Crosslinking of tRNA containing a long extra arm to elongation factor Tu by *trans*-diamminedichloroplatinum(II)

Nils-Jørgen Rasmussen, Friedrik P.Wikman and B.F.C.Clark
Department of Biostructural Chemistry, Institute of Chemistry, University of Aarhus, Denmark

Received March 29, 1990; Revised and Accepted July 17, 1990

ABSTRACT

A tRNA containing a long extra arm, namely *E. coli* $tRNA_1^{Leu}$ has been crosslinked to elongation factor Tu, with the crosslinking reagent *trans*-diamminedichloroplatinum(II). The nucleotide involved in the crosslinking was identified to be a guanosine in the variable region at position 47F or 47G.

INTRODUCTION

The interaction between elongation factor Tu (EF-Tu) and aminoacyl tRNAs has been intensively studied by many different techniques. As part of the ternary complex aa-tRNA:EF-Tu:GTP, the tRNA is carried to the ribosome to add one aminoacid to the growing polypeptide chain. Since the factor is capable of interacting with all elongator tRNAs, it must recognize some of their common features. Apart from interaction with the 3-CCA end and the aminoacyl residue, it has been shown that a large part of the tRNA is in contact, or at least placed in the vicinity of the protein factor(1). The tRNA regions that could interact with the factor are the stem region comprised of the amino acid stem and the T-stem, and also the area around the variable loop(1). The latter finding was rather surprising because there is a great variation of size in this region. Thus we found it important to investigate the interaction of EF-Tu and a tRNA with a long extra arm.

The crosslinking reagent *trans*-diamminedichloroplatinum(II) (*trans*-DDP) has previously been successfully used in crosslinking experiments with nucleic acid-protein complexes(2,3). Earlier experiments on the aa-tRNA:EF-Tu:GTP complex have revealed some areas of the molecules that are positioned sufficiently close to allow crosslinking(4,5). In this work we have used a tRNA with a long extra arm, namely $tRNA_1^{Leu}$, and also improved the method as to allow determination of the actual crosslinking nucleotide. Using 2-dimensional PAGE for purification of oligonucleotides we obtained enough pure material for identification of the crosslinking nucleotide as a guanosine in the extra arm at position 47F or 47G. Taking into account, the proposed conformation of the long extra arm(6), we find that these results are in agreement with earlier footprinting results and also give support to the crosslinking experiments performed by Ofengand *et al.*(7).

MATERIALS AND METHODS

Pure *E. coli* B $tRNA_1^{Leu}$ was obtained from Subriden RNA, Rolling Bay, Washington, USA. *E. coli* EF-Tu was prepared according to Leberman *et al.*(8) and crude *E. coli* synthetases according to Bruton(9). α-[^{32}P]ATP (410 Ci/mmol), γ-[^{32}P]ATP (3000 Ci/mmol), [^{3}H]ADP (18 Ci/mmol), [^{3}H]GDP (11.7 Ci/mmol), [^{3}H]CDP (15.2 Ci/mmol), [^{3}H]UDP (12.5 Ci/mmol), [^{3}H]leucine (80 Ci/mmol) and T_4 polynucleotide kinase (EC 2.7.1.78) were all from Amersham, UK. Nucleotidyl transferase (EC 2.7.1.40) was a gift from H. Sternbach, Göttingen, FRG. RNase A (EC 3.1.4.22) and RNase T_2 (EC 3.1.27.1) were from SIGMA, London, UK. RNase T_1 (EC 3.1.27.3) from Sankyo, Japan. RNase B. cereus (EC 3.1.27.x), RNase Phy M (EC 3.1.27.x) and RNase U_2 (EC 3.1.27.4) from P-L Biochemicals, FRG. Pyruvate kinase (EC 2.7.1.40), non radioactive 5'-tri and diphosphates ATP, CTP, GTP, GDP, CDP, UDP, ADP (3',5'-), 1-O-octyl-β-D-glucopyranoside and phosphoenolpyruvate were from Boehringer Mannheim, FRG. Leucine, (2)[N-morpholino]-ethanesulfonic acid, trizma base, 2-mercaptoethanol, dithiothreitol and *trans*-diamminedichloroplatinum(II) from Sigma, London, UK. En3hance spray was obtained from NEN, USA. Diethylpyrocarbonate, dimethylsulfate and sodiumcacodylate from Fluka, FRG. Aniline (distilled), acrylamide and N,N'-methylenebisacrylamide from BDH, UK. Ammoniumpersulfate and N,N,N',N'-tetramethylethylenediammine from Bio-Rad, Ca., USA. Electrophoresis grade urea were purchased from Schwartz/Mann, N.Y., USA. Silane A174 from Pharmacia Fine Chemicals, Sweden. Ultrogel AcA 44 and 54 from LKB, Sweden. Separon Hema DEAE was obtained from Tessek, Aarhus, Denmark. All other chemicals were from Merck, Darmstadt, FRG.

Formation of the crosslinked complex

3'-[^{32}P]-labelling of tRNA, aminoacylation and ternary complex formation was as described(4). Pt-crosslinking of ternary complex and xTC purification was as in(4) with minor changes.

10% saturated *trans*-DDP was added to the purified TC (ex. 6000 pmoles TC_1^{Leu} 1–2 mM) and incubated at RT (21–22°C) in the dark for 15'. After incubation the material was adjusted to 0.15 M NaCl and 0.05% OGP, loaded on a 500 μl Separon Hema DEAE column and eluted as described. The eluates were counted, and the fractions containing the crosslinked complex xTC were combined.

RNase digestion and isolation of crosslinked oligonucleotides

The crosslinked ternary complex was digested with RNase A in the required concentration (0.10–2.50 u/pmoles xTC/μl) for 15'

at RT. The digestion was stopped by chilling on ice, and the sample was made 0.15 mM in NaCl and 6M in urea. The oligonucleotides still crosslinked to EF-Tu were isolated by chromatography at RT on a Ultrogel AcA 54 column (50×0.6 cm). Running buffer was 12.5 mM KH_2PO_4 pH 7.2, 0.15 M NaCl and 6 M urea. The fractions containing the crosslinked oligonucleotides were identified by monitoring the eluate at 254 nm, and the combined fractions were dialysed against 3×1 l H_2O at 4°C, and lyophilized.

