Role of modified nucleosides in tRNA: Effect of modification of the 2-thiouridine derivative located at the 5'-end of the anticodon of yeast transfer RNA₂^{Lys}

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ABSTRACT

Yeast tRNA₂^{Lys} codes preferentially for AAA and contains a 2-thiouridine derivative (U*) at the 5'-position of the anticodon. Removal of the 2-thio group from U* by treatment with CNBr did not affect the amino acid accepting activity of the modified tRNA₂^{Lys}. CNBr treated tRNA₂^{Lys} was active in protein synthesis but with a much reduced efficiency. Although the modified tRNA₂^{Lys} was recognized by elongation factor (EF) T, the EFT dependent binding to ribosomes of tRNA₂^{Lys} (CNBr) was markedly decreased.

INTRODUCTION

Elucidation of the primary structures of different transfer RNAs from bacterial and eukaryotic sources shows the presence of a number of modified bases in non-varient position of the tRNA (1,2). The modified nucleoside 2-thiouridine and its derivatives have been shown to be present in the 5'-position of the anticodon of a number of tRNAs. Thus, tRNA₂^{Glu} from *Escherichia coli* contains 2-thio-5-methylaminomethyluridine (3), tRNA₁^{Gln} from *E. coli* contains an uncharacterized derivative of s²U (4). 2-Thio-5-carboxymethyluridine methyl ester is present in tRNA₃^{Glu} (5,6) and tRNA₂^{Lys} (7) from yeast. 2-Thio-5-methyluridine is present in tRNA₃^{Glu} and tRNA₂^{Lys} from rat liver (8). An uncharacterized 2-thiouridine derivative has been reported to be present in a bacteriophage T₄ coded tRNA^{Leu} (9).

The presence of the 2-thiouridine at the "wobble" position (10) of the anticodon suggests specific function(s) of this particular modified nucleoside in codon-anticodon interaction. It has been observed that all of these tRNA containing 2-thiouridine derivatives recognize codon N₁N₂A. Thus, trinucleotide stimulated ribosomal binding studies have shown that both tRNA₂^{Glu} from *E. coli* (11) and tRNA₃^{Glu} from yeast (5) preferentially recognize GAA as against GAG. Similarly, *E. coli* tRNA₁^{Gln} preferentially recognizes CAA (4) and T₄ - coded tRNA^{Leu} recognized UUA only (12). It has been suggested (6) that the presence of sulfur at the 2-position of uridine prevents its hydrogen bonding with guanosine

Chemical modification of thiouridine or its derivatives in tRNA with

CNBr was used by Saneyoshi and Nishimura (13) to study the role of these thio-uridine derivatives in tRNA function. Treatment of unfractionated *E. coli* tRNA with CNBr decreased the levels of acceptance of the amino acids glutamic acid, glutamine and lysine (13). It is now known that both tRNA^{Glu}₂ and tRNA^{Gln}₁ contains s²U derivatives (3,4). The s²U derivatives in the 5'-end of the anticodon of these tRNAs, therefore, are involved in the recognition of the tRNA with cognate aminoacyl-tRNA synthetases. Studies from Söll's laboratory (14,15) showed that CNBr treated tRNA^{Glu} and tRNA^{Gln} from *E. coli* have a decreased affinity for the cognate aminoacyl-tRNA synthetases and are therefore, very poorly aminoacylated. They, however, showed that tRNA^{Glu} or tRNA^{Gln} lacking s²U derivatives, isolated from a mutant of *E. coli* grown under sulfur deficient condition, can be aminoacylated as well as normal tRNA^{Glu} or tRNA^{Gln} containing s²U derivatives (14,15). It is suggested that the anticodon region of both tRNA^{Glu} and tRNA^{Gln} is involved in the recognition process with the aminoacyl-tRNA synthetases but the presence of s²U derivatives itself in the anticodon of these tRNA is not essential for their interaction with the aminoacyl-tRNA synthetases.

