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Selective Oxidation of Polynucleotides with Monoperphthalic Acid[1]

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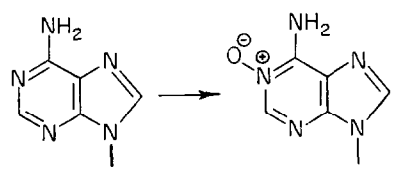
AMP can be oxidized to AMP-1-*N*-oxide by monoperphthalic acid at pH 7.0 and 20°C in aqueous buffer on the monomer as well as on the polymer level. No reaction occurs if nitrogen 1 is protected, e.g., by hydrogen bonding. The reaction is used for structural studies of ribopolynucleotides. Application to homoribopolynucleotides and rRNA showed that no unzipping and chain breaks occur and that side reactions with CMP and GMP do not disturb the evaluation of the AMP oxidation.

In yeast $tRNA^{Phe}$ 4 out of 17 AMPs are oxidized: the 3′-terminal AMP (No. 76) and three AMPs in the anticodon loop (No. 35, 36, 38), indicating that this molecule has a very compact structure with these two regions sticking out.

In yeast $tRNA_2^{Ser}$, containing 16 AMPs of which 8 are not base-paired in the cloverleaf, at 20°C 6 AMPs and at 40°C 8 AMPs are oxidizable. In contrast, in the dimer of yeast $tRNA_2^{Ser}$ only one AMP per tRNA could be oxidized, namely, the 3′-terminal AMP.

INTRODUCTION

During the translation process of the genetic message, highly specific protein–nucleic acid interactions between tRNA and enzymes or other cellular components occur, which can hardly be understood in the absence of a well-defined three-dimensional structure of tRNA (*1*). One possible approach to the study of tertiary structure in the tRNA molecule is to explore those chemical reactions which are selective toward certain exposed functional groups in the molecule. Apart from the selectivity, the necessary conditions for such reactions are that they require mild conditions which do not alter the subtle three-dimensional structure of the molecule and that they are analytically easy to follow. The oxidation of adenosine to adenosine-1-*N*-oxide with



[1] Abbreviations (according to IUPAC-IUB). tRNA = transfer ribonucleic acid, rRNA = ribosomal ribonucleic acid, U = uridine, C = cytidine, A = adenosine, G = guanosine, hU = dihydrouridine, ψ = pseudouridine, I = inosine, T = ribothymidine, m^1A = 1-methyl-adenosine, m^5C = 5-methyl-cytidine, m^2G = N^2-methyl-guanosine, m^7G = 7-methyl-guanosine, m_2^2G = N^2-dimethyl-guanosine, Cm = 2′-*O*-methyl-cytidine, Gm = 2′-*O*-methyl-guanosine, N* = minor nucleoside in the anticodon loop of yeast $tRNA^{Phe}$, AMP = pA = adenosine 5′-phosphate, Ap = adenosine 3′-phosphate, poly A = homopolymer of pA, poly A·poly U = double helical polynucleotide of poly A and poly U.

monoperphthalic acid at room temperature and neutral pH seems to meet these requirements (*2–5*). This reaction can also be carried out at the polymer level. The positions of the oxidized AMPs in the polymer can be determined by known sequencing methods. In this paper the scope and limitations of the method of *N*-oxidation of polynucleotides with monoperphthalic acid are explored and the method is applied to structural studies on ribosomal RNA (*4*) and transfer RNA (*3*).

EXPERIMENTAL PROCEDURES

Homoribopolymers. Poly A and poly U (prepared in our laboratory by Dr. H. Sternbach) of approximately equal chain length were each obtained by chromatography on Sephadex G-100 columns (3.5 × 100 cm, 0.1 *M* Tris buffer, pH 8.0, 300-droplet fractions) pooling those fractions with an identical elution volume. After desalting on a Sephadex G-25 column rechromatography was carried out using the same conditions. The fractions used eluted slightly earlier than tRNA used as marker (Fig. 1).

Ribosomal RNA. 70S ribosomes were isolated from exponentially grown *E. coli* B (T2-r) (*6*). After being washed four times in 0.01 *M* Tris buffer, pH 7.4, containing 0.005 *M* magnesium acetate, they exhibited an absorbancy ratio of $A_{259}/A_{280} = 2.1$. A 5–20% sucrose gradient (*7*) revealed only the presence of 70S ribosomes. The 30S and 50S subunits were isolated by suspending 70S ribosomes in 0.01 *M* phosphate buffer, pH 7.0, containing 0.001 *M* Mg^{2+} followed by differential centrifugation (*6*). The purity of the 30S and 50S ribosomal subunits was also checked in a 5–20% sucrose gradient. A mixture of 23S and 16S rRNA was isolated from 70S ribosomes (*8*). Pure 23S and 16S rRNA were prepared from the subunits. 5S rRNA was isolated by slightly modifying the method of Reynier et al. (*9*). One milliliter of a solution of 20 mg rRNA (23S, 16S and 5S) in 0.05 *M* NaCl and 1% methanol (v/v) was placed on top of a Sephadex G-100 column (185 × 3 cm) and eluted with the same solvent. Under these conditions 5S rRNA is partially separated from 23S and 16S rRNA. The fractions containing mainly the 5S rRNA were precipitated with ethanol and rerun on the same column; thus, pure 5S rRNA was obtained. The entire isolation was carried out at 4°C.

