Altered Topography of 16S RNA in the Inactive Form of *Escherichia coli* 30S Ribosomal Subunits†

James J. Hogan and Harry F. Noller*

ABSTRACT: We have studied the topography of 16S RNA in the inactive form of the 30S ribosomal subunit (Ginsburg, I., et al. (1973) *J. Mol. Biol. 79,* 481), using the guanine-specific reagent kethoxal. Oligonucleotides surrounding reactive guanine residues were isolated and quantitated by means of diagonal electrophoresis and sequenced. Comparison of these results with experiments on active or reactivated subunits reveals the following: (1) Most of the sites which are reactive in active 30S subunits are much more reactive (average 13-fold) in inactive subunits. Upon reactivation, these sites return to a less reactive state. Thus, a reversible increase in accessibility of specific 16S RNA sites parallels the reversible loss of protein synthesis activity of 30S subunits. (2) The number of kethoxal-reactive sites in inactive subunits is about twice that of active subunits. The nucleotide sequences and locations of the additional accessible sites in inactive subunits have been determined. (3) Sites that can be located in the 16S RNA sequence are distributed throughout the RNA chain in inactive subunits, in contrast to the clustering observed in active subunits. (4) The sites of kethoxal substitution are single stranded. Yet, of the 30 sites that can be located, 23 were predicted to be base paired in the proposed secondary structure model for 16S RNA (Ehresmann, C., et al. (1975), *Nucleic Acids Res. 2,* 265).

Conformational changes have often been reported to accompany ribosome function (Sherman & Simpson, 1969; Chuang & Simpson, 1971; Schreier & Noll, 1971; Gupta et al., 1971; Huang & Cantor, 1972; Chang, 1973; Paradies et al., 1974; Dahlberg, 1974; Chapman & Noller, 1977). The most extensively studied characterization of a ribosomal conformational change has been carried out by Zamir and her co-workers, who showed that inactivation of 30S and 50S ribosomal subunits by depletion of magnesium or monovalent cations involves transitions to altered conformational states (for a review, see Zamir et al., 1974). Studies on the transition involving the 30S subunit show that the reactivity of specific ribosomal proteins toward *N*-ethylmaleimide is distinctly different for the active and inactive forms (Ginsburg et al., 1973). In studies on the comparison of the reactivity of active and inactive 30S and 70S ribosomes, Ginsburg & Zamir (1975) suggested that the conformational state of the inactive form of the 30S subunit may be related to the conformation of the 30S subunit in the active 70S ribosome.

We have recently used reactivity of 16S RNA toward kethoxal as a probe of 16S RNA topography in 30S and 70S ribosomes (Noller, 1974; Chapman & Noller, 1977). Here, we use this technique to compare the reactivity of specific sites in 16S RNA in active and inactive 30S subunits. In contrast to the protein studies, we find that sites in 16S RNA become much more reactive in inactive subunits than in active subunits, whereas 16S RNA becomes significantly less reactive in 70S ribosomes. Furthermore, many previously inaccessible 16S RNA sites become reactive toward kethoxal upon inactivation of 30S subunits. Nucleotide sequence analysis of oligonucleotides surrounding these sites enables us to locate regions of increased reactivity in the 16S RNA chain, providing new information about the conformational state of inactive 30S particles.

Materials and Methods

Growth and Labeling of Bacterial Cells. Escherichia coli strain MRE 600 were grown at 37 °C on a low-phosphate medium (Garen & Levinthal, 1960) supplemented with all 20 amino acids (20 μg/mL). After 30 min of growth, 20 mCi of carrier-free [^{32}P]orthophosphate (ICN) was added to 75 mL of medium and the cells were harvested in late log phase by a short low-speed centrifugation. Cells were opened by the lysozyme freeze-thaw method of Ron et al. (1966) in the presence of buffer A. The crude lysate was centrifuged for 30 min at 16 000 rpm (Sorvall RC-2B, SS-34 rotor) and the supernatant was layered onto 4 mL of 1.1 M sucrose in buffer B. After centrifugation at 55 000 rpm (IEC B-60 ultracentrifuge, A321 rotor) for 24 h at 6 °C, the 70S ribosome pellet was resuspended by gentle rotary shaking for 1 h at 0 °C in 1 mL of buffer C. The resuspended ribosomes were layered on a 35-mL 5–20% sucrose gradient in buffer C and centrifuged for 13.5 h at 20 000 rpm (Beckman L3-40 centrifuge, SW 27 rotor) at 2 °C. Fifteen-drop fractions were collected and the fractions corresponding to the 30S peak were pooled, the magnesium ion concentration was raised to 10 mM by addition of 1 M $MgCl_2$, and 0.65 volume of ethanol (0 °C) was added. Precipitated subunits were recovered by centrifugation at 10 000 rpm for 30 min (Sorvall RC-2B, HB-4 rotor) at 0 °C, and resuspended in buffer C.