We have previously reported (16) that of the two major species of tRNA^{Lys} from baker's yeast, one, tRNA^{Lys}₁, recognized both the codons AAA and AAG while the other species tRNA^{Lys}₂ containing a 2-thio-5-carboxymethyluridine derivative (U*) recognized AAA preferentially. Elucidation of the primary structure of tRNA^{Lys}₂ from baker's yeast by Madison *et al* (7) showed that a s²U derivative is present at the 5'-end of the anticodon. In this report we present the results of our investigations on the function of U* in aminoacylation and codon-anticodon interaction of tRNA^{Lys}₂. Replacement of the sulfur by oxygen in U* brought about by CNBr treatment of tRNA^{Lys}₂ does not affect the extent of aminoacylation of this tRNA by homologous aminoacyl-tRNA synthetases. The efficiency of ribosomal binding and polylysine synthesis by tRNA^{Lys}₂ is, however, markedly reduced after CNBr treatment. A preliminary report of these studies have been presented (17).

MATERIALS AND METHODS

The purification of tRNA^{Lys}₁ and tRNA^{Lys}₂ has been described earlier (16). Assays for poly(A) or poly [r-(A-A-G)] directed polylysine synthesis, triplet dependent ribosomal binding of lysyl-tRNAs, formation of the ternary complex [EFT-GTP-Lys-tRNA^{Lys}], aminoacylation of tRNA^{Lys} and chromatographic separation of T₁ digestion products of tRNA^{Lys} have been described earlier (16,18). Modification of tRNA^{Lys} with CNBr was carried out according to the procedure of Walker and RajBhandary (19). 15.0 A₂₆₀ Units of tRNA^{Lys} in 1.1 ml

of 0.2 M potassium phosphate (pH 8.5) was mixed with a freshly prepared solution of CNBr to a final concentration of 0.5 mg of CNBr/ml and was heated at 100° for 4 min. The mixture was chilled, excess CNBr was dialyzed and the tRNA was recovered by ethanol precipitation. Hydrolysis of tRNA or oligonucleotide fragments and analysis of the modified bases on a high pressure ion exchange column connected to a UV analyzer recording absorbance at 254 nm, was done as described earlier (20).

RESULTS

CNBr Treatment. Treatment of *E. coli* tRNA^{Tyr} and tRNA^{Met}_f with CNBr at pH 8.5 and at 100° specifically converted s⁴U into U (19). To find out if CNBr can remove the S in the 2-position of s²U or s²U derivatives, we treated an authentic sample of s²U with CNBr. The UV spectra of the reaction product was different from that of s²U and matched with the UV spectra of U. The elution volume of the CNBr treated s²U on a high pressure cation exchange was changed to that corresponding to U (20). An authentic sample of U* was also treated with CNBr and chromatographed in the high pressure system. Figure 1 shows the elution profiles of U*, U* treated with CNBr and a mixture of U* and U* treated with CNBr. The modified U*,

Fig. 1: High pressure chromatography of (A) U*, (B) U* (CNBr) and (C) a mixture of U* and U* (CNBr). The chromatography was done as described under Materials and Methods using system I at a flow rate of 2.53 ml/hr.

presumably 5-carboxymethyl uridine or its methyl ester, is eluted earlier than U*. A minor peak moving between U* and the modified U* was also observed and it could be an intermediate product of the CNBr reaction. The modification of U* with CNBr

thus appears to be complete under these conditions used.

CNBr Treatment of tRNA₂^{Lys}. Both tRNA₁^{Lys} and tRNA₂^{Lys} were treated with CNBr as described earlier. The nucleoside composition of the modified tRNAs were analyzed by the high pressure chromatographic system. The relevant portions of the elution profiles are shown in Figure 2. The peak corresponding to U* present in tRNA₂^{Lys} (Fig. 2C) disappeared after CNBr treatment (Fig. 2D) but there was no change in the corresponding portion of the elution profile for tRNA₁^{Lys}, which did not contain any U*, before and after CNBr treatment (Fig. 2A and 2B).