Transfer RNA. Yeast $tRNA^{Phe}$ and $tRNA_2^{Ser}$ were isolated from crude baker's yeast tRNA (Boehringer, Mannheim, Germany) by combined extraction (*10*) and chromatography on benzoylated DEAE-cellulose (*11*) and DEAE-Sephadex (*12, 13*). Chargeability was 1400 pmoles with $tRNA^{Phe}$ and 1490 pmoles with $tRNA_2^{Ser}$ per OD_{260} unit. Charging and purity criteria are the same as described (*14–16*). Different batches of the specific tRNAs contained between 20 and 50% of the 3′-terminal adenosine. If necessary the 3′-terminus was restored (*17, 18*).

Monoperphthalic acid. Monoperphthalic acid (200–400 μmole/ml) was prepared according to Payne (*19*) and stored in ether at −20°C. Its concentration was determined by adding an aliquot to a 20% KI solution and titrating with thiosulfate. Prior to each oxidation monoperphthalic acid was brought into aqueous solution by transferring 1–2 ml of the ether solution to 1 ml water followed by removal of the ether with a gentle stream of air. The solution was then adjusted to pH 7.0 with 2 *N* NaOH.

N-Oxidation. By a standard procedure, to 2 mg RNA (6 μmoles of nucleotides) dissolved in 0.15 ml water were added 0.1 ml 2 *M* phosphate buffer, pH 7.0, and 0.25 ml aqueous solution of monoperphthalic acid (in different experiments 15–60 μmoles). Thus, the reaction mixture had a total volume of 0.5 ml, a phosphate buffer concentration of 0.4 *M*, and contained a 10–40-fold excess of reagent. The reaction was carried out in the dark since AMP-1-*N*-oxide is sensitive to light (*20*). The presence of excess of monoperphthalic acid was checked at the end of each experiment.

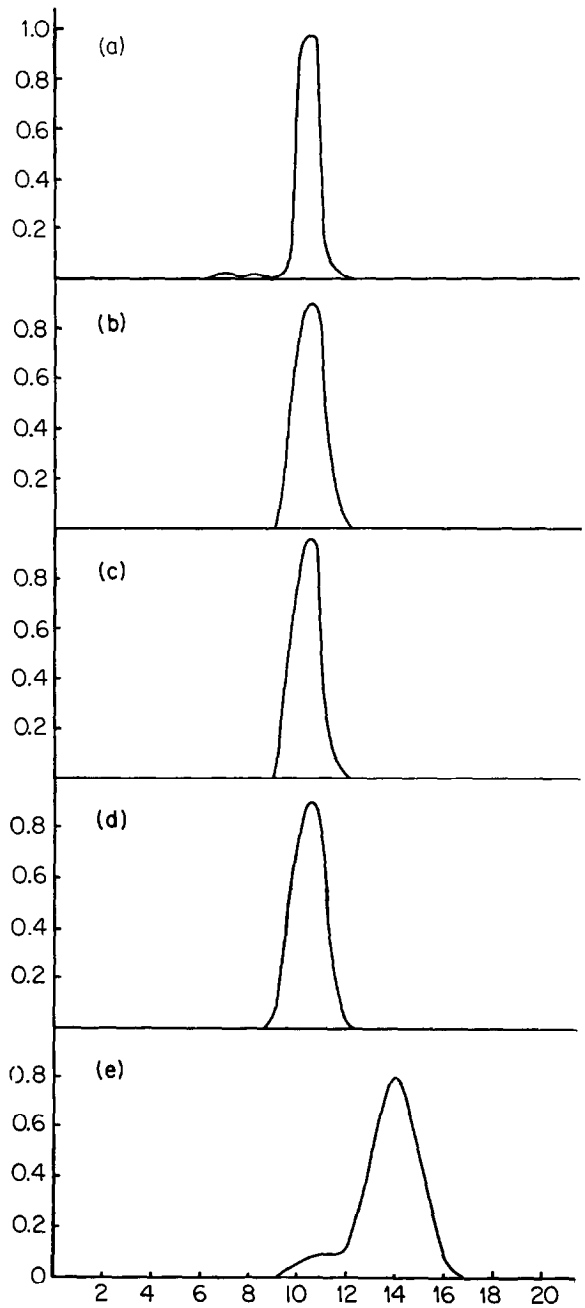


FIG. 1. Ordinate: uv absorption ($OD_{260\,nm}$). Abscissa: Fractions. Chromatography of homoribopolynucleotides before and after *N*-oxidation on a Sephadex G-100 column (3.5 × 100 cm) in Tris buffer, pH 8.0. Fraction size 300 droplets. The void volume of the column is 6 fractions. (a) poly A (20 $OD_{260\,nm}$ units), (b) poly U (20 $OD_{260\,nm}$ units), (c) poly A-1-*N*-oxide, 84% oxidized (20 $OD_{260\,nm}$ units, *N*-oxidation at pH 7.0, 20°C, 10-fold molar excess of reagent), (d) 1:1 complex of poly A·poly U of equal chain length (*N*-oxidation conditions as above), <2% oxidized (20 $OD_{260\,nm}$ units), (e) yeast tRNA bulk (30 $OD_{260\,nm}$ units).

Removal of monoperphthalic acid. The reaction mixture was freed of the monoperphthalic acid by Sephadex G-50 chromatography. The column (20.5 × 2.0 cm) was eluted with water. When necessary the oxidized sample was concentrated by either alcohol precipitation or lyophilization.