Poly(U)-Directed tRNA Binding. tRNA (Calbiochem) was charged according to Nirenberg and Leder (1964) with [^{14}C]phenylalanine (460 mCi/mmol). Poly(U)-directed binding of [^{14}C]phenylalanyl-tRNA to 30S ribosomes was carried out with 30 μg of 30S, 50 μg of poly(U), and 50 μg of [^{14}C]phenylalanyl-tRNA in 100 μL of buffer D. After incubation for 1 h at 0 °C samples were diluted with 5 mL of buffer D at 0 °C. The mixtures were filtered on Millipore HAWP filters followed by two 0 °C washes with buffer D. Filters were dried and counted in 5 mL of 0.84% Butyl-PBD (Beckman) in toluene.

Inactivation and Reactivation of 30S Ribosomes. The 30S subunits (0.4 mg/mL in buffer C) were inactivated by dialysis in 0.2-mL aliquots for 20 h at 6 °C with three changes against

† From the Thimann Laboratories, University of California, Santa Cruz, California 95064. *Received September 12, 1977.* Supported by U.S. Public Health Service Grant No. 17129 from the National Institute of General Medical Sciences.

0006-2960/78/0417-0587$01.00/0

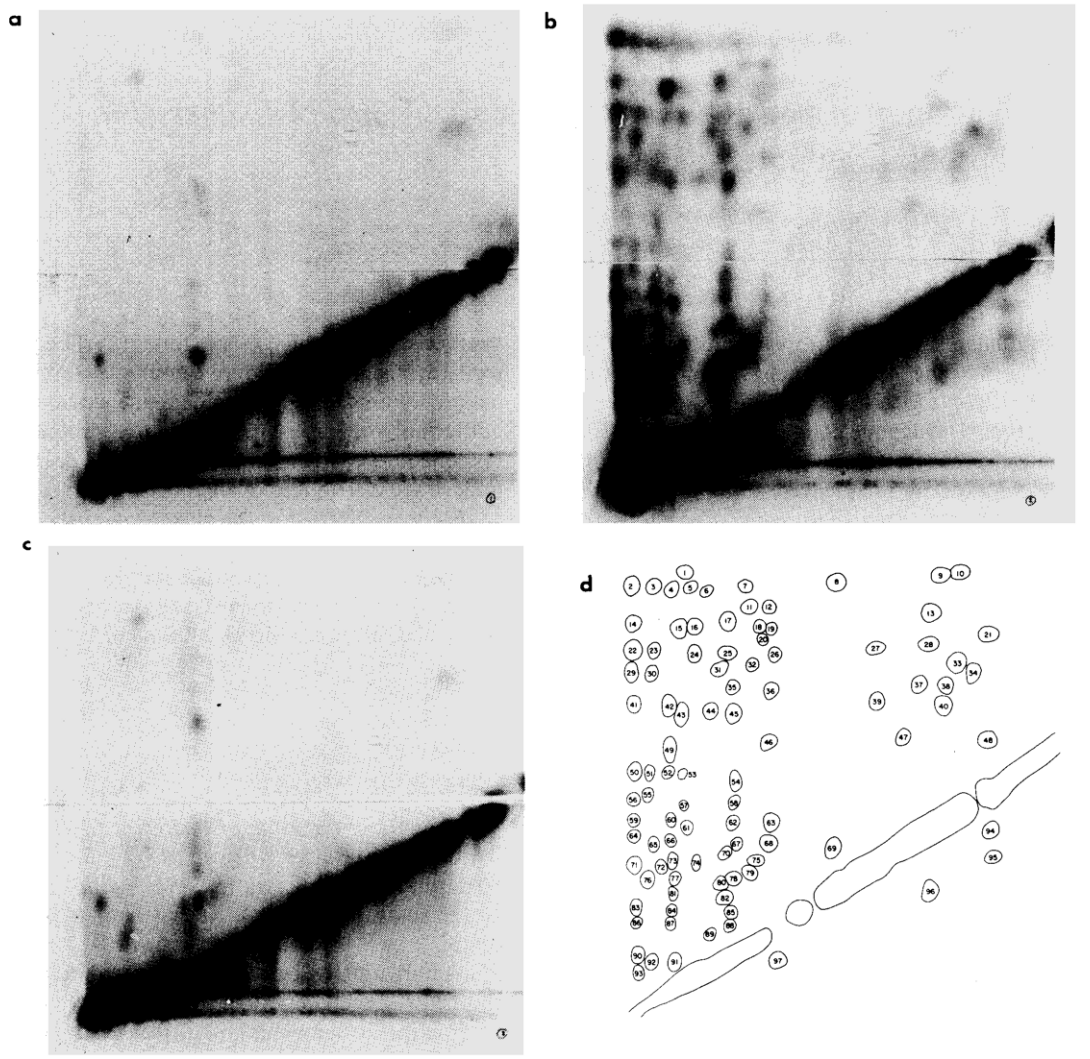

FIGURE 1: Autoradiographs of diagonal electropherograms of ^{32}P-labeled 16S RNA from (a) activated, (b) inactivated, (c) inactivated then reactivated, 30S ribosomal subunits. Oligonucleotide numbers are shown in d. Autoradiography was for 7 h with Kodak XR-5 film. Each electropherogram contained 42×10^6 cpm of ^{32}P-labeled 16S RNA.