Fig. 2: Detection of modification of U* by CNBr treatment of tRNA₂^{Lys} using high pressure cation exchange chromatography. (A) and (B) show portions of the profiles of analysis of tRNA₁^{Lys} before and after CNBr treatment respectively; (C) and (D) are corresponding figures for tRNA₂^{Lys}. System II with a flow rate of 6.0 ml/hr. was used.

The oligonucleotides derived from tRNA₂^{Lys} and tRNA₂^{Lys} (CNBr) by digestion with RNase T₁ were compared (Fig. 3). The results showed that the elution profiles were similar except for the peak 17. In the case of tRNA₂^{Lys} (CNBr) peak 17* eluted earlier and partially overlapped with the peak 16*. Oligonucleotide present in peak 17* was separated from peak 16* by paper chromatography of the last peak, which contained a mixture of the both. The nucleoside compositions of the oligonucleotides present in peak 17 and peak 17* were determined by the high pressure chromatographic system (Fig. 4). In the case of peak 17 the following nucleosides were present in the molar proportions shown: G-1.00, U-2.95, A-1.10, C-2.90, t⁶A-0.84 and U*-0.92. So peak 17 must contain a decanucleotide of the composition (U₃, C₃, A, t⁶A, U*)G. Since both t⁶A and U* were present in the same oligonucleotide, peak 17 might be considered as the anticodon fragment of tRNA₂^{Lys}. The composition of fragment 17 indeed agreed with that of the T₁ fragment containing the anticodon of tRNA₂^{Lys} (7,21).

Fig. 3: DEAE-cellulose chromatography of complete T_1 ribonuclease digest of CNBr untreated and treated $tRNA_{2}^{Lys}$. (A) 60.0 A_{260} units of $tRNA_{2}^{Lys}$ and (B) 65.0 A_{260} units of $tRNA_{2}^{Lys}$ (CNBr) were used.

Fig. 4: Nucleoside analysis of the presumed anticodon containing oligonucleotide obtained from chromatography of the T_1 digest of (A) $tRNA_{2}^{Lys}$ and (B) $tRNA_{2}^{Lys}$ (CNBr). System II with a flow rate of 7.2 ml/hr was used

Oligonucleotide 17* did not contain any U* and had the following compositions: G-1.00, U-3.35, A-1.15, C-2.75 and t^6A -0.82. Evidently this fragment contained the anticodon and had the same nucleoside composition as fragment 17 except for the absence of U*. The increased amount of U detected could be due to the coelution of the CNBr modification product of U* with U. The CNBr treatment of $tRNA_2^{Lys}$ thus appears to have specifically and completely modified the U* present at the 5'-end of the anticodon.

Function of $tRNA_2^{Lys}$ (CNBr): Recognition by Aminoacyl-tRNA Synthetases.
The lysine acceptance of $tRNA_2^{Lys}$ (CNBr) was tested using aminoacyl tRNA synthetase from yeast. It accepted about 65% of lysine as compared to untreated $tRNA_2^{Lys}$ under optimal conditions of charging of the latter. When a more appropriate control tRNA was used, namely, a sample of $tRNA_2^{Lys}$ which had gone through the entire procedure of CNBr treatment in the absence of CNBr, the reduction in the amount of charging was found not to be due to CNBr treatment. The control tRNA and the CNBr treated tRNA were charged almost to the same extent as shown in Fig. 5. However, when heterologous synthetase preparation from *E. coli* and

Fig. 5: Lysine charging to CNBr treated and untreated $tRNA_2^{Lys}$ by aminoacyl tRNA synthetase from yeast, *E. coli* and wheat embryo. Incubations were for 20 min. Amounts of different synthetase preparations used were yeast, 0.40 mg protein/ml; *E. coli*, 1.20 mg/ml and wheat embryo, 0.54 mg protein/ml.

wheat embryo were used, less lysine was charged to $tRNA_2^{Lys}$ (CNBr) as compared to control $tRNA_2^{Lys}$ (Fig. 5). The kinetics of charging of the control and CNBr treated $tRNA_2^{Lys}$ using yeast synthetase are shown in Fig. 6. It appears that $tRNA_2^{Lys}$ (CNBr) is charged less efficiently than $tRNA_2^{Lys}$ for the first 5 minutes.