5′-[^{32}P]-labelling of oligonucleotides and reversion of the crosslink

5′-end labelling was performed according to the method reported by Silberklang(10). Immediately after the T_4 kinase reaction 10 μl of 2M thiourea was added and incubation was proceeded at 37°C for 10'. The reaction was stopped by chilling on ice and extracted twice with 1 vol. phenol saturated with 0.3M NH_4OAc. 2 μl carrier-tRNA (5 mg/ml), 20 μl 0.3 M NH_4OAc and 500 μl cold ethanol was added, and the RNA was precipitated at −70°C.

Isolation of individual oligonucleotides

Individual oligonucleotides for sequencing were isolated by 2-dimensional PAGE essentially as reported by Lockard *et al.*(11) apart from recycling of the buffer in the first dimension, and the use of 1 mM EDTA in the second dimension. A film was exposed of the gel with radioactive position markers. This film was used for cutting out oligonucleotides from the gel. Extraction of the gel slices was with 2×50 μl 0.3 M NH_4OAc. After extraction, 5 μl carrier tRNA (0.2 mg/ml) and 500 μl cold ethanol was added, and the oligonucleotides were allowed to precipitate at −20°C overnight. Small oligonucleotides were precipitated at −70°C for several days. The oligonucleotides were dried and redissolved in 10 μl H_2O and the radioactivity was estimated by Cerenkov counting.

Sequence analysis

For determining the 5′-ultimate nucleoside in each oligonucleotide a complete RNase T_2 digest was performed, followed by thin layer chromatography on Cel300 PEI (20×20 cm sheets) in the buffers reported by Gupta & Randerath(12). The plates were run with radioactive nucleotide monophosphates as markers, and sprayed with EN3HANCE, before exposure.

Chemical sequencing of oligonucleotides was done according to Peattie(13). Enzymatic sequencing was done essentially as reported by Donis-Keller *et al.*(14,15) except that the digestion buffer for the RNase U_2 reaction was without dye markers.

RESULTS AND DISCUSSION

Deacylation of tRNA in ternary complex

In order to make sure that the ternary complex is intact in the course of platinum crosslinking, the rate of deacylation of the tRNA in the ternary complex was measured under similar conditions as used for the subsequent platination step.

In their report on the relative affinities of aminoacyl-tRNAs for EF-Tu:GTP, Louie *et al.*(16) showed that $tRNA_1^{Leu}$ has a five times higher dissociation constant than $tRNA^{Phe}$ at 4°C thus probably well under 10 nM based on the dissociation constants determined by fluorescence by Abrahamson *et al.*(17). While neither deacylated tRNA nor denatured Leu-$tRNA^{Leu}$ interact with EF-Tu:GTP(18,19), a dissociation constant of that order of magnitude should be low enough for keeping the charged $tRNA_1^{Leu}$ in ternary complex. This effectively protects against deacylation(20). Our deacylation measurements, using the nitrocellulose assay(16−18), resulted in radically longer halflives of the charged tRNA when compared to reported deacylation rates for 'free' aminoacyl-tRNAs. These results are indicative for the persistence of a ternary complex (data not shown).

Pt-crosslinking of ternary complex and xTC purification

The crosslinking of $tRNA_1^{Leu}$ was found to occur at somewhat lower *trans*-DDP concentration than found with $tRNA^{Phe}$. Addition of 0.15 M NaCl following the crosslinking step blocks further platinum crosslinking. The excess platinum is then removed by the extensive low salt elution of the Separon Hema DEAE column. Non crosslinked ternary complex dissociates at these salt concentrations and EF-Tu is eluted at 0.2 M salt while the tRNA elution peak lies around 0.55−0.7 M (data not shown), however noticeable spilling does occur around 0.45 M where the xTC elutes. The pooled xTC may therefore contain some additional free tRNA and perhaps small amounts of non crosslinked ternary complex. However any non crosslinked RNA is removed at the subsequent gel filtration step as described below.

RNase digestion of xTC and isolation of crosslinked oligonucleotides

In order to locate the crosslinking site(s) in the tRNA the pooled xTC was subjected to Pancreatic RNase A digestion at varying concentrations. Limited RNase digestion will create mixtures of oligonucleotides, some still being crosslinked to EF-Tu. Depending on the extent of digestion the oligonucleotides will be of variable length, the shortest occurring when most heavily digested. At each RNase concentration used, a pool of fragments of variable length still crosslinked to EF-Tu is thus also produced. In total these will theoretically produce a 'funnel shaped' set of oligonucleotides around the nucleotide where the crosslink occurs or if more crosslinking sites exist, a set of funnels of oligonucleotides. RNase A was chosen because it is a very potent RNase.

Isolation of the crosslinked oligonucleotides was performed by chromatography on an AcA 54 column. Ribonucleases almost inevitably create problems when trying to remove or alternatively inactivate them(21), and the problems are especially grave when using RNase A. In order to circumvent this obstacle the isolation of crosslinked oligonucleotides was done under denaturing conditions adding 6 M urea immediately after digestion. This greatly reduces the activity of RNase A and running the AcA 54 column under these conditions keeps the activity suppressed until the crosslinked oligonucleotides have been isolated. The use of 0.15 M NaCl and 6 M urea in the elution buffer also prevents any unspecific recrosslinking(3) and eliminates any noncovalently bound fragments in the exclusion limit peak containing the EF-Tu with crosslinked oligonucleotides.

5′-[^{32}P]-labelling of oligonucleotides and isolation of individual oligonucleotides

When labelling oligonucleotides using T_4 polynucleotide kinase the labelling intensity does not necessarily reflect the actual amounts of the different oligonucleotides present in the incubation mixture(22). However in these experiments only the 20 or so most intensively labelled oligonucleotides contained sufficient radioactivity to enable subsequent sequencing.