Separation of mononucleotides. The polynucleotides were hydrolyzed for 12–14 hr in 0.3 *N* KOH at 37°C, then chilled to 0°C and neutralized with perchloric acid. The precipitated $KClO_4$ was removed by centrifugation at 1000 rpm for 10 min. From the

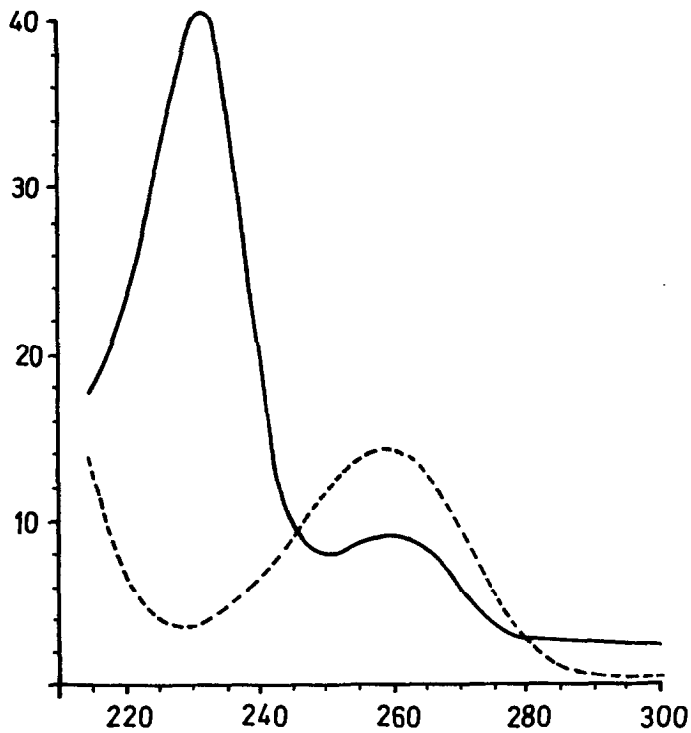


FIG. 2. Ordinate: Absorption ($\epsilon \cdot 10^3$). Abscissa: Wavelength (nm). uv Spectra of AMP (---) and AMP-1-*N*-oxide (——) (*20*).

supernatant fraction thus obtained the 2′(3′)-nucleotides were separated on a Dowex 50-H^+ column (200–400 mesh, 4 × cross-linked) according to Katz and Comb (*21*). The quantitative determination of the nucleotides had to be slightly modified, since 2′(3′)-GMP and 2′(3′)-AMP-1-*N*-oxide are eluted as a single peak. The 2′(3′)-GMP and AMP-1-*N*-oxide fraction was adjusted to pH 7.0 with 0.05 *M* Tris buffer and determined according to Holiday (*22*). The molar extinction coefficients used for these calculations are summarized in Table 1; Fig. 2.

Sequence determinations. 400 OD_{260}-units of oxidized tRNA in 6 ml of 0.02 *M* phosphate buffer, pH 7.2, were hydrolyzed with 300 units of T_1-RNase (EC 2.7.7.26.; Sankyo, Tokyo, Japan) and 500 μg of pancreatic RNase (EC 2.7.7.16.; Boehringer, Mannheim, Germany) at 37°C for 7–9 hr. If desired, 2 units of alkaline phosphatase (EC 3.1.3.1.; Boehringer, Mannheim, Germany) were added. The methods of separating the oligonucleotides are specified in the legends to the figures. The oligonucleotides were freeze-dried and hydrolyzed with 0.3 *N* KOH. The solution was neutralized and

TABLE 1

ULTRAVIOLET SPECTRAL DATA OF NUCLEOTIDES

Nucleotide	Wavelength (nm)	Extinction coefficient (1/moles·cm)	Solvent
2′(3′)-UMP	260	9.600[a]	0.05 *N* HCl
2′(3′)-AMP	257	14.900[a]	0.05 *N* HCl
2′(3′)-AMP	279	3.550[a]	0.05 *N* HCl
2′(3′)-CMP	279	13.000[a]	0.05 *N* HCl
2′(3′)-CMP	257	5.600[a]	0.05 *N* HCl
2′(3′)-GMP	257	11.800[a]	0.05 *N* HCl
2′(3′)-GMP	252	13.700[b]	0.05 *M* Tris buffer, pH 7.0
2′(3′)-GMP	232	5.470[b]	0.05 *M* Tris buffer, pH 7.0
2′(3′)-AMP-1-*N*-oxide	232	40.600[c]	0.05 *M* Tris buffer, pH 7.0
2′(3′)-AMP-1-*N*-oxide	252	8.000[c]	0.05 *M* Tris buffer, pH 7.0

[a] Data taken from Katz and Comb (*21*).
[b] Data taken from Pabst Laboratories, Milwaukee, Wisconsin, Circular OR 10.
[c] Data taken from Cramer and Schlingloff (*20*).

adjusted to pH 7.0 by addition of Tris buffer. The nucleotides were then hydrolyzed with alkaline phosphatase. Alternatively the oligonucleotides were split by digestion with snake venom phosphodiesterase (EC 3.1.4.1.; Boehringer, Mannheim, Germany) and alkaline phosphatase in 0.02 *M* Tris buffer pH 7.0. The nucleosides were separated either by paper chromatography (Schleicher & Schüll 2043 b, Dassel, Germany) in the system ethanol:1 *M* aqueous ammonium acetate (8:2) (system A) or on thin-layer plates of silica gel (Merck, Darmstadt, Germany) using water-saturated butanol as solvent (system B). For quantitative estimation the spots were cut out, eluted with double-distilled water, and measured uv spectroscopically.