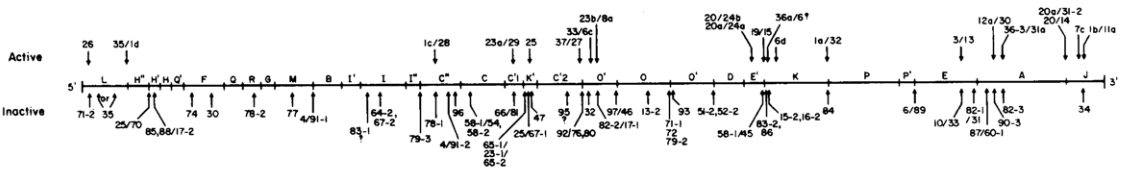

FIGURE 2: Distribution of kethoxal-reactive sites in the 16S RNA chain. Arrows indicate the position of kethoxal substitution in the active and inactive forms of the 30S ribosomal subunit. The numbering systems for oligonucleotides are from Noller (1974) and Chapman & Noller (1977) for active subunits, and from this paper for inactive subunits. Arrangements of lettered sections is according to Ehresmann et al. (1975).

300 mL of buffer E (Ginsburg et al., 1973). Subunits were reactivated by incubation for 20 min at 40 °C in buffer F.

Kethoxal Treatment. Active and reactivated ^{32}P-labeled 30S subunits in buffer F, and inactive subunits in buffer E were adjusted to 50 mM potassium cacodylate, pH 7.0, by addition of a 1 M stock solution. One-tenth volume of a 37 mg/mL solution of kethoxal (Nutritional Biochemical Corp.) in 20% EtOH was then added, and the reaction allowed to proceed for 1 h at 37 °C. The 30S ribosomes were then precipitated with 0.65 volume ethanol (0 °C) after adjusting the magnesium concentration to at least 10 mM by addition of 1 M $MgCl_2$. The 30S ribosomes were pelleted by centrifugation at 10 000 rpm for 30 min (Sorvall RC-2B, HB-4 rotor) at 0 °C.

Extraction of 16S RNA and Diagonal Electrophoresis. After kethoxal treatment the 30S ribosomes were resuspended in buffer G and extracted three times with water-saturated phenol. Phenol layers were reextracted twice with buffer G and the combined aqueous layers were extracted with ether and precipitated with 2 volumes of ethanol (0 °C). After incubation at −20 °C for 2 h, the RNA was centrifuged at 10 000 rpm for 30 min (Sorvall RC-2B, HB-4 rotor) at 0 °C. The RNA precipitate was redissolved in 0.24 M NH_4OAc and ethanol precipitated twice more. The final precipitate was redissolved in 0.2 mL of water, and 42 million counts/min taken for each diagonal. The samples were lyophilized and resuspended in 10 μL of 0.1 mg/mL RNase T_1 (Sankyo), 0.5 mg/mL alkaline phosphatase (Sigma) in buffer H. They were digested for 1 h at 37 °C and electrophoresed on a 110-cm sheet of DEAE

TABLE I: Oligonucleotide Sequences.