Taking into account the extreme number of oligonucleotides

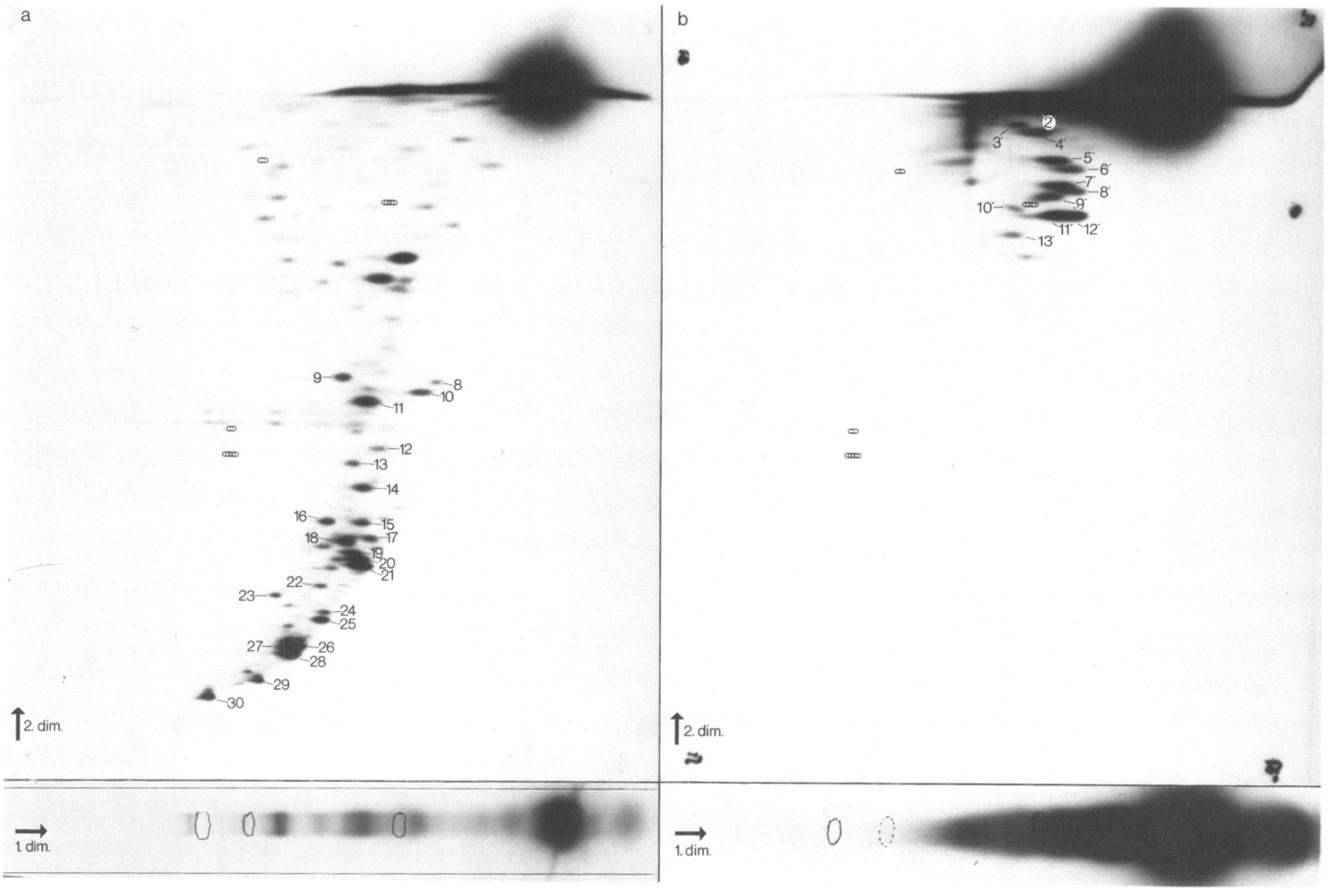

Figure 1. Two-dimensional PAGE of 5-end labelled oligonucleotides. The numbered fragments have been subjected to sequencing. Experimental details as described in methods. a: limited RNase A digested xTC_f^{Leu} (0.1 u/pmole xTC/μl). b: extensively RNase A digested xTC_f^{Leu} (2.5 u/pmole xTC/μl).

that may at this stage be crosslinked to EF-Tu, especially when only a limited digestion of tRNA has been performed, it is of crucial importance to have a highly discriminating method for isolating the individual oligonucleotides. Here 2 dimensional pH-shift PAGE is very suitable. This method utilizes the fact that the different nucleotide species in an oligonucleotide, have different net charges at some pH values. Running a first dimension at pH 3.5 will result in marked differences in the migration speed of oligonucleotides of the same length but with different C+A/U+G ratios. The first dimension gel is then followed by a conventional pH 8.3 second dimension gel, where the oligonucleotides are separated according to length. For a review see Wachter & Fiers(23).

In order to make reproducible 2D PAGE it proved necessary to circulate the buffer for the first dimension to avoid any pH gradient to build up during electrophoresis. This also eliminated streaking of fragments in the second dimension impairing the isolation of sufficiently pure oligonucleotides for sequencing.

Sequencing of oligonucleotides

The sequencing of two sets of oligonucleotides from crosslinking of aa-$tRNA_f^{Leu}$ in ternary complex with EF-Tu:GTP has been successfully completed (fig. 1).

The 5'-end group analysis, served as a fixpoint when establishing the location of the labelled RNA fragments. Examples of these used for sequencing crosslinked oligonucleotides from xTC_f^{Leu} are shown in fig. 2. As would be expected from the specificity of RNase A the predominant 5'-terminal group was adenine when only slightly digested, however when more heavily digested, guanine turned out to be the terminal group, almost without exception.

One modified nucleotide in $tRNA_f^{Leu}$ had a distinctly altered mobility compared to the mobilities of unmodified and normally occurring modified nucleotides. It was recognized by the subsequent sequencing of the fragment to be the nonidentified G* at position 37, 3'-proximal to the anticodon(24,25).

For the overall sequencing of oligonucleotides the chemical modification or Peattie method was chosen. The great advantage of this method lies in the fact that the reactions are almost insensitive towards second order interactions(13) and far less sensitive compared to the enzymatic sequencing method. Thus this method should be most suited for determining fractions of tRNAs with the inherent possibility of very strong interactions.

In order to pin down exactly where the funnel shaped cluster was positioned in the sequence it proved necessary to perform an enzymatic sequencing. The result showed that at least one fragment contained GGAC while the remaining contained either GGA, GAC or GG. This strongly suggest that all oligonucleotides are from the 3'-side of the extra arm, the only other possible site for some of the oligonucleotides being the 5'-side of the D arm where a GGAA stretch exists.

