

Characterization of functionally active subribosomal particles from *Thermus aquaticus*

(ribosome/rRNA/ribosomal proteins/peptidyl transferase)

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ABSTRACT Peptidyl transferase activity of *Thermus aquaticus* ribosomes is resistant to the removal of a significant number of ribosomal proteins by protease digestion, SDS, and phenol extraction. To define the upper limit for the number of macromolecular components required for peptidyl transferase, particles obtained by extraction of *T. aquaticus* large ribosomal subunits were isolated and their RNA and protein composition was characterized. Active subribosomal particles contained both 23S and 5S rRNA associated with notable amounts of eight ribosomal proteins. N-terminal sequencing of the proteins identified them as L2, L3, L13, L15, L17, L18, L21, and L22. Ribosomal protein L4, which previously was thought to be essential for the reconstitution of particles active in peptide bond formation, was not found. These findings, together with the results of previous reconstitution experiments, reduce the number of possible essential macromolecular components of the peptidyl transferase center to 23S rRNA and ribosomal proteins L2 and L3. Complete removal of ribosomal proteins from *T. aquaticus* rRNA resulted in loss of tertiary folding of the particles and inactivation of peptidyl transferase. The accessibility of proteins in active subribosomal particles to proteinase hydrolysis was increased significantly after RNase treatment. These results and the observation that 50S ribosomal subunits exhibited much higher resistance to SDS extraction than 30S subunits are compatible with a proposed structural organization of the 50S subunit involving an RNA “cage” surrounding a core of a subset of ribosomal proteins.

Peptidyl transferase, the enzymatic activity responsible for catalysis of peptide bond formation, was shown to be an integral part of the large ribosomal subunit more than 30 years ago (1). However, despite decades of research, it is still not known which molecular components of the large subunit, containing 23S and 5S rRNAs associated with more than 30 ribosomal proteins, contribute directly to the catalytic reaction. Genetic studies have shown that mutants lacking individual large ribosomal subunit proteins are viable, indicating that at least 10 different ribosomal proteins are dispensable for peptidyl transferase activity (2). Extraction of ribosomal proteins with high concentrations of salt showed that a significant fraction of ribosomal protein could be removed without drastic effect on peptidyl transferase (3–5). Finally, reconstitution of large ribosomal subunits from individual components with omission of one or more proteins demonstrated that no more than a limited number of proteins, L2, L3, and L4, can possibly be involved in the catalysis of peptide bond formation (6, 7). However, no isolated protein, or mixture of proteins, has ever

been shown to catalyze the peptidyl transferase reaction (see ref. 8 for review).

Evolutionary arguments (reviewed in ref. 9), the discovery of ribozymes (10, 11), and a growing appreciation that rRNA plays an important functional role in translation have focused attention on 23S rRNA as a prime candidate for this catalytic role (12, 13). *In vitro* reconstitution has demonstrated that 23S rRNA is essential for the peptidyl transferase activity of 50S subunits (6). Subsequent tRNA affinity labeling and footprinting experiments together with studies of antibiotic-resistance mutations implicated domain V of 23S rRNA as an element of the peptidyl transferase center (8, 14). Nevertheless, most attempts to demonstrate catalytic activity for isolated rRNA were unsuccessful (see, however, refs. 15 and 16). One possible explanation for this failure is that the active conformation of the RNA might be lost during protein extraction. Accordingly, rRNA from thermophilic organisms, which would be expected to have a more robust structure, might have a better chance to maintain its functional conformation upon removal of ribosomal proteins. Indeed, peptidyl transferase activity of ribosomes from the thermophilic bacteria *Thermus aquaticus* is resistant to vigorous protein-extraction procedures, including treatment with proteinase K in the presence of SDS followed by phenol extraction, but is highly sensitive to treatment with ribonuclease (17). To define the upper limit for the number of macromolecular components that are required for peptidyl transferase activity, we analyzed the properties and composition of active particles obtained after treatment of *T. aquaticus* 50S ribosomal subunits with proteinase K, SDS, and phenol (KSP particles, for proteinase K, SDS, and phenol). We found that the extracted particles contained 23S rRNA and 5S rRNA associated with eight ribosomal proteins and sedimented at 50S, indicative of a compactly folded structure. Conditions leading to removal of the remaining proteins caused dissociation of 5S rRNA and a shift to a slower sedimentation value along with complete loss of peptidyl transferase activity.