Oligonucleotide no.	Sequence	Oligonucleotide no.	Sequence	Oligonucleotide no.	Sequence
1	UG_{OH}	34	$A^*A^*C(C,U)G_{OH}$	65-1	UAGp
2	Gp	35	$AACG_{OH}$	65-2	$AU(C,U)G_{OH}$
3	Gp	36	$CCAG_{OH}$	66	UAGp
4	Gp	37	$CCAG_{OH}$	67-1	$AU(C,U)G_{OH}$
5	Gp	38	AAGp	67-2	$UUACCCG_{OH}$
6	Gp	39	nd	68	CUGp
7	Gp	40-1	CCG_{OH}	69	$(U,C_2)Gp$
8	Gp	40-2	ACG_{OH}	70	$UAAUG_{OH}$
9-1	Gp	40-3	AAG_{OH}	71-1	$AAUUG_{OH}$
9-2	UCG_{OH}	40-4	AAGp	71-2	$AUCAUG_{OH}$
10	Gp	41-1	AAGp	72	$AAUUG_{OH}$
11	UAG_{OH}	41-2	$AAAUG_{OH}$	73-1, 73-2	UAGp + (C,U)AGp
12-1	UAG_{OH}	42	AAGp	74	$AC(C_2U_2)G_{OH}$
12-2	$UCCG_{OH}$	43-1	ACG_{OH}	75	CUAGp
13-1	AUG_{OH}	43-2	nd	76	$AUAC(C_2U)G_{OH}$
13-2	$UACG_{OH}$	44	AAG_{OH}	77	$AC(U_2C_2)ACG_{OH}$
14-1	CGp	45	$AAAUG_{OH}$	78-1	$UAAUACG_{OH}$
14-2	AUG_{OH}	46	AG_{OH}	78-2	$AU(U,C_3)AG_{OH}$
15-1	AUG_{OH}	47	$AAUACCG_{OH}$	79-1, 79-2	CUAGp + $AAUUG_{OH}$
15-2	$UCCCG_{OH}$	48	AG_{OH}	79-3	$CUAAC(C_2U)G_{OH}$
16-1	AUG_{OH}	49-1	AG_{OH}	80	$AUAC(C_2U)G_{OH}$
16-2	$UCCCG_{OH}$	49-2	nd	81	$AAU(U,C_2)AG_{OH}$
17-1	AUG_{OH}	50	UUG_{OH}	82-1	AUCAGp
17-2	$C(C,U)G_{OH}$	51-1	UUG_{OH}	82-2	UAAACGp
18	AUG_{OH}	51-2	$(AU,AC_2,C_2)G_{OH}$	82-3	$UC^*AC(A,C)CAUG_{OH}$
19	AUG_{OH}	52-1	UUG_{OH}	83-1, 83-2	UAAAGp + AAAUGp
20	$CAUG_{OH}$	52-2	$(AU,AC_2,C_2)G_{OH}$	84	$AUAAACUG_{OH}$
21-1	$UCCG_{OH}$	53	UUG_{OH}	85	AAACUGp
21-2	$(U,C_2)AG_{OH}$	54	$AAAUCCCCG_{OH}$	86	AAAUGp
22	AGp	55	UUG_{OH}	87	AAUACGp
23-1	AGp	56	UGp	88	AAACUGp
23-2	$CCAG_{OH}$	57	UGp	89	$A(C_3U_2A)AAAG_{OH}$
24	AGp	58-1	UGp	90-1	UUGp
25	AGp	58-2	$AAAUCCCCG_{OH}$	90-2	UCUGp
26	AGp	59	UGp!	90-3	C(C,U)UGp
27	AGp	60-1	UGp!	91-1	$AAUAUUG_{OH}$
28	AGp	60-2	$U(C_3U)G_{OH}$	91-2	$AAUUACUG_{OH}$
29	ACGp	61-1	UGp!	92	AUUAGp
30	$CCAUCG_{OH}$	61-2	$C(U_2C_2)G_{OH}$	93	AAUUGp
31	$AAUG_{OH}$	62	UGp!	94	UAGp
32	$UCCACG_{OH}$	63	UGp!	95	C(U,C)AGp
33	$AAUCG_{OH}$	64-1	nd	96	UAAAGp
		64-2	$UUACCCG_{OH}$	97	$C(C_2U)UGp$

paper (Whatman DE-81) in 7% formic acid at 1400 V for 20 h. Unblocking and redigestion were performed as described by Noller (1974). The strips (4 × 46 cm) containing the first dimension separation were sewn onto DE-81 sheets (46 × 58 cm) and electrophoresed at right angles to the first dimension at 600 V for 12 h in 7% formic acid. They were then dried and autoradiographed with Kodak XR-5 film for 7 h to locate the oligonucleotides. The spots were cut out and counted in a gas-flow counter.

RNA Sequence Determination. RNA sequence methods were described previously (Barrell, 1971; Noller, 1974). Oligonucleotides were repurified before sequencing as previously described (Noller, 1974). Overdigestion with RNase T_1 (Woese et al., 1976; Herr & Noller, 1978) was done in 10 μL of 10 mg/mL RNAse T_1, 0.01 M Tris-HCl (pH 7.8), 0.001 M EDTA in a sealed capillary tube for 16 h at 37 °C.

Buffers: (A) 0.02 M Tris-HCl (pH 7.5)–0.1 M NH_4Cl–0.01 M $MgCl_2$–0.5 mM EDTA–6 mM β-mercaptoethanol; (B) 0.02 M Tris-HCl (pH 7.5)–0.5 M NH_4Cl–0.01 M $MgCl_2$–0.5 mM EDTA–6 mM β-mercaptoethanol; (C) 0.01 M Tris-HCl (pH 7.5)–0.1 M NH_4Cl–1 mM $MgCl_2$–1 mM β-mercaptoethanol; (D) 0.01 M Tris-HCl (pH 7.2)–0.05 M NH_4Cl–0.01 M $MgCl_2$; (E) 0.01 M Tris-HCl (pH 7.2)–0.1 M NH_4Cl–0.5 M $MgCl_2$–1 mM β-mercaptoethanol; (F) 0.05 M Tris-HCl (pH 7.2)–0.2 M NH_4Cl–0.02 M $MgCl_2$–2 mM dithiothreitol; (G) 0.2 M NaOAc (pH 5.0)–0.02 M sodium borate–0.02 M EDTA; (H) 0.01 M Tris-HCl (pH 7.8)–0.1 mM $ZnCl_2$–0.02 M potassium borate.