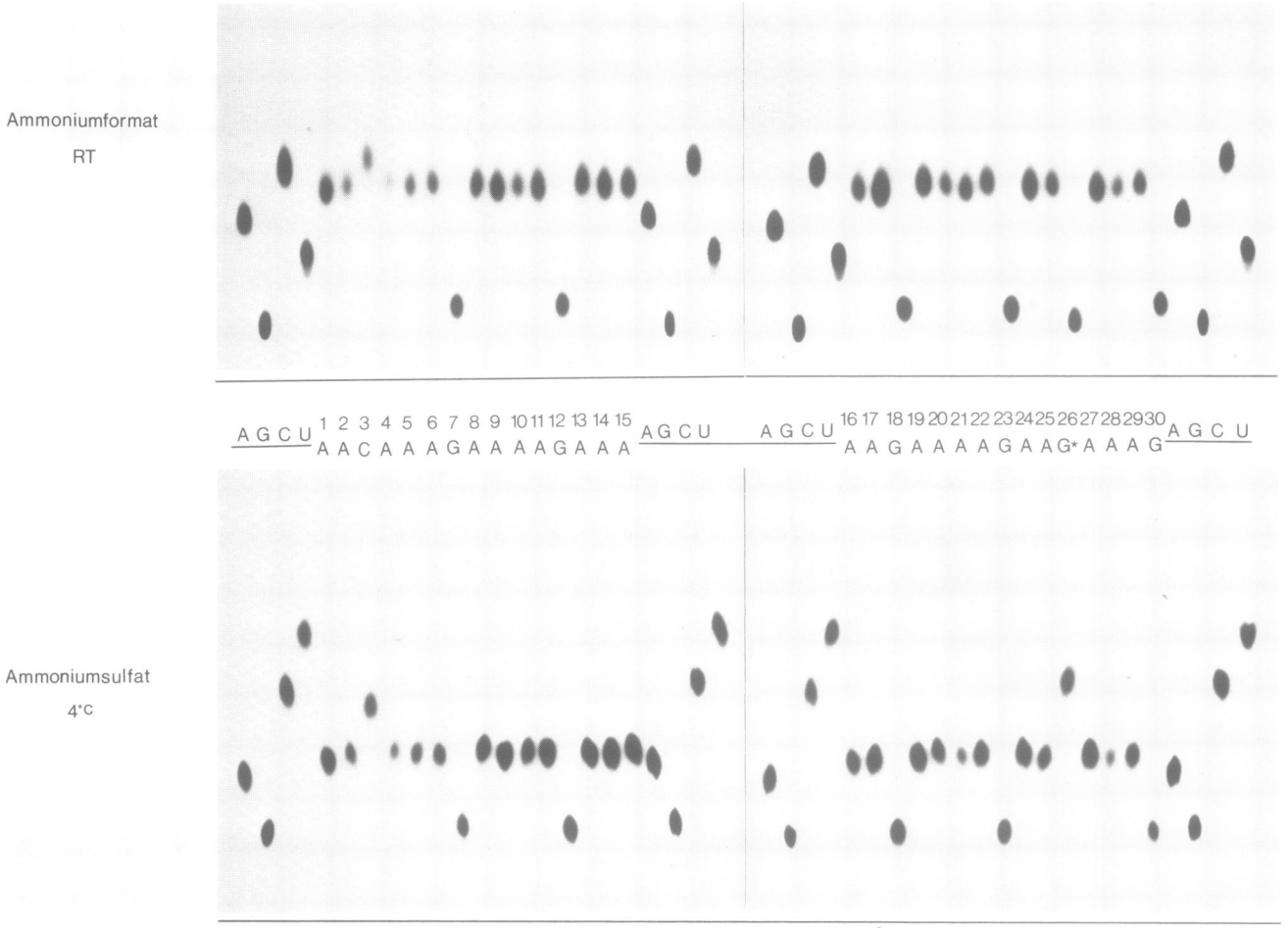

Figure 2. Thin layer chromatographs of 3',5'-diphosphate nucleosides from completely RNase T_2 digested 5'-end labelled oligonucleotides. Oligonucleotides are from the limited RNase A digested xTC_1^{Leu} experiment (fig. 1a). Experimental conditions as described in methods. The numbers in the figures (1−30) are used for identification of the parent oligonucleotide. AGCU depict standard 3'-diphosphate nucleosides.

Returning to the slightly RNase A digested crosslinking experiment it is clear that some oligonucleotides were from the 5' half of the tRNA. These fragments, that appear as weak spots on figure 1a, can all be explained as having been cut off longer oligonucleotides or intact tRNA after the AcA 54 column by small amounts of RNase possibly during the 5'-end labelling procedure where the crosslinked oligonucleotides are kept at 37°C for a prolonged time. Alternatively a cleaved tRNA or fragment may still even under 6M urea denaturing conditions be bound by the strong secondary and tertiary interactions in tRNA (or even by interstrand *trans*-DDP crosslinks(26)) to the rest of the complex containing the EF-Tu crosslinked oligonucleotide.

That these fragments were 5'-labelled to a considerably lower extent than the sum of possible offspring fragments strongly indicate that they were present in much smaller amounts than the oligonucleotides in the main funnelshaped cluster. This only emphasizes, that in order to avoid too many ambiguous oligonucleotides, the best strategy is to sequence only the longest fragments in a given experiment, and instead do several crosslinking experiments followed by increased RNase cleavage.