MATERIALS AND METHODS

Cell Growth and Ribosome Isolation. *T. aquaticus* was grown in a modified Castenholz TYE medium (18) containing 1× Castenholz salts, 1× Nitsch's trace elements, 4 g/liter yeast extract, 8 g/liter bacto-peptone, and 2 g/liter NaCl (17). Cells were grown at 70°C with shaking to $A_{600} = 0.6$, harvested, and stored at –70°C. For preparation of ³⁵S-labeled ribosomes, cells were grown in medium containing 1× Castenholz salts, 10× Nitsch's trace elements, 0.2 g/liter bacto-peptone, and 2 μCi/ml of a mixture of [³⁵S]methionine and cysteine (specific activity, 1,000 Ci/mmol; American Radiolabeled Chemicals, St. Louis). Ribosomes and ribosomal subunits were prepared as described in ref. 17.

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Preparation of KSP Particles. KSP particles were prepared by treatment of 50S subunits with proteinase K in the presence of SDS, followed by phenol extraction essentially as described (17). Briefly, 5 A_{260} of *T. aquaticus* 50S subunits were resuspended in 500 μ l of buffer A (25 mM Tris-HCl, pH 7.5/150 mM NH_4Cl /5 mM MgCl_2) containing 1 mg/ml proteinase K and 0.5% SDS and incubated for 1 hr at 37°C. An equal volume of neutralized phenol was added, vortexed for 45 min at 4°C, and centrifuged in a microcentrifuge at 4°C for 3 min. The phenol phase was removed and the aqueous phase was extracted four times with chloroform. KSP particles were precipitated with three volumes of ethanol for 1 hr at -75°C, pelleted in a microcentrifuge for 10 min at 4°C, and resuspended in 50 μ l of buffer A.

Particles were purified by sedimentation in a sucrose gradient (10–40%) in buffer containing 25 mM Tris-HCl, pH 7.5/250 mM NH_4Cl /10 mM MgCl_2 /6 mM 2-mercaptoethanol in an SW 41 rotor (Beckman) for 15 hr at 26,000 rpm at 4°C. Gradients were fractionated through an ISCO flow spectrophotometer. Peak fractions were combined, precipitated with three volumes of ethanol, resuspended in 50 μ l of buffer A, and stored at -75°C.

Peptidyl Transferase Assay. Peptidyl transferase activity was assayed by using a peptidyl transferase reaction (19) with a full-length formyl-[^3H]Met-tRNA as a donor substrate (20). In a typical assay, 7.4 pmol (0.2 A_{260}) of 50S subunits or KSP particles was incubated with 2.5 pmol (25,000 cpm) formyl-[^3H]Met-tRNA in 40 μ l buffer containing 20 mM Tris-HCl, pH 8.0/400 mM KCl/20 mM MgCl_2 /1 mM puromycin. Reactions were initiated by addition of 20 μ l cold methanol and incubated for 20 min at 0°C. To terminate the reaction, 10 μ l 4 M KOH was added and incubated for an additional 20 min at 37°C. After the addition of 200 μ l 1 M KH_2PO_4 , reaction mixtures were extracted with 1 ml of ethyl acetate. Five hundred microliters of the ethyl acetate extract was mixed with 10 ml scintillation mixture and counted. Under these conditions, the amount of formyl-methionyl-puromycin formed was linear with respect to ribosome concentration within the amounts used in these experiments.

Analysis of Proteins. Proteins from 50S subunits and KSP particles were isolated by acetic acid extraction according to ref. 21. Two volumes of the extraction mixture, containing glacial acetic acid and 1 M MgCl_2 in 20:1 (vol/vol) ratio, were added to the sample with mixing. Samples were shaken at 4°C for 45 min and spun in a microcentrifuge at 4°C for 15 min. Proteins were precipitated from supernatants with 10 volumes of cold acetone for 2 hr at -75°C, dissolved in a minimal volume of gel loading buffer, and resolved by two-dimensional gel electrophoresis (22) or by SDS-gel electrophoresis (23). Proteins were visualized by Coomassie blue or silver staining. For sequencing, 50S subunit proteins resolved by two-dimensional gel were transferred to poly(vinylidene difluoride) membrane (Millipore) and sequenced in the protein-sequencing facility of the University of Illinois.

