 78, 2082–2085.
[4] Rich, A. (1977) Acc. Chem. Res. 10, 388–396.
[5] Kim, S. H. (1979) in: Transfer RNA: Structure, Properties, and Recognition (Schimmel, P. R. et al. eds) pp. 83–100, Cold Spring Harbor Laboratory, NY.
[6] Reuben, M. A., Kuznezov, I. J. and Wickstrom, E. (1979) Biochim. Biophys. Acta 565, 219–223.
[7] Peattie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 1760–1764.
[8] Donis-Keller, H., Maxam, A. M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527–2538.
[9] Bruce, A. G. and Uhlenbeck, O. C. (1978) Nucleic Acids Res. 5, 3665–3677.
[10] Cowan, P. M. and McGavin, S. (1955) Nature 176, 501–503.
[11] Schimmel, P. R. and Flory, P. J. (1967) Proc. Natl. Acad. Sci. USA 58, 52–59.
[12] Stryer, L. and Haugland, R. P. (1967) Proc. Natl. Acad. Sci. USA 58, 719–726.
[13] Clark, B. F. C. (1978) in: Transfer RNA (Altman, S. ed) pp. 14–47, MIT Press, Cambridge MA.
[14] Ehrenberg, M., Rigler, R. and Wintermeyer, W. (1979) Biochemistry 18, 4588–4599.
[15] Ladner, J. E. (1978) in: Biochemistry of Nucleic Acids II, (Clark, B. F. C. ed) vol. 17, pp. 1–18, University Park Press, Baltimore MD.