Fig. 6: Kinetics of lysine charging to CNBr treated and untreated tRNA₂^{Lys} with yeast enzyme. Synthetase concentration was 0.40 mg protein/ml and tRNA concentration was 0.42 A₂₆₀ unit/ml.

Participation in Protein Synthesis: Ribosomal Binding. Since CNBr treated tRNA₂^{Lys} could be charged quite efficiently with lysine, its participation in the process of protein synthesis was investigated. Elongation factor (EF) T dependent ribosomal binding of [¹⁴C] Lys-tRNA₂^{Lys} (CNBr) was measured using the triplet ApApG and poly(A) as templates. Data shown in Table 1 compare the extents of ribosomal binding of CNBr treated and untreated tRNA₂^{Lys}. CNBr

Table 1
Ribosomal Binding of Different Lys-tRNA^{Lys}

tRNA added	CNBr treatment	pmoles of Lys-tRNA ^{Lys} added	pmoles of Lys-tRNA ^{Lys} bound in presence of		
			-template	+poly(A)	+ApApG
Lys ₁	-	12.0	0.36	3.60	2.95
	+	10.6	0.49	2.35	2.05
Lys ₂	-	15.5	2.71	12.36	4.83
	+	16.9	1.14	2.88	1.48

The reaction mixtures contained 1.4 A₂₆₀ units of high salt washed ribosomes, 0.1 mM GTP, 0.02 mg of EF-T, 0.23 A₂₆₀ unit of poly(A) or 0.05 A₂₆₀ units of ApApG in 0.05 ml and incubated at 25° for 20 min.

treated and untreated tRNA₁^{Lys} was used as control. As shown previously (16) binding of Lys-tRNA₁ was stimulated almost equally by ApApG and poly(A). As expected, in this case treatment with CNBr had no effect on the pattern of stimulation or the efficiency of binding. Binding of Lys-tRNA₂^{Lys} was stimu-

lated much more by poly(A) than by ApApG since AAA was the preferred codon in this case (16). CNBr treatment of tRNA₂^{Lys} drastically reduced its efficiency of binding to the ribosomes. The extent of ribosomal binding was reduced to about 18% by CNBr treatment of tRNA₂^{Lys} but still AAA was the preferred codon. Thus, the modification of U* in tRNA₂^{Lys} by CNBr treatment has decreased its capacity for ribosomal binding without altering the codon recognition pattern.

Polylysine Synthesis. The ability of polylysine synthesis of CNBr treated and untreated tRNA₁^{Lys} and tRNA₂^{Lys} were tested using poly(A) and poly [r(A-A-G)] as templates. Data presented in Table 2 shows that the results were similar to those obtained in the ribosomal binding studies. For tRNA₁^{Lys}, CNBr treatment did not have any effect either on the coding properties or on the efficiency of polylysine synthesis. With tRNA₂^{Lys}, however, there was a pronounced effect on the efficiency which was reduced by about 60%. But again the overall pattern of codon recognition remained unchanged and AAA was the more efficient codon.

Table 2

Polylysine Synthesis using CNBr treated and Untreated Lysyl-tRNAs

tRNA Used	CNBr Treatment	Amount of [¹⁴ C] lysine polymerized in 30 min. (pmoles/ml)				
		-mRNA	+poly(A)		+poly [r-(A-A-G)]	
			% input		% input	
Lys ₁	-	12.8	94.8	27.6	48.0	12.0
	+	13.7	68.2	21.0	47.7	13.0
Lys ₂	-	17.9	81.6	41.6	25.4	5.0
	+	8.0	35.0	16.0	10.0	1.7

Poly(A) concentration was 2.3 A₂₆₀ units/ml and poly [r-(A-A-G)] concentration was 15.5 nmoles/ml. The concentrations of [¹⁴C] Lys-tRNA₁^{Lys}, CNBr untreated and treated were 297 pmoles/ml and 262 pmoles/ml respectively; the corresponding figures for [¹⁴C] Lys-tRNA₂^{Lys} were 153 pmoles/ml and 167 pmoles/ml respectively.