Results

Activation, inactivation, and reactivation of 30S ribosomal subunits were performed as described by Ginzburg et al. (1973). Ribosome activity was monitored by nonenzymatic binding of [^{14}C]Phe-tRNA directed by poly(U). In agreement with the findings of Zamir and co-workers, we find that depletion of monovalent cations or magnesium gives a 15- to 20-fold reduction in tRNA binding activity by 30S subunits. Heat treatment of the particles in the presence of these ions restores them to nearly full activity (data not shown).

To examine the effects of the inactivation and reactivation process on the topography of 16S RNA, we subjected ^{32}P labeled 30S subunits to kethoxal modification (Noller, 1974), after (a) activation, (b) inactivation, or (c) inactivation followed by activation. The modified RNA was extracted from

TABLE II: Location of Kethoxal-Reactive Sites in Inactive 30S Ribosomal Subunits.

Oligonucleotide[a] no.	Sequence and location[b] of reactive guanine	Location[c] in 16S RNA	Proposed secondary[d] structure (Ehresmann et al., 1975)	Sequence[e] conserved in prokaryotes (Woese et al., 1975)	Reactive[f] in active 30S	Protected[f] by 50S
71-2	K GAUCAUG	L	ds	−	+	
35	K GAACG	L	ds		+	
25/70	K GAGUAAUG	H″–H′		+	−	
85, 88/17-2	K GAAACUGC(C,U)G	H′		+	−	
74	K GAC(C_2,U_2)G	F	ds	−	−	
30	K GCCAUCG	F	ss	−	−	
78-2	K GAU(U,C_3)AG	R		−	−	
77	K GAC(U_2,C_2)ACG	M	ds	+	−	
4/91-1	K GGAAUAUUG	M–B	ds	+	−	
83-1	K GUAAAG	I (or C″)	ds	++/+	−	
64-2, 67-2	K GUUACCCG	I	ds	−	−	
79-3	K GCUAAC(U,C_2)G	I″–C″	ds	−	−	
78-1	K GUAAUACG	C″	ds	++	+	−
4/91-2	K GGAAUUACUG	C″	ds	+	−	
96	K GUAAAG	C″ (or I)		++/+	−	
58-1/54, 58-2	K GUGAAAUCCCCG	C	ds	−	−	
66/81	K GUAGAAUUCCAG[f]	C′1	ss	−	+	+
25/67-1, 65-1/23-1/65-2	K K GUAGAGAUCUG[f]	K′	ds; ds	−	+	+
47	K GAAUACCG	K′	ds	−	−	
95	K GC(U,C)AG	C′2 (or L)		++/−	−	
92/76, 80	K GAUUAGAUACCCUG[f]	C′2–D′		++; ++	+; +	+; +
32	K GUCCACG	D′	ds	+	+	−
82-2/17-1	K GUAAACGAUG	D′	ds	++	+	−
97/46	K GC(C_2,U)UGAG	D′–0	ds	−	−	
13-2	K GUACG	0	ds		−	
71-1, 72, 79-2, 93	K K GAAUUG	0–0′		++	−	
51-2, 52-2	K G(AC_2,AU,C_2)G	0′–D	ss	−	−	
58-1/45, 83-2, 86	K K GUGAAAUG	E′–K	-; ds	++/−[g]	+	+
15-2, 16-2	K GUCCCG	K	ds	++	+	+
84	K GAUAAACUG	K–P		−	+	−
6/89	K GGA(C_3,U_2,A)AAAG	P′–E	ds	−	−	
10/33	K GGAAUCG	E	ds	++	+	−
82-1/31	K GAUCAGAAUG	E–A	ds	++	−	

TABLE II: *(Continued)*

Oligonucleotide[a] no.	Sequence and location[b] of reactive guanine	Location[c] in 16S RNA	Proposed secondary[d] structure (Ehresmann et al., 1975)	Sequence[e] conserved in prokaryotes (Woese et al., 1975)	Reactive[f] in active 30S	Protected[f] by 50S
87/60-2	K GAAUACGU(U,C_3)G	A	ss	++; ++	−	
90-3	K GCCUUG[f]	A	ss	+	+	
82-3	K GUC*AC(A,C)CAUG[f]	A	ss	+	+	+
34	K GA*A*CCUG[f]	J	ss	+	+	+