RESULTS

N-*Oxidation of polynucleotides.* With poly A, the reaction at pH 7.0 and 20°C yields 84% AMP-1-*N*-oxide within 3 hr and does not proceed any further with 10-fold molar excess; to oxidize the remaining unreacted AMP one has to use a 30-fold excess. In a 1:1 mixture of the unfractionated homopolymers poly A and poly U 13% of the AMP is oxidizable, using a 10-fold excess of monoperphthalic acid per mole of AMP. However, in a 1:1 mixture of poly A and poly U of identical elution volume in Sephadex G-100 chromatography (Fig. 1) <2% of the AMPs are oxidizable. Poly C is 73% oxidized to the 3-*N*-oxide, while in the presence of poly G or poly I only 16% and <1%, respectively, of *N*-oxide are formed (Table 2) (*23*). In poly C·poly G, also 15% GMP reacts with monoperphthalic acid, yielding some non-uv-absorbing and three uv-absorbing substances, so far not further characterized (*23*). CMP-3-*N*-oxide generated during the reaction is quantitatively transformed to non-uv-absorbing material on alkaline hydrolysis (*24*). Sephadex G-100 chromatography (Fig. 1) of oxidized and non-oxidized homoribopolynucleotides showed that no chain breaks had occurred during the *N*-oxidation, since both fractions appear quantitatively at exactly the same position (Fig. 1). Since 5S rRNA and tRNA are clearly separated on this column, internal splitting of poly A into large fragments would shift the peak. Mono- or oligonucleotides

TABLE 2

EXTENT OF *N*-OXIDATION OF POLYNUCLEOTIDES (pH 7.0, 20°C, 7.5 hr)

Polymer oxidized	Nucleotide oxidized	Excess of reagent	Extent of *N*-oxidation (%)
Poly A	AMP	10	84
		30	98
Poly A·poly U (1:1)[a]	AMP	10	13
Poly A·poly U (1:1)[b]	AMP	10	< 2
Poly C	CMP	10	73
Poly C·poly G[c]	CMP	10	16
	GMP		15
Poly C·poly I[c]	CMP	10	Undetectable
	IMP		Undetectable

[a] Poly A longer than poly U, unfractionated.
[b] Poly A and poly U fractionated by Sephadex G 100 chromatography (Fig. 1).
[c] Unfractionated.

split off from the terminus of the chain would have been eluted and recognized in the late fractions.

N-*Oxidation of ribosomal RNA.* The reaction of monoperphthalic acid with rRNA (pH 7.0, 20°C, 20-fold molar excess of reagent) or tRNA (pH 7.0, 20°C, 10-fold molar excess of reagent) reaches its maximum level within 5–7.5 hr (Fig. 3). Because of the differences in uv absorption of AMP and AMP-1-*N*-oxide, the quotient A_{232}/A_{259}

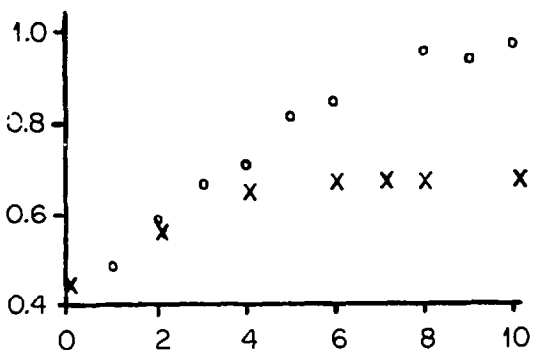


FIG. 3. Ordinate: Degree of *N*-oxidation (A_{232}/A_{259}). Abscissa: Time (hr). Time course of *N*-oxidation at pH 7.0 and 20°C of rRNA (23S + 16S) (○○○) (20-fold excess of reagent per AMP) and $tRNA^{Phe}$ (×××) (10-fold excess of reagent per AMP).

gives an approximate measure of the oxidation of AMP. Therefore, this ratio is used to follow the reaction qualitatively. When quantitative data are given, the amount of oxidation was determined also by nucleotide analysis. Figure 4 shows that in the case of 5S rRNA about 20-fold excess of reagent is necessary to obtain maximal oxidation. The same results are obtained for 23S and 16S rRNA, whereas in the case of tRNA a

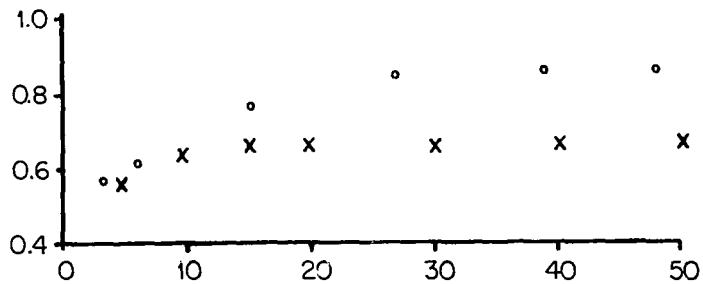


FIG. 4. Ordinate: Degree of *N*-oxidation (A_{232}/A_{259}). Abscissa: μmoles monoperphthalic acid/2 mg RNA (6 μmoles nucleotides). Saturation of *N*-oxidation of 5S rRNA (○○○) and $tRNA^{Phe}$ (×××) at pH 7.0, 20°C and 7.5 hr with excess of reagent. So 15 and 30 μmoles of monoperphthalic acid correspond to a 10- and 20-fold excess of reagent per AMP, respectively.

10-fold excess of reagent is sufficient. The nucleotide compositions of several RNAs before and after *N*-oxidation are summarized in Table 3. Since monoperphthalic acid does not react with UMP under the conditions applied, the 2′(3′)-UMP found after oxidation was used as a standard. Since 5S rRNA (*25*) displays a two-step melting profile, in which the first step has a T_m of 47°C, it was also oxidized at this temperature.