The lower efficiency of CNBr treated tRNA₂^{Lys} could be due to the presence of some inhibitory agent generated during the CNBr treatment. This possibility was, however, ruled out by the fact that mixing of Lys-tRNA₂^{Lys} (CNBr) or tRNA₂^{Lys} (CNBr) into reaction mixture containing [¹⁴C] Lys-tRNA₂^{Lys} did not decrease the polylysine synthesis as directed by poly(A). The kinetics of poly(A) directed polylysine synthesis using CNBr treated and untreated tRNA₂^{Lys} shows that although tRNA₂^{Lys} (CNBr) was less efficient than tRNA₂^{Lys}, the time course were similar in both the cases (Fig. 7). The observed decreased efficiency of tRNA₂^{Lys} thus appears not due to its lower rate of transfer of lysine to the growing poly-

Fig. 7: Kinetics of poly(A) directed polylysine synthesis using [¹⁴C] Lys-tRNA₂^{Lys} and [¹⁴C] Lys-tRNA₂^{Lys} (CNBr). Amounts of poly(A) used was 2.3 A₂₆₀ units/ml, amounts of [¹⁴C] Lys-tRNA₂^{Lys} and [¹⁴C] Lys-tRNA₂^{Lys} (CNBr) used were 250 pmoles/ml. For each point on the graph the value for the amount of lysine polymerized in the absence of poly(A) has been subtracted.

peptide chain. Also the optimum Mg⁺² ion concentration was the same (15 mM) in both cases.

EFT-GTP-Lys-tRNA^{Lys} Formation. The reduced efficiency of EF-T dependent ribosomal binding of tRNA₂^{Lys} (CNBr) could be due to the decreased capacity of formation of the ternary complex [EFT-GTP-Lys-tRNA^{Lys}]. Equivalent amounts of [¹⁴C] Lys-tRNA₂^{Lys} and [¹⁴C] Lys-tRNA₂^{Lys} (CNBr) were used for complex formation with EF-T and [³H] GTP (Fig. 8). All of the input Lys-tRNA^{Lys} was used for complex formation in the case of both CNBr treated and untreated tRNA^{Lys}. Thus, the reduced efficiency of Lys-tRNA₂^{Lys} (CNBr) in protein synthesis is not reflected at the step of the ternary complex [EFT-GTP-tRNA^{Lys}] formation.

DISCUSSION

To study the role of 2-thiouridine derivatives present in the 5'-position of the anticodon we have modified the U* present at the 5'-position of the anticodon of yeast tRNA₂^{Lys} with CNBr using Walker and RajBhandary's procedure (19). This procedure removed the sulfur of s⁴U and replaced it with oxygen to produce U (19). Using s²U we established that CNBr under this condition also removed the sulfur from s²U and modified 2-thio-5-carboxymethyluridine methyl ester, presumably to 5-carboxymethyluridine methyl ester. Analysis of the oligonucleotide fragments obtained by RNase T₁ digestion of CNBr modified and unmodified tRNA₂^{Lys} showed that U* was modified completely and specifically by the procedure used. Singhal (23) also reported the replacement of the sulfur by oxygen in the s²U

derivative present at the 5'-position of the anticodon of $\text{tRNA}_2^{\text{Glu}}$ using CNBr.