[a]Oligonucleotide numbers refer to Table I. [b]The positions of kethoxal-reactive guanines are indicated by the letter K. [c]Location of oligonucleotide sequences in the 16S RNA chain is based on the sequence of Ehresmann et al. (1975). [d]Secondary structure proposed by Ehresmann et al. (1975) is shown by ds or ss, depending on whether the kethoxal-reactive site is proposed to be base-paired or unpaired, respectively, in their model. [e]Phylogenetic conservation of nucleotide sequences is based on the work of Woese et al. (1975). Sequences containing an oligonucleotide found in the 16S RNA of at least 24 of the 27 prokaryotic species studied are indicated by ++; between 14 and 23 by +; fewer than 14 by −. [f]Results of Noller (1974) and Chapman and Noller (1977). [g]There are two copies of the sequence AAAUG in 16S RNA, only one of which is highly conserved (Woese et al., 1975). It is not clear whether or not the kethoxal-reactive sequence is the conserved one.

the particles and subjected to a diagonal electrophoresis procedure in order to identify RNA sequences originating from the sites of kethoxal modification (Noller, 1974). Since kethoxal reacts only with single stranded, exposed guanine residues, the method affords a highly sensitive map of the surface topography of the 16S RNA within the ribosome. Figure 1 shows the result of diagonal mapping of the 30S subunits treated in this way. Heat activated subunits (Figure 1a) give a diagonal pattern corresponding to that observed previously for active 30S subunits (Noller, 1974; Chapman & Noller, 1977). Inactivated subunits, in contrast, show a greatly enhanced reactivity toward kethoxal, reflected in a greater reactivity of sites which are weakly reactive in active subunits, as well as the appearance of numerous spots not detected in active subunits (Figure 1b). Reactivation of inactive subunits (Figure 1c) restores the diagonal pattern to nearly that of the active subunits.

The spots appearing on the diagonal pattern from inactive 30S subunits were eluted, repurified, and subjected to nucleotide sequence analysis. These results are shown in Tables I (see paragraph at the end of this paper concerning supplementary material) and II. As expected, many new sequences, not previously detected in diagonal patterns of active subunits, were identified. In addition, most of the accessible sites in active subunits were also found to be accessible in inactive subunits. Some of the sites previously shown to be accessible in active subunits were not detected in inactive subunits, notably the sequences CUUGCUG (35/1d), UCGUCAG (20/24b, 20/24a), UCGU*AACAAG_{OH} (20/14), and UAACCG (7c), using the nomenclature of Chapman & Noller (1977). The probable location of the modified sites in the 16S RNA chain, based on the sequence results of Ehresmann et al. (1975), is shown in Figure 2. Included in Figure 2 are the locations of sites modified in active subunits, for comparison.

In addition to previously noted discrepancies between our sequence results (Chapman & Noller, 1977) and those of Ehresmann et al. (1975), we find an additional one among the sites exposed during inactivation. Oligonucleotide 78-2 was found to have the sequence AU(U,C_3)AG, in contrast to the sequence AU(U,C_2)AG reported by Ehresmann et al. (1975). Woese and co-workers (Uchida et al., 1974) find the sequence AUCCCUAG, in agreement with our findings.

Quantitation of ^{32}P-labeled spots from the diagonal maps shows that inactivation of 30S subunits is accompanied by a 13-fold enhancement of reactivity, on the average. Some sites have an enhanced reactivity as high as 50-fold greater than in active subunits (Table III). Reactivation of inactive subunits greatly restores the topography characteristic of the active subunits, in accord with the restoration of tRNA binding ability (Table III).

Discussion

Inactivation of 30S ribosomal subunits induced by depletion of magnesium ions and monovalent cations has been shown to alter the reactivity of several 30S ribosomal proteins with *N*-ethylmaleimide (Ginsburg et al., 1973; Ginsburg & Zamir, 1975). Here, we show that the reactivity of guanine residues in 16S RNA with kethoxal is dramatically altered upon inactivation (Figure 1 and Table III). Thus, a major disruption of the "active" topography of the 16S RNA accompanies loss of biological activity. This result suggests that proper functioning of the 30S subunit depends on maintaining the correct conformation of 16S RNA. The general result is that most sites become 10- to 20-fold more reactive toward kethoxal in the inactive form, and almost completely regain their inaccessibility upon reactivation (Figure 1 and Table III). Such an alteration in RNA topography could result from one or more of the following mechanisms: (1) conformational changes in 16S RNA; (2) disruption of RNA–protein interactions; or (3) conformational changes in ribosomal proteins, which could directly or allosterically unmask sites in 16S RNA. Our experiments do not distinguish between these possibilities.