TABLE 3

NUCLEOTIDE ANALYSIS OF RNAs BEFORE AND AFTER *N*-OXIDATION (pH 7.0, 20°C, 7.5 hr)[a]

RNA	Excess of reagent	UMP (%)	GMP (%)		CMP (%)		AMP (%)		AMP-1-*N*-oxide (%)
			Before	After	Before	After	Before	After	
23S rRNA	20	20.8	32.8	28.7	22.2	20.1	24.1	11.8	8.7
16S rRNA	20	21.8	33.4	31.8	21.6	21.6	23.2	15.1	7.3
5S rRNA	20	17.6	34.9	34.0	28.9	21.8	18.7	9.5	7.0
5S rRNA	30	17.6	34.9	28.4	28.9	22.4	18.7	10.1	5.6
5S rRNA (47°C, 1.5 hr)	30	17.6	34.9	20.7	28.9	16.2	18.7	5.2	8.7
$tRNA^{Phe}$	10	24.3	26.2	27.4	20.9	21.0	18.8	16.7	3.6
$tRNA^{Phe}$	40	24.3	26.2	25.3	20.9	20.1	18.8	16.2	4.1

[a] The procedure was carried out as described under Methods. The amount of UMP, which is completely resistant to the reagent, was kept the same before and after *N*-oxidation and the other nucleotides after *N*-oxidation related to it.

Reaction was complete within 90 min after which 8.7% AMP-1-*N*-oxide was formed in comparison with 5.6% at 20°C. Besides the increase in AMP oxidized a sharp increase in attack on CMP and GMP was also noted. These results have been briefly communicated and discussed by Cramer and Erdmann (*4*) with regard to the total amount of base-pairing in rRNA and to models proposed for the structure of 5S rRNA.

N-Oxidation and nucleotide analysis of yeast $tRNA^{bulk}$, $tRNA^{Phe}$, and $tRNA^{Ser}$. Table 4 shows the amount of oxidizable AMPs of yeast $tRNA^{bulk}$, $tRNA^{Phe}$, $tRNA^{Ser}$, and the dimer of $tRNA^{Ser}$ (*26*) at 20°C and 40°C. *N*-Oxidation of minor nucleosides was not systematically explored. The number of AMP residues oxidized at 20°C is always

lower than the number of unpaired AMPs in the cloverleaf model (*27*). In the case of $tRNA^{bulk}$ and $tRNA^{Ser}$ two additional AMPs are oxidized at 40 °C.

TABLE 4

N-OXIDATION OF AMP IN YEAST tRNA
(pH 7.0, 7.5 hr, 10-fold molar excess of reagent)

tRNA	Number of AMPs per tRNA molecule			
	Total	Not base-paired in clover-leaf	*N*-oxidized at 20°C[a]	*N*-oxidized at 40°C
Bulk			4.1 (27%)	6 (36%)
Phe	17	9 (44%)	4 (22%)	4 (22%)
Ser_2	16	8 (50%)	6 (38%)	8 (50%)
Ser_2/dimer	16	8 (50%)	1 (8%)	—

[a] Analyzed by nucleotide analysis and uv-spectroscopy.

N-*Oxidation of yeast* $tRNA^{Phe}$. Figure 5 compares the oligonucleotide pattern of a combined T_1-RNase and pancreatic RNase digest of $tRNA^{Phe}$ before and after *N*-oxidation. The dark peaks are those which appear after oxidation: the first (fractions 3–8) consists only of adenosine-1-*N*-oxide, while in the original $tRNA^{Phe}$ adenosine is found; this shows that the 3′-terminal adenosine has reacted quantitatively. After oxidation two new oligonucleotides are found. Fractions 76–82 contain a dinucleotide. After alkaline hydrolysis and phosphatase treatment the two nucleosides can be separated by thin-layer chromatography on silica gel using system B. One is adenosine-1-*N*-oxide, the other is different in spectral and chromatographic properties from all nucleotides found in unoxidized $tRNA^{Phe}$. The second AMP-1-*N*-oxide containing oligonucleotide (fractions 125–136) is fairly resistant to snake venom and spleen phosphodiesterase. On alkaline hydrolysis and subsequent dephosphorylation with phosphatase it yielded a dinucleoside phosphate isolated by paper chromatography (system A) which, according to its uv-spectral properties, consisted of an equimolar ratio of adenosine-1-*N*-oxide and guanosine, as calculated by the procedure of Holiday (*22*). In addition to the dinucleoside phosphate 1 mole of adenosine-1-*N*-oxide was found and in different experiments either one or two very weak uv-absorbing spots could be detected by paper chromatography (system A). According to the work of RajBhandary (*28*) on the primary structure, these data show that fractions 125–136 contain the oxidation product of the anticodon plus nucleotide N*p (*29*) (GmpApApN*p). It is resistant against diesterases because of the minor nucleotides on its 3′ and 5′ end. On alkaline hydrolysis it generates 1 mole AMP-1-*N*-oxide, 1 mole N*′p and the dinucleotide $Gmpox^1Ap$. The anticodon containing hexanucleotide of the unoxidized material (fractions 150–157) does not appear after *N*-oxidation. If low amounts of pancreatic RNase are used, an AMP-1-*N*-oxide-containing nucleotide is eluted as a broad peak in the region of the hexanucleotide, and the amount of the two other AMP-1-*N*-oxide-containing oligonucleotides is low. Redigestion of the hexanucleotide with higher amounts of pancreatic RNase results in its splitting into the two AMP-1-*N*-oxide-containing oligonucleotides. Thus, we conclude that on *N*-oxidation the minor nucleoside N* next to the anticodon is altered to a nucleoside, which is now susceptible to pancreatic digestion. This is in accordance with the data published by RajBhandary