Transfer RNA₂^{Lys} (CNBr) was aminoacylated by homologous synthetase to more than 90% as compared to the control tRNA which had gone through all the steps

Fig. 8: Complex formation between EFT, GTP, and CNBr untreated and treated Lys-tRNA₂^{Lys} as assayed by gel filtration on Sephadex G-100. 250 pmoles of [¹⁴C] Lys-tRNA₂^{Lys} and 235 pmoles of [¹⁴C] Lys-tRNA₂^{Lys} (CNBr) were used for the incubations with 5 nmoles of [³H] GTP and 200 µg of EFT. —o—o— shows [³H] radioactivity and —x—x—x shows [¹⁴C] radioactivity.

of CNBr treatment in the absence of CNBr. The control tRNA was about 65% as active as the original purified tRNA₂^{Lys}. This loss of acceptor activity could be due to partial denaturation and loss of specific conformation during the treatment at alkaline pH. In contrast, Agris *et al* (15) observed a large loss of acceptor activity of control tRNA₂^{Glu} when it was treated according to the procedure of Walker and RajBhandary but in the absence of CNBr.

Heterologous synthetases from *E. coli* and wheat embryo, however, recognized the control tRNA₂^{Lys} better than tRNA₂^{Lys} (CNBr). Therefore it seems that the presence of sulfur in U* is helpful for recognition of tRNA₂^{Lys} by the heterologous synthetases.

Saneyoshi and Nishimura (13) observed a complete loss of amino acid accepting ability by tRNA₂^{Glu} upon treatment with CNBr under conditions which produced a cyanate derivative of thiouridine. But Agris *et al* (14) showed

that tRNA^{Glu} treated with CNBr under the condition of Saneyoshi and Nishimura, can be charged to about 70% of that of untreated tRNA^{Glu} if higher amounts of purified glutamyl-tRNA synthetase were used. Also Seno *et al* (15) showed that CNBr treatment of tRNA^{Glu} decreased its affinity for the synthetases. We, however, observed that tRNA^{Lys}₂ treated with CNBr under the conditions of Saneyoshi and Nishimura (13) accepted about 70% lysine as compared to the acceptance by the proper control tRNA (data not presented). The modification of a 2-thiouridine to either 2-thiocyanouridine or uridine in a tRNA molecule thus does not necessarily result in the loss of its amino acid accepting activity. It appears, therefore, that the s²U derivative present at the 5'-end of the anticodon may not be involved in the recognition of the tRNA^{Lys} by its cognate aminoacyl synthetase. Hence the loss of amino acid acceptance by a tRNA on treatment with CNBr does not *a priori* prove the presence of a s²U derivative at the 5' end of the anticodon as has been claimed by others (13, 22).

The efficiency of tRNA^{Lys}₂ in the process of protein synthesis was markedly reduced by the CNBr treatment. This was manifested both in template stimulated ribosomal binding and in poly(A) and poly [r-(A-A-G)] directed polylysine synthesis. In all these experiments limiting amounts of Lys-tRNA^{Lys} were used and the per cent of input lysine utilized for ribosomal binding or polylysine synthesis was measured as the index of efficiency of the tRNA. There was no difference in this respect between untreated tRNA^{Lys}₂ and control mock CNBr treated tRNA^{Lys}₂. The efficiency of tRNA^{Lys}₂ was also reduced to the same extent by CNBr treatment following the procedure of Saneyoshi and Nishimura (13) (unpublished observation). These authors (13) in contrast, observed a complete loss of ribosomal binding on CNBr treatment of Lys-tRNA from *E. coli* and Agris *et al* (14) obtained similar results with tRNA^{Glu}. In our hands, however, tRNA^{Lys}₂ (CNBr) though less efficient is far from completely inactive either in ribosomal binding or in polylysine synthesis. The reduced ribosomal binding is not reflected in the extent of EFT-GTP-Lys-tRNA^{Lys} formation which shows that like the Lys-tRNA synthetase, EFT also recognizes unmodified and CNBr modified tRNA^{Lys}₂ equally well. Some later steps in the process of protein synthesis are probably affected. It is possible that the modification of U* changes the conformation of tRNA^{Lys}₂ in such a way that its binding to the proper site on ribosomes is affected.