Ginsberg & Zamir (1975) noted that the reactivity of several 30S subunit proteins is similar in inactive 30S particles and in 70S ribosomes and suggested that the structure of the 30S subunit in the inactive form may be related to its structure in the 70S ribosome. Our findings, on the contrary, show that the kethoxal reactivity of 16S RNA in the inactive 30S subunit is very much greater at almost all sites than is the 16S RNA in 70S ribosomes (Chapman & Noller, 1977). By the criterion of kethoxal activity, the inactive 30S subunit appears to be a much more "open" structure than the active 30S subunit, while the 30S subunit coupled with the 50S subunit becomes more "closed."

TABLE III:[a] Relative Reactivities of 16S RNA Sites in Active, Inactive, and Reactivated 30S Ribosomal Subunits.

Oligo-nucleotide no.	Rel reactivities Inact./act.	Rel reactivities React./active	Oligo-nucleotide no.	Rel reactivities Inact./act.	Rel reactivities React./act.	Oligo-nucleotide no.	Rel reactivities Inact./act.	Rel reactivities React./act.
1	10.4	3.7	35	14.5	3.6	69	3.5	1.3
2	34.7	1.7	36	7.0	1.3	70	8.1	1.3
3	34.3	2.0	37	3.2	0.7	71	23.2	1.7
4	17.2	1.9	38	5.0	1.0	72	14.5	2.5
5	16.9	2.0	39	3.1	0.9	73	15.2	2.0
6	7.8	0.9	40	5.2	1.4	74	9.7	1.4
7	2.6	0.8	41	15.5	1.4	75	11.0	2.5
8	7.7	1.2	42	27.0	1.9	76	12.8	1.6
9	4.3	1.1	43	45.5	3.5	77	17.2	2.2
10	2.5	0.9	44	7.5	1.3	78	1.4	1.0
11	20.8	3.0	45	6.4	2.6	79	13.9	0.7
12	7.3	1.4	46	5.7	1.2	80	5.9	2.6
13	9.1	1.7	47	2.5	0.9	81	16.9	3.3
14	20.8	1.5	48	1.6	0.9	82	5.1	1.3
15	10.7	1.4	49	19.6	1.3	83	15.8	1.2
16	22.7	1.7	50	20.0	1.4	84	8.5	1.8
17	21.7	4.0	51	20.4	1.9	85	4.8	1.5
18	25.6	2.9	52	7.9	1.8	86	19.6	2.0
19	13.7	3.1	53	4.8	2.3	87	8.0	2.2
20	4.7	2.0	54	9.4	1.6	88	4.2	1.2
21	6.1	1.3	55	22.2	1.6	89	1.9	0.7
22	41.6	1.6	56	14.5	0.7	90	21.3	1.3
23	37.0	1.9	57	6.5	1.6	91	12.7	3.2
24	20.0	1.8	58	6.1	1.4	92	9.2	1.0
25	17.9	6.6	59	25.5	1.7	93	16.9	1.5
26	13.2	2.8	60	20.4	2.4	94	2.4	1.6
27	2.0	0.4	61	17.5	4.2	95	3.4	1.2
28	9.7	2.2	62	11.9	3.7	96	3.0	1.4
29	8.6	0.8	63	6.4	1.8	97	2.4	1.0
30	50.0	2.7	64	25.6	2.1			
31	20.0	3.3	65	25.6	1.5			
32	11.9	1.7	66	12.5	1.9			
33	1.6	0.7	67	9.9	2.6			
34	4.8	1.1	68	7.9	2.0			

[a] Relative reactivity is expressed as the ratio of the reactivity in two experiments. Molar yields are not presented since many of the spots contained multiple oligonucleotides or impurities, which were later resolved upon repurification.

The total number of reactive guanines in inactive subunits can be estimated to be on the order of 70 to 80 (Tables I and II), about two-thirds of the total number of guanines estimated to be unpaired in the 30S subunit (Noller, 1974). Of these, 41 can be tentatively located in the 16S RNA chain (Figure 2) based on the sequence of Ehresmann et al. (1975). This is about twice the number of accessible sites found in active subunits (Noller, 1974; Chapman & Noller, 1977). Comparison of the distribution of kethoxal-reactive sites in the 16S RNA chain between active and inactive subunits (Figure 2) shows that these sites are distributed throughout the RNA chain in inactive subunits, in contrast to the clustering of reactive sites in active subunits.

Kethoxal reacts only with single-stranded RNA (Litt, 1969). Thus, it is a convenient tool for the assessment of secondary structure models. We have previously noted discrepancies between the reactivity of specific sites in active 30S subunits (Noller, 1974; Chapman & Noller, 1977) and the secondary structure model for 16S RNA proposed by Ehresmann et al. (1975). The additional sites revealed by inactivation of 30S subunits provide additional information about secondary structure. As summarized in Table II, of the 30 single-stranded sites which can be located in the primary sequence of Ehresmann et al. (1975), 23 were predicted by the latter authors to be in double-stranded regions; i.e., 77% of the assignable sites are in disagreement with their proposed secondary structure. It cannot be ruled out that some disruption of secondary structure occurs during inactivation. However, the conditions of inactivation (0.5 mM $MgCl_2$, 0.1 M NH_4Cl, 0 °C) are significantly milder than those used for isolation of 16S RNA. Furthermore, it is probable that the structure of 16S RNA in the free state differs from that in the 30S subunit.