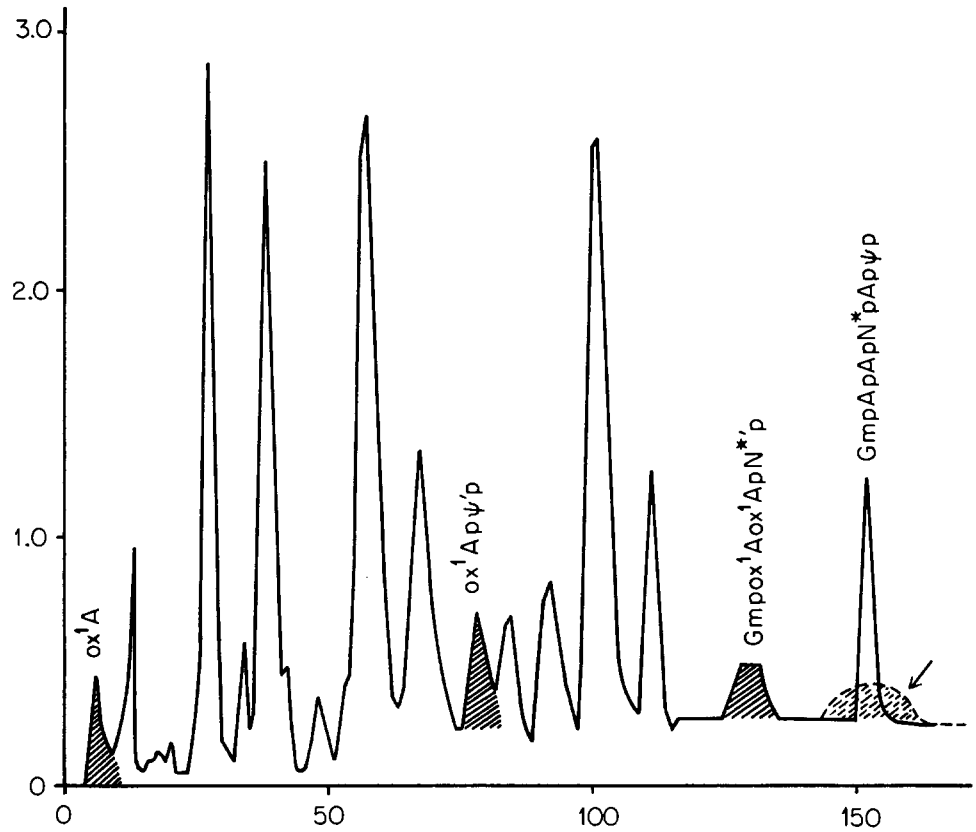


FIG. 5. Ordinate: Concentration ($OD_{260\,nm}$). Abscissa: Fractions. Elution pattern of yeast $tRNA^{Phe}$ after *N*-oxidation and pancreatic $+T_1$ RNase digestion on DEAE-cellulose (45 × 1.5 cm, carbonate form) modified according to RajBhandary (*28*) with two gradients (first: 300 ml each of 0.01 *M*, 0.08 *M*, and 0.17 *M* ammonium carbonate, second (starting from fraction 75): 300 ml each of 0.15 *M* and 0.55 *M* ammonium carbonate). Fraction size 10 ml. The arrow marks the dark peak appearing on digestion with very little amounts of pancreatic RNase (ox^1A = adenosine-1-*N*-oxide; ψ' and N*' are oxidation products of ψ and N*).

(*28*). Furthermore, the dinucleotide generated should be $ox^1Ap\psi'p$. Since ox^1ApX is found, the ψ adjacent to the anticodon loop must be oxidized to a so far unknown product.

In summary, out of 17 AMPs in the primary structure of yeast $tRNA^{Phe}$ only the AMPs 35, 36, 38 (anticodon loop) and 76 (3'-terminus) are oxidizable (Fig. 6).

N-*Oxidation of yeast* $tRNA_2^{Ser}$. The 3' terminal A is oxidized since the only nucleoside generated by alkaline and pancreatic hydrolysis is adenosine-1-*N*-oxide. The elution patterns of a pancreatic digest of oxidized and unoxidized $tRNA_2^{Ser}$ (*30*) show drastic differences, especially with respect to the fragments of the anticodon loop. Therefore, the anticodon loop must be heavily attacked. In contrast to $tRNA^{Phe}$, unambiguous localization of the adenosine-1-*N*-oxide residues was difficult.

The elution patterns of oxidized and unoxidized dimers of yeast $tRNA_2^{Ser}$ (*26*) are identical except for the 3'-terminal adenosine, which is oxidized to adenosine-1-*N*-oxide. This emphasizes that the andicodon loop, in the monomer form heavily attacked by the oxidant, is fully resistant and, therefore, must be buried in the dimer.