While the 5'-ends of the oligonucleotides are unambiguously determined, the situation is somewhat different when it comes to the 3'-end. This is due to the positioning of the label at the 5'-end. The band that represents the 3'-end nucleotide is thus the uncleaved mainband. The only resort is to compare the migration length of this very strong band with fortuitously occurring longer fragments with the same 5'-end and run on the same gel. Thus the determination of the 3'-ends of the longer fragments is of a speculative nature based on the specificity of RNase A. Actually the repeated occurrence of groups of two oligonucleotides with the same 5'-end but 3'-ends being either the intact 3'-end of the tRNA or deprived of the terminal adenine could be explained as being identical fragments but either with or without platinum still being bound to the fragment(4). When it comes to the extensively RNase A digested experiment where the determination of length of the individual fragments is much easier, a tightly bound platinum seems indeed to be the only possible explanation. Note that in all double spots the relative position of the two is always the same. This is also indicative of where the crosslink to EF-Tu occurs: Contrary to the oligonucleotides from the 3'-half, only # 15 and # 16 of the sequenced fragments assigned to the 5'-half of the $tRNA_1^{Leu}$ seem to be identical. Either a very weak crosslinking site to EF-Tu exists in this region or more likely this double spot is due to an additional single ligand platinum binding site or simply due to a badly determined 3'-end.

CONCLUSION

Crosslinking techniques have been widely used for identification of neighbouring areas or contact points between RNA and

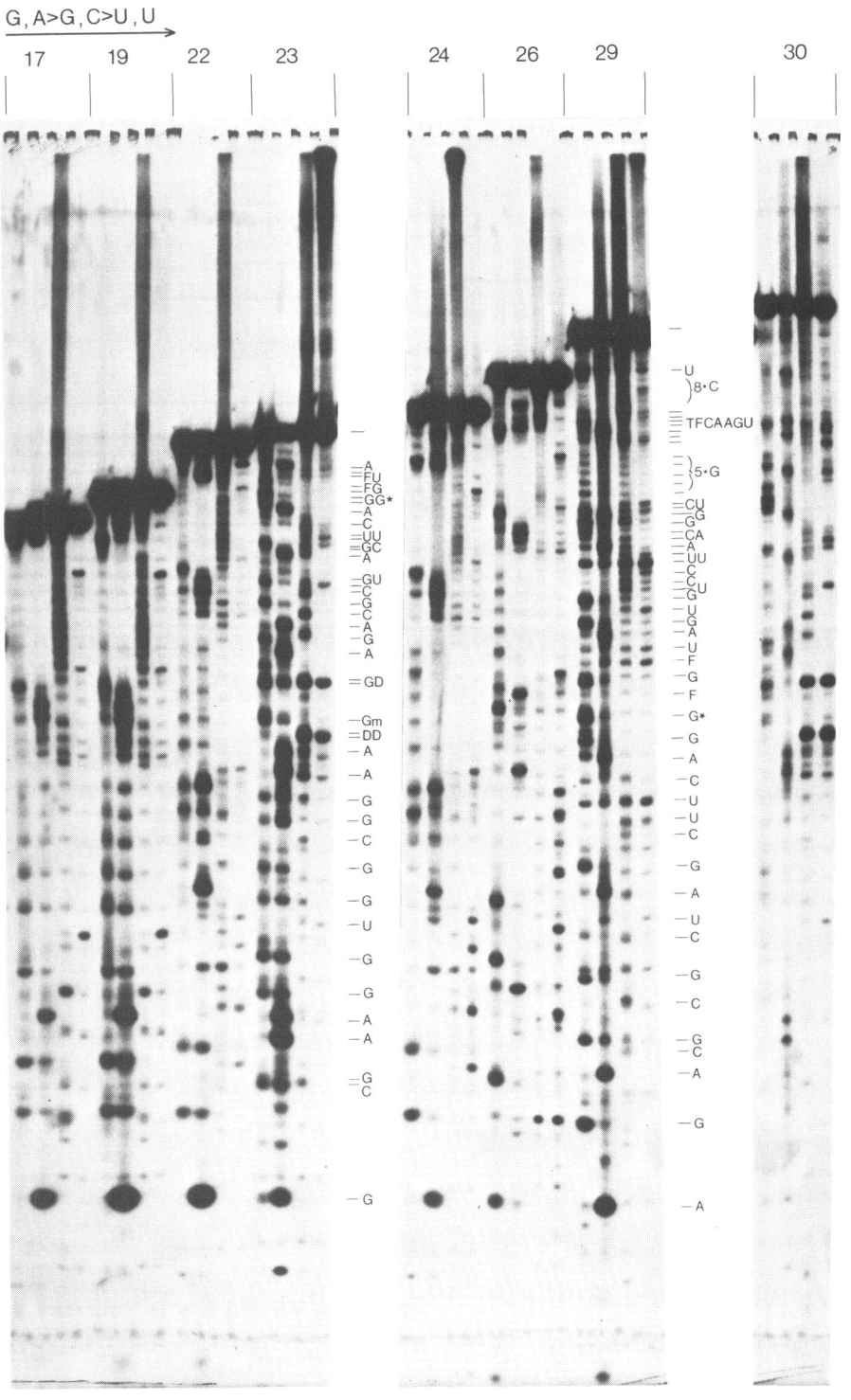

Figure 3. Autoradiogram of a chemical sequencing gel used to analyze the tRNA fragments isolated on fig. 1a. For each oligonucleotide the order of base specific modifications is as indicated at the top. The numbers at each set of reactions identify the oligonucleotides as cut out from the two-dimensional PAGE gel. Experimental details as in methods.

proteins within ribonucleoprotein complexes. Two types of complexes have been extensively studied; aminoacyl-tRNA synthetase:tRNA(3,27,28) and the ribosome(29) but also the ternary complex aa-tRNA:EF-Tu:GTP has been investigated by means of various crosslinking approaches(4,7,30).