Woese et al. (1975) have reported a comparative analysis of the ribonuclease T_1 oligonucleotides from 16S RNA in 27 species of prokaryotes. Although Table II contains only 54 of the approximately 500 T_1 oligonucleotides in 16S RNA, 18 of the 28 "universal" oligonucleotide sequences are in kethoxal-reactive sites. This observation is in agreement with previous conclusions that the most highly conserved sequences in 16S RNA are in accessible, single-stranded sites, rather than in double-stranded structure or in inaccessible sequences which could include binding sites for ribosomal proteins (Woese et al., 1975; Chapman & Noller, 1977). This lends further support to the idea that these sequences are functionally important, and not merely "structural" or assembly sites. Clarification of the specific roles of these ribosomal RNA sequences will be a complex task, requiring a variety of novel approaches.

Acknowledgment

We wish to thank R. Katze for discussions.

Supplementary Material Available

Sequence data to Table I (10 pages). Ordering information is given on any current masthead page.

References

Barrell, B. G. (1971) *Proced. Nucleic Acid Res. 2,* 751-779.

Bear, D. G., Ng, R., Van Derveer, D., Johnson, N. P., Thomas, G., Schleich, T., & Noller, H. F. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 1824-1828.

Chang, F. N. (1973) *J. Mol. Biol. 78,* 563-568.

Chapman, N. M. & Noller, H. F. (1977) *J. Mol. Biol. 109,* 131-149.

Chuang, D., & Simpson, M. V. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68,* 1474-1478.

Czernilofsky, A. P., Kurland, C. G., & Stöffler, G. (1975) *FEBS Lett. 58,* 281-283.

Dahlberg, A. (1974) *J. Biol. Chem. 249,* 7673-7678.

Dahlberg, A. E. & Dahlberg, J. E. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72,* 2940-2944.

Ehresmann, C., Stiegler, P., Mackie, G. A., Zimmerman, R. A., Ebel, J. P., & Fellner, P. (1975) *Nucleic Acids Res. 2,* 265-278.

Garen, A. & Levinthal, C. (1960) *Biochim. Biophys. Acta 38,* 470-483.

Ginzburg, I., & Zamir, A. (1975) *J. Mol. Biol. 93,* 465-476.

Ginzburg, I., Miskin, R., & Zamir, A. (1973) *J. Mol. Biol. 79,* 481-494.

Gupta, S. L., Waterson, J., Sopori, M. L., Weissman, S. M., & Lengyel, P. (1971) *Biochemistry 10,* 4410-4421.

Herr, W., & Noller, H. F. (1978) *Biochemistry 17,* 307-315.

Huang, K. & Cantor, C. R. (1972) *J. Mol. Biol. 67,* 265-275.

Kenner, R. A. (1973) *Biochem. Biophys. Res. Commun. 51,* 932-938.

Kolb, A., Hermoso, J. M., Thomas, J. O., & Szer, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74,* 2379-2383.

Litt, M. (1969) *Biochemistry 8,* 3249-3253.

Nirenberg, M. & Leder, P. (1964) *Science 145,* 1399-1407.

Noller, H. F. (1974) *Biochemistry 13,* 4694-4703.

Noller, H. F. & Chaires, J. B. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 3115-3118.

Paradies, H. H., Franz, A., Pon, C. L., & Gualerzi, C. (1974) *Biochem. Biophys. Res. Commun. 59,* 600-607.

Ron, E. Z., Kohler, R. E., & Davis, B. D. (1966) *Science 153,* 1119-120.

Schreier, M. H., & Noll, H. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68,* 805 -809.

Sherman, M. I., & Simpson, M. V. (1969) *Proc. Natl. Acad. Sci. U.S.A. 64,* 1388-1395.

Szer, W., Hermoso, J. M., & Boublik, M. (1976) *Biochem. Biophys. Res. Commun. 70,* 957-964.

Uchida, T., Bonen, L., Schaup, H. W., Lewis, B. J., Zablen, L., & Woese, C. (1974) *J. Mol. Evol. 3,* 63-77.

Woese, C. R., Fox, G. E., Zablen, L., Uchida, T., Bonen, L., Pechman, K., Lewis, B. J., & Stahl, D. (1975) *Nature (London) 254,* 83-86.

Woese, C. R., Sogin, M., Stahl, D., Lewis, B. J., & Bonen, L. (1976) *J. Mol. Evol. 7,* 197-213.

Zamir, A., Miskin, R., Vogel, Z., & Elson, D. (1974) *Methods Enzymol. 30F,* 406-426.