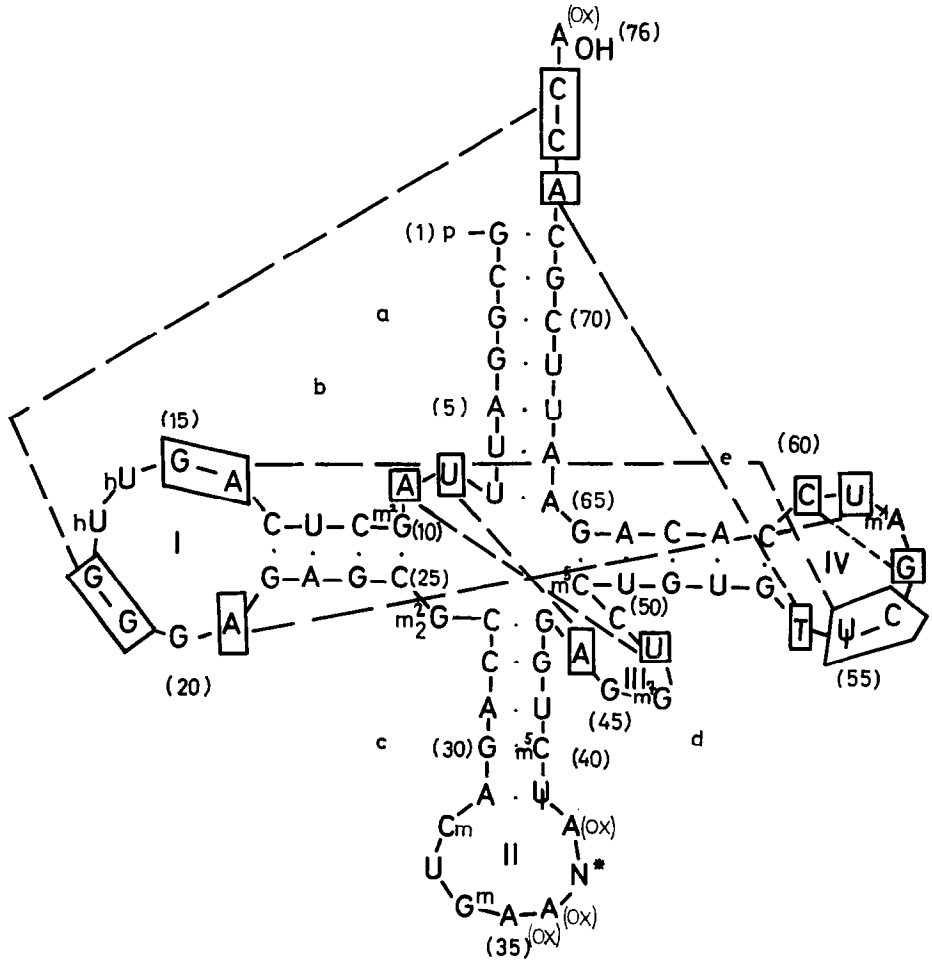


FIG. 6. Cloverleaf arrangement of yeast $tRNA^{Phe}$ with the *N*-oxidized AMPs marked by (○×) containing schematically the additional base-pairing proposed by Cramer et al. (*1*, *3*); coordinates of the model available on request.

DISCUSSION

The reaction of AMP to AMP-1-*N*-oxide, in monomeric or polymeric state, with monoperphthalic acid is carried out at 20°C and pH 7.0 in phosphate buffer during a few hours. Under these conditions the stability of tertiary structure can be assumed. AMP-1-*N*-oxide is the only detectable oxidation product of AMP in homopolymers as well as in natural ribonucleic acids. As shown from the sequential studies a particular AMP in accessible position is quantitatively oxidized. Thus, the reaction is specific and quantitative with respect to a particular adenosine position in a polynucleotide. The by-products generated from the oxidation of CMP and GMP do not interfere with the analytical evaluation of the AMP oxidation. The reaction can be followed easily at the polymer level by the change of the quotient A_{232}/A_{259} (Fig. 2, Table 1) and after total hydrolysis at the mononucleotide level by quantitative determination of the

nucleotides (*21*, *22*), thus furnishing a convenient analytical procedure on a small scale. The specificity of the reaction for exposed regions is further demonstrated by the more than 98% suppression of the oxidation in the case of the poly A·poly U and the more than 99% suppression of the oxidation in the case of the poly I·poly C (Table 2). The absence of any unzipping of base-paired regions is indicated by the fact that in the double-stranded polynucleotides and ribosomal RNA a plateau of oxidation is reached. Neither prolonged reaction time (Fig. 3) nor excess of reagent (Fig. 4) alters the saturation value of AMP-1-*N*-oxide. Chain breaks did not occur during the reaction of homopolyribonucleotides since oxidized poly A exhibited the same mobility on Sephadex G-100 columns as the non oxidized samples (Fig. 1). In $tRNA^{Phe}$ unspecific chain breaks would have become apparent in the oligonucleotide pattern.

In yeast $tRNA^{Phe}$, both at 20°C and 40°C, only the anticodon loop and the 3′-terminus react. This is in accordance with other data on $tRNA^{Phe}$ indicating that this species has a rather compact structure (*1*, *3*). In contrast, yeast $tRNA_2^{Ser}$ has two more AMPs accessible to the reagent at 20°C in addition to the anticodon and the 3′-end. Two further AMPs become available at 40°C in $tRNS_2^{Ser}$. This suggests that $tRNA_2^{Ser}$ has a more open structure than $tRNA^{Phe}$. In the dimer of $tRNA_2^{Ser}$ only the 3′-terminal adenosine is oxidized. The dimer can still be charged and is shielded against enzymatic digestion (*26*). This supports the idea of an association of the anticodon arms to form an antiparallel dimer and is consistent with recent X-ray data (*31*).