The codon recognition pattern of tRNA^{Lys}₂ was practically unchanged by CNBr treatment. Yoshida *et al* (6) observed that tRNA^{Glu}₃ from yeast preferentially recognizing GAA (5) contained U* at the 5' end of the anticodon. They postulated that the substitution of oxygen at the 2-position of uridine by sulfur did not allow the non-standard base pairing between G and U and hence codon GAG was not recognized (6).

If this was the case then chemically replacing the sulfur by oxygen with CNBr treatment should relieve the restrictive recognition of N_1N_2A codon only. $tRNA_2^{Lys}$ having U^* at the 5'-end of the anticodon recognized the codon AAA much better than AAG (16) but the data presented have shown that even after treatment with CNBr under conditions which converted the thiouridine to uridine, the codon recognition pattern was unchanged. Even for $tRNA_2^{Lys}$ (CNBr), AAA was the preferred codon though the overall efficiency for protein synthesis was reduced. This indicates that the structural specificity in a group of tRNA, with 2-thiouridines in their anticodon, for recognizing N_1N_2A preferentially to N_1N_2G may not lie entirely on the presence of the thioketo group on the uridine moiety. The substituent at the 5 position of the uridine may also play a positive role.

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REFERENCES

1. Nishimura, S. (1972) Progress in Nucleic Acid Research and Molecular Biology (Davidson, J.N. and Cohn, W.E., Eds.) 12, 49-85.
2. Barrell, B.G. and Clark, B.F.C., Handbook of Nucleic Acid Sequence, Joynson-Bruvers, England, 1974.
3. Ohasi, Z., Harada, F. and Nishimura, S. (1972) FEBS Letters 20, 239-241.
4. Folk, W.R. and Yaniv, M. (1972) Nature New Biology 237, 165-166.
5. Sekiya, T., Takeishi, K. and Ukita, T. (1969) Biochim. Biophys. Acta 182, 411-426.
6. Yoshida, M., Takeishi, K. and Ukita, T. (1971) Biochim. Biophys. Acta 228, 153-166.
7. Madison, J.T., Boguslawski, S.J. and Teetor, G.H. (1972) Science 176, 687-689.
8. Kimura-Harada, F., Saneyoshi, M. and Nishimura, S. (1971) FEBS Letters 13, 335-338.
9. Pinkerton, T.C., Paddock, G. and Abelson, J. (1972) Nature New Biology 240, 88-90.
10. Crick, F. (1966) J. Mol. Biol. 19, 548-555.
11. Ohasi, Z., Saneyoshi, M., Harada, F., Hara, H. and Nishimura, S. (1970) Biochem. Biophys. Res. Commun. 40, 866-872.
12. Scherberg, N.H. and Weiss, S.B. (1972) Proc. Nat. Acad. Sci. U.S.A. 69, 1114-1118.
13. Saneyoshi, M. and Nishimura, S. (1971) Biochim. Biophys. Acta 246, 123-131.
14. Agris, P.F., Söll, D. and Seno, T. (1973) Biochemistry 12, 4331-4337.
15. Seno, T., Agris, P.F. and Söll, D. (1974) Biochim. Biophys. Acta 349, 328-338.
16. Sen, G.C. and Ghosh, H.P. (1973) Biochim. Biophys. Acta 308, 106-116.
17. Sen, G.C. (1974) Proc. Can. Fed. Biol. Soc. 17, 349.
18. Ghosh, K. and Ghosh, H.P. (1972) J. Biol. Chem. 247, 3369-3375.

19. Walker, R.T. and RajBhandary, U.L. (1972) J. Biol. Chem. 247, 4879-4892.
20. Sen, G.C. and Ghosh, H.P. (1974) Anal. Biochem. 58, 578-591.
21. Madison, J.T., Boguslawski, S.J. and Teetor, G.H. (1974) Biochemistry 13, 518-523.
22. White, B.N. (1975) Biochim. Biophys. Acta 395, 322-328.
23. Singhal, R.P. (1974) Biochemistry 13, 2924-2932.