When choosing a crosslinker, some features that are of importance for the outcome of the experiment must be taken into consideration. The nature of the chemical bond influences the method for determination of the crosslinking site, i.e. the sequencing of the oligonucleotide or the peptide under investigation. If the crosslinker makes a covalent bond, compounds that differ from the native nucleotides or aminoacids are created. These compounds must be identified independently. Another restraint is the size of the crosslinker. Classical

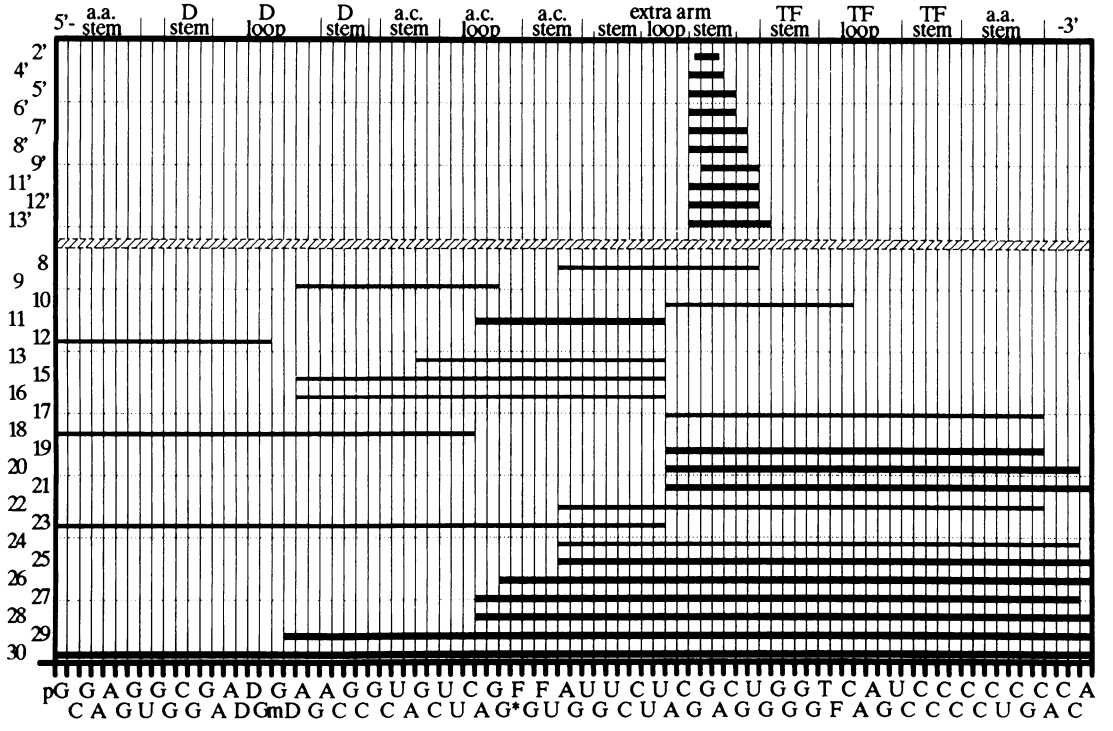

Figure 4. Sequenced oligonucleotides from two sets of *trans*-DDP crosslinked TC_1^{Leu}. Oligonucleotide numbers at the left. The upper fragments (2'-13') were from an extensively RNase A digested (2.5 u/pmole TC/μl) experiment (fig. 1b). The lower fragments (8–30) from a more limited RNase A digested (0.1 u/pmole TC/μl) experiment (fig. 1a). Fragments smaller than 4 nucleotides could theoretically originate in other parts of the tRNA, see discussion. For clarity they have only been depicted in the funnel.

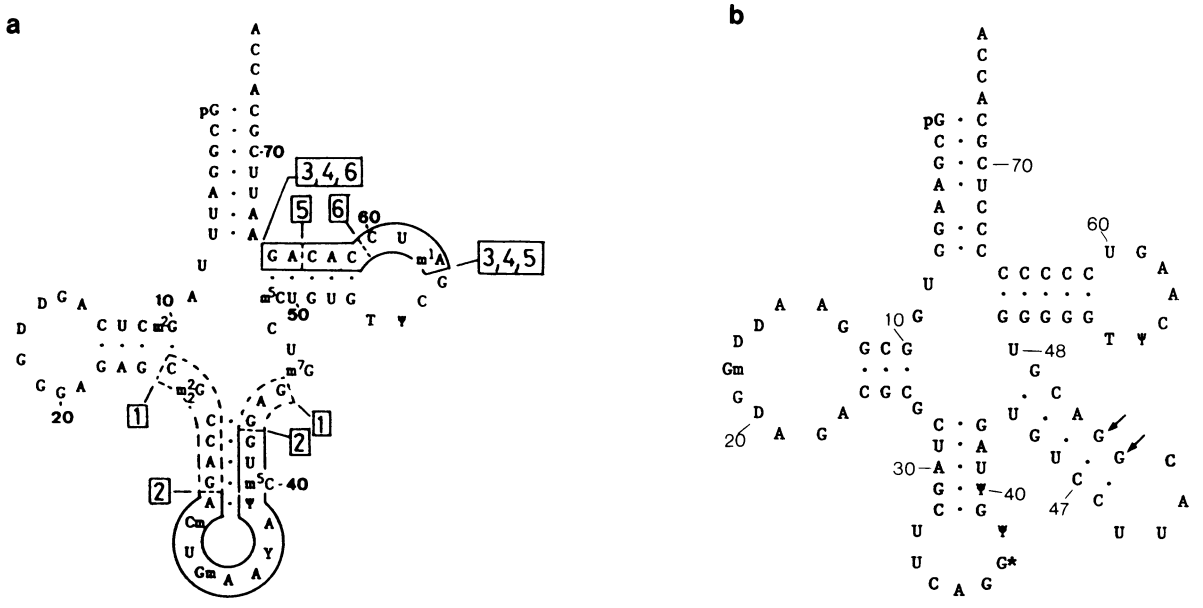

Figure 5. a: The *yt*RNA^{Phe} cloverleaf structure. Fragments in boxes were found crosslinked to EF-Tu by Wikman *et al.*(4). b: The $tRNA_1^{Leu}$ cloverleaf structure with the *trans*-DDP crosslinking site indicated with arrows.

crosslinkers normally have two reactive groups, separated by a spacer. Furthermore the spacer is often equipped with a cleaveable bond, i.e. a disulphide bond. All this adds to the length of the crosslinker that typically is 20Å or longer. This makes the interpretation of the results difficult, as the crosslinked elements could be situated far from each other.

Crosslinking by *trans*-DDP does not suffer from the above mentioned limitations. It is a square planar molecule with a maximum span of 7 Å between the two exchangeable chloride ligands. In addition, the chemical bond is a transition metal-ligand bond, and can therefore easily be cleaved by exchange with other stronger binding ligands.