Our data show that the method of *N*-oxidation with monoperphthalic acid is general and meets the requirements of a mild and selective reaction applicable for the study of the tertiary structure of nucleic acids. The individual RNAs studied gave characteristic results, thus indicating the capacity of the reagent to differentiate between subtle differences in structure. The reaction is limited, however, if highly sensitive molecules are investigated, e.g., $tRNA^{Ser}$ compared to $tRNA^{Phe}$. These two tRNAs exhibit different stability in structure, e.g., on phosphorolyzing with polynucleotide-phosphorylases (*32*). As a consequence, reaction conditions such as time, temperature, and excess of reagent have to be worked out for each species.

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REFERENCES

1. F. CRAMER, *Progr. Nucl. Acid Res. Mol. Biol.* **11**, 391 (1971).
2. F. CRAMER AND H. SEIDEL, *Biochim. Biophys. Acta* **91**, 14 (1964); H. SEIDEL AND F. CRAMER, *Biochim. Biophys. Acta* **108**, 367 (1965).
3. F. CRAMER, H. DOEPNER, F. VON DER HAAR, E. SCHLIMME, AND H. SEIDEL, *Proc. Nat. Acad. Sci. U.S.A.* **61**, 1384 (1968); F. CRAMER, V. A. ERDMANN, F. VON DER HAAR, AND E. SCHLIMME, *J. Cell. Physiol.* **74**, 163 (Suppl.) (1969).
4. F. CRAMER AND V. A. ERDMANN, *Nature, London* **218**, 92 (1968).
5. J. GANGLOFF AND J. P. EBEL, *Bull. Soc. Chim. Biol.* **50**, 2335 (1968).
6. A. TISSIÈRES, J. D. WATSON, D. SCHLESSINGER, AND B. R. HOLLINGWORTH, *J. Mol. Biol.* **1**, 221 (1959).
7. R. J. BRITTEN AND R. B. ROBERTS, *Science* **131**, 32 (1960).
8. C. G. KURLAND, *J. Mol. Biol.* **2**, 83 (1960).
9. M. REYNIER, M. AUBERT, AND R. MONIER, *Bull. Soc. Chim. Biol.* **49**, 1205 (1967).
10. J. X. KHYM, *Biochemistry* **2**, 401 (1963); J. X. KHYM, *J. Biol. Chem.* **240**, Pc 1488 (1965).

11. I. GILLAM, S. MILLWARD, D. BLEW, M. V. TIGERSTROM, E. WIMMER, AND G. M. TENER, *Biochemistry* **6**, 3043 (1967).
12. J. D. CHERAYIL AND R. M. BOCK, *Biochemistry* **4**, 1174 (1965).
13. F. VON DER HAAR, unpublished results.
14. M. H. MAKMAN AND G. L. CANTONI, *Biochemistry* **4**, 1434 (1965).
15. E. SCHLIMME, F. VON DER HAAR, AND F. CRAMER, *Z. Naturforsch. B*-**24**, 631 (1969).
16. V. NEUHOFF, F. VON DER HAAR, E. SCHLIMME, AND M. WEISE, *Hoppe Seyler's Z. Physiol. Chem.* **350**, 121 (1969).
17. P. LEBOWITZ, P. L. IPATA, M. H. MAKMAN, H. H. RICHARDS, AND G. L. CANTONI, *Biochemistry* **5**, 3617 (1966).
18. F. CRAMER, F. VON DER HAAR, AND E. SCHLIMME, *Fed. Eur. Biochem. Soc. Lett.* **2**, 136 (1968).
19. G. B. PAYNE, *J. Org. Chem.* **24**, 1354 (1959).
20. F. CRAMER AND G. SCHLINGLOFF, *Tetrahedron Lett.* 1964, 3201.
21. S. KATZ AND D. G. COMB, *J. Biol. Chem.* **238**, 3065 (1963).
22. E. R. HOLIDAY, *Biochem. J.* **30**, 1795 (1936).
23. E. LADURNER, Dissertation, Techn. Univ. Braunschweig 1969.
24. F. FITTLER, Dissertation, Techn. Hochschule Darmstadt 1964.
25. G. G. BROWNLEE, F. SANGER, AND B. G. BARRELL, *Nature, London* **215**, 735 (1967).
26. A. ADAMS AND H. G. ZACHAU, *Eur. J. Biochem.* **5**, 556 (1968); H. G. ZACHAU, *Eur. J. Biochem.* **5**, 559 (1968).
27. R. W. HOLLEY, J. APGAR, G. A. EVERETT, J. T. MADISON, M. MARQUISEE, S. H. MERRILL, J. R. PENSWICK, AND A. ZAMIR, *Science* **147**, 1462 (1965).
28. U. L. RAJBHANDARY AND S. H. CHANG, *J. Biol. Chem.* **243**, 598 (1968).
29. K. NAKANISHI, N. FURUTACHI, M. FUNAMIZO, D. GRUNBERGER, AND I. B. WEINSTEIN, *J. Amer. Chem. Soc.* **92**, 7617 (1970).
30. H. G. ZACHAU, D. DÜTTING, AND H. FELDMANN, *Hoppe Seyler's Z. Physiol. Chem.* **347**, 212 (1966).
31. S. H. KIM AND A. RICH, *Science* **162**, 1381 (1968); **166**, 1621 (1969).
32. M. N. THANG, B. BELTCHEV, AND M. GRUNBERG-MANAGO, *Eur. J. Biochem.* **19** (1971) 184; B. BELTCHEV, M. N. THANG, AND C. PORTIER, *Eur. J. Biochem.* **19**, 194 (1971).