Preparation and Analysis of rRNA. rRNA was prepared from 50S ribosomal subunits or KSP particles by extraction with guanidine thiocyanate-phenol mixture according to ref. 24. RNA pellets were dissolved in 90% formamide, heated 1 min at 90°, and loaded onto a 6% denaturing polyacrylamide gel. Gels (20 \times 20 cm) were run at 20 W and stained with ethidium bromide.

SDS Treatment of 30S and 50S Ribosomal Subunits. 30S or 50S ribosomal subunits (150 μ g) were incubated for 1 hr at 40°C in 100 μ l buffer B (25 mM Tris-HCl, pH 7.5/20 mM MgCl_2 /150 mM NH_4Cl) with or without 0.5% SDS. The reaction mixtures were loaded onto 10–30% sucrose gradients (in buffer B) and centrifuged at 4°C in a SW 60 rotor for 2 hr at 55,000 rpm. The peaks were collected and concentrated by using Centricon 100 microconcentrators. The material was precipitated with 4 volumes of cold acetone, dissolved in 20 μ l

SDS loading buffer (25 mM Tris-HCl, pH 6.5/6 M urea/1% 2-mercaptoethanol/10% glycerol/1% SDS/0.005% bromophenol blue), heated for 2 min at 90°C, and analyzed by electrophoresis in 15% polyacrylamide/SDS/urea gel (25). The gel was stained with Coomassie blue.

RNase and Protease Treatment of KSP Particles. Two A_{260} units of the particles were incubated for 15 min at 37°C in 30 μ l of buffer A with or without RNase A (final concentration, 10 μ g/ml). This was followed by addition of SDS and proteinase K to final concentrations of 0.5% and 2 mg/ml, respectively, and incubation for 30 min at 37°C. Untreated control KSP particles were incubated for 45 min at 37°C in buffer A. At the end of the incubation, two-thirds of the reaction mixture was mixed with 10 μ l of SDS loading buffer and proteins were analyzed by gel electrophoresis in 15% polyacrylamide/SDS gel (23). The gel was stained with Coomassie blue.

Sequencing of 5S rRNA. To confirm the identity of the fast-migrating RNA band on the polyacrylamide gel, it was eluted from the gel and sequenced by the reverse transcriptase/dideoxy method using the primer d(CCCGCACCGACTACTCTCC), which is complementary to the 3' end of *T. aquaticus* 5S rRNA (26).

RESULTS

50S subunits of *T. aquaticus* ribosomes sediment as a single peak during sucrose gradient centrifugation (Fig. 1A). Treatment of the subunits with proteinase K in the presence of SDS followed by phenol extraction converted them into KSP particles, which formed four peaks on a sucrose gradient. The two major peaks sedimented at about 50S and 80S, while two minor peaks had sedimentation coefficients of ca. 15S and 30S (Fig. 1B). Peptidyl transferase assays showed that only the more rapidly sedimenting 50S and 80S peaks (KSP50 and KSP80 particles, respectively) possessed catalytic activity (Table 1). Repeated phenol extractions increased progressively the slow-sedimenting, inactive peaks, 15S and 30S, at the expense of the active KSP50 and KSP80 particles (Fig. 1C and D). The total peptidyl transferase activity of unfractionated KSP particles

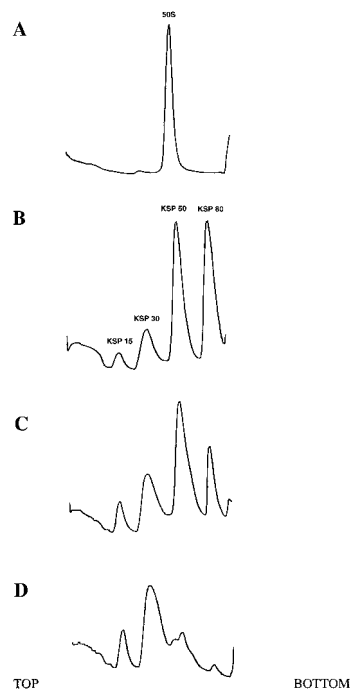


FIG. 1. Sucrose gradient centrifugation of 50S ribosomal subunits before (A) and after treatment with proteinase K, SDS, and one (B), two (C), or four (D) phenol extractions.

Table 1. Specific activities of 50S subunits and KSP particles

Ribosomal particles*	Peptidyl transferase activity, cpm†
50S subunits	8,780 ± 120
KSP15	210 ± 40
KSP30	200 ± 40
KSP50	3,910 ± 890
KSP80	3,620 ± 700
No ribosomes	260‡ ± 40

*50S subunits or KSP particles (