Binding of *trans*-DDP to proteins occurs at neutral pH to sulphur atoms of cysteines and methionines and to the unprotonated imidazole ring of histidines(31,32). Platinum binding sites on RNA are also specific. The order of reactivity has been found by competitive reactions of the four nucleoside monophosphates to be GMP > AMP > > CMP > UMP(33), and binding occurs at N7 in guanine, N7 and N1 in adenine and at N3 in cytosine under neutral conditions. Due to the predilection of *trans*-DDP for sulphur ligands, tRNAs containing rare nucleosides with thiogroups, were avoided in this study in order to reduce the risk of unspecific crosslinks. This restriction limits the choice of *E. coli* tRNAs.

No *trans*-DDP crosslinking experiment of ribonucleoprotein complexes has up to now pinpointed individual nucleotides where the crosslink to the protein occurs. *E. coli* 16S rRNA fragments ranging 14−40 nucleotides have been identified crosslinked to initiation factor IF3 originating from the central region (819−859) and the 3′-part of the molecule(22). Crosslinked 5−23 bases long oligonucleotides from the 3′-half of *yeast* $tRNA^{Val}$ complexed with cognate valyl-tRNA synthetase have likewise been identified(3).

We have here demonstrated the advantages of doing several RNase digestions and the necessity of 2 dimensional PAGE, in order to isolate effectively the individual oligonucleotides for sequencing.

As previously mentioned the ternary complex aa-tRNA:EF-Tu:GTP has also been subjected to *trans*-DDP crosslinking studies(4). Aminoacylated *yeast* $tRNA^{Phe}$ in complex with *E. coli* EF-Tu:GTP was crosslinked under similar conditions yielding 40% crosslinked species. Here the yields were considerably lower (5%) either because the crosslinking reaction was not allowed to proceed for the same length of time (15' versus 1h at RT) or more likely the difference could be explained by slightly less favourable positions of crosslinkable groups. In the $ytRNA^{Phe}$:EF-Tu:GTP complex, oligonucleotides encompassing the 3′-half of the T arm and the anticodon arm were found crosslinked, the latter unexpectedly since this region is not believed to be in direct contact with the EF-Tu.

The crystal structure of four tRNAs are now known. All tRNAs have the same overall structure with only small variations. Even truncated tRNA species from mammalian mitochondria like bovine mitochondrion $tRNA^{Ser}_{AGY}$ lacking the whole D arm have been shown to fit into a model closely resembling the *yeast* $tRNA^{Phe}$ structure(34) (fig. 5a).

Regrettably no tRNA species encompassing a long extra arm has as yet provided crystals of sufficient quality for detailed X-ray analysis (e.g. $tRNA^{Leu}$(35,36)), but in all the structures determined so far the region with the variable loop does not notably influence the tertiary structure of the rest of the molecule. Dock-Bregeon *et al.*(6) have recently proposed a solution structure of *yeast* $tRNA^{Ser}$. $ytRNA^{Ser}$ has an extra arm that is only one nucleotide shorter than that of $tRNA^{Leu}_{1}$. In their model the extra arm is situated on the outer side of the L-shaped tRNA molecule, largely in the same plane of the L-shape but bend somewhat towards the one side of the tRNA that we earlier have shown to be protected by EF-Tu(1).

The crosslinking site found in this study of $tRNA^{Leu}_{1}$ is in agreement with the information presented above. The two guanosines at positions 47F and 47G are situated next to the extra loop in the 3′-side of the stem of the extra arm (fig. 5b).

As far as adduction of *trans*-DDP this area may resemble the G42-G43-A44 stretch in *yeast* $tRNA^{Phe}$ where the two guanines are part of normal Watson-Crick base pairs in the anticodon stem and A44 pair with m^2_2G26(37). The G43 is as previously noted capable to monofunctionally adduct *trans*-DDP and studies of this GGA stretch in the high resolution model of the $ytRNA^{Phe}$ makes it probable that the other replaceable ligand can reach out of the double helix thus making a crosslink to a protein possible.

The position of the crosslink in the extra arm rises some questions when comparing the result with the findings of other investigators on what regions of the tRNA are in close proximity of the EF-Tu.

Numerous experiments have demonstrated the importance of the aminoacyl group and the backbone of the single stranded 3′-end of the aa-tRNA for entering a ternary complex with EF-Tu:GTP. Of special interest for this work is the fact that all sites in the variable loop have been shown to be protected by EF-Tu. However it has been pointed out that in order to allow changes in this region , the interaction of the two macromolecules may not in every case be very close. Indeed the very existence of a long extra arm supports this. Also the spatial arrangement of the extra arm according to Dock-Bregeon *et al.*(6) allows for interaction with a large protein on one side of the tRNA.

The last experiments to be mentioned here are the crosslinking experiments performed by Kao *et al.*(7). They have successfully crosslinked *E. coli* $tRNA^{Phe}$ bearing a 20 Å long photoaffinity probe on its X47 (acp^3U47) residue to EF-Tu. When the NAK probe was substituted by a shorter NAG probe (14 Å) the crosslink completely disappeared indicating that the two crosslinkable sites are at least 14 Å apart. While the X47 nucleoside is adjacent to the semiinvariable purine 48 in the variable loop it may also be considered positioned at the root of an hypothetical protruding extra arm. The *trans*-DDP crosslinking site reported here in $tRNA^{Leu}_{1}$ is positioned in the other end of an extra stem of five basepairs, it is thus located at a distance of 13−18 Å from this crosslinking site.

Thus one can compare the platinum crosslinking of the extra arm in $tRNA^{Leu}_{1}$ to EF-Tu with crosslinking of $tRNA^{Phe}$ to EF-Tu by a bifunctional reagent spanding a similar length of 13−18 Å.

ACKNOWLEDGEMENTS

This work was supported by grants from the Danish Natural Science Research Councils FTU and Biotechnology Research programs.

REFERENCES

1. Wikman, F., Siboska, G. E., Petersen, H. U. and Clark, B. F. C., (**1982**), *EMBO J.*, **1**, 1095−1100.
2. Baudin, F., Romby, P., Romaniuk, P. J., Ehresmann, B. & Ehresmann, C., (**1989**), *Nucleic Acids Res.*, **17**, 10035−10046.
3. Tukalo, M. A., Kubler, M.-D., Kern, D., Mougel, M., Ehresmann, C., Ebel, J.-P., Ehresmann, B. and Giegè, R., (**1987**), *Biochemistry*, **26**, 5200−5208.
4. Wikman, F. P., Romby, P., Metz, M. H., Reinbolt, J., Clark, B. F. C., Ebel J.-P., Ehresmann, C. and Ehresmann, B. (**1987**) *Nucleic Acids Res.* **15**, 5787−5801.
5. Metz-Boutigue, M.-H., Reinbolt, J., Ebel, J.-P., Ehresmann, C. & Ehresmann, B., (**1989**), *FEBS Letters*, **245**, 194−200.
6. Dock-Bregeon, A. C., Westhof, E., Gieg, R. & Moras, D., (**1989**), *J. Mol. Biol.* **206**, 707−722.
7. Kao, T.-H., Miller, D. L., Abo, M. and Ofengand, J. (**1983**) *J. Mol. Biol.* **166**, 383−405.
8. Leberman, R., Antonsson, B., Giovanelli, R. Guariguata, R., Schumann, R. and Wittinghofer, A. (**1980**) *Anal. Biochem.* **104**, 29−36.

9. Bruton, C. J., Jakes, R. & Atkinson, T. (**1975**) *Eur. J. Biochem.* **59**, 327–333.
10. Silberklang, K., Gillum, A. M. and RajBhandary, U. L. (**1977**) *Nucleic Acids Res.* **4**, 4091–4108.
11. Lockard, R. E., Alzner-Deweerd, B., Heckman, J. E., MacGee, J., Tabor, M. W. and RajBhandary, U. L. (**1978**) *Nucleic Acids Res.* **5**, 37–56.
12. Gupta, R. C. and Randerath, K. (**1979**) *Nucleic Acids Res.* **6**, 3443–3458.
13. Peattie, D. (**1979**) *Proc. Nat. Acad. Sci. USA*, **76**, 1760–1764.
14. Donis-Keller, H., Maxam, A. M. and Gilbert, W. (**1977**) *Nucleic Acids Res.* **4**, 2527–2538.
15. Donis-Keller, H. (**1980**) *Nucleic Acids Res.* **8**, 3133–3142.
16. Louie, A., Ribeiro, S., Reid, B. R. and Jurnak, F. (**1984**) *J. Biol. Chem.* **259**, 5010–5016.
17. Abrahamson, J. K., Laue, T. M., Miller, D. L. and Johnson, A. E. (**1985**) *Biochemistry*, **24**, 692–700.
18. Jerez, C., Sandoval, A., Allende, J., Henes, C. and Ofengand, J. (**1969**) *Biochemistry*, **8**, 3006.
19. Chen, C.-M. (**1971**) *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **30**, (Abstract) 1283.
20. Beres, L. and Lucas-Lenard, J. (**1973**) *Biochemistry*, **12**, 3998–4005.
21. D'Alessio, J. M. (**1982**) *Gel electrophoresis of nucleic acids* (Rickwood, D. and Hames, B. D., ed.) pp. 173–197, IRL Press, Oxford, Washington DC, USA.
22. Ehresmann, C., Moine, H., Mougel, M., Dondon, J. Grunberg-Manago, M., Ebel, J.-P. and Ehresmann, B. (**1986**) *Nucleic Acids Res.* **12**, 4803–4821.
23. de Wachter, R. and Fiers, W. (**1982**) *Gel electrophoresis of nucleic acids* (Rickwood, D. and Hames, B. D., ed.) pp. 77–116, IRL Press, Oxford, Washington DC, USA.
24. Blank, H. U., and Söll, D. (**1971**) *Biochem. Biophys. Res. Comm.* **43**, 1192.
25. Sprinzl, M., Hartmann, T., Meissner, F., Moll, J. and Vorderwülbecke, T. (**1987**) *Nucleic Acids Res.* **15**, R53-R188.
26. Roberto, J. J. and Petsko, J. M., (**1972**) *Nature (London)*, **235**, 282–284.
27. Ofengand, J. (**1982**) *Protein Biosynthesis in Eukaryotes* (Perez-Bercoff, P. ed.) pp. 1–67, Plenum, N.Y.
28. Kisselev, L. L. (**1985**) *Prog. Nucleic. Acid Res. Mol. Biol.* **32**, 237–266.
29. Noller, H. F. and Lake, J. A. (**1984**) *Membrane Structure and Function* (Bittar, E. ed.) **6**, pp. 218–297, Wiley, N.Y.
30. Johnson, A. E., Miller, D. L. and Cantor, C. R. (**1978**) *Proc. Nat. Acad. Sci. USA*, **75**, 3075–3079.
31. Petsko, G. A., Philips, D. C., Williams, R. J. P. and Wilson, I. A. (**1978**) *J. Mol. Biol.* **120**, 345–360.
32. Petsko, G. A. (**1985**) *Methods Emzymol.* **114**, 147–157.
33. Mansy, S., Chu, G. Y. H., Duncan, R. E. and Tobias, R. S. (**1978**) *J. Am. Chem. Soc.* **100**, 607.
34. de Bruijn, M. H. L. and Klug, A. (**1983**) *EMBO J.* **2**, 1309–1321.
35. Young, J. D., Bock, R. M., Nishimura, S., Ishikura, H., Yamada, Y., RajBhandary, U. L., Labanauskas, M. and Connors, P. G. (**1969**) *Science*, **166**, 1527–1530.
36. Labanauskas, M., Connors, P. G., Young, J. D., Bock, R. M., Anderegg, J. W. and Beeman, W. W. (**1969**) *Science*, **166**, 1530–1532.
37. Jack, A., Ladner, E. and Klug, A. (**1976**) *J. Mol. Biol.* **108**, 619–649.

Abbreviations

acp^{3}U: 3-(3-amino-3-carboxypropyl)uridine
EF-Tu: elongation factor Tu
NAG: 2-nitro-4-azidophenylglycine
NAK: 6-(2-nitro-4-azidophenylamino)caproate
OGP: 1-O-octyl-β-D-glucopyranoside
PAGE: polyacrylamide gel electrophoresis
PEI: polyethyleneimine
TC: ternary complex: aa-tRNA:EF-Tu:GTP
trans-DDP: *trans*-diamminedichloroplatinum(II)
xTC: TC crosslinked with *trans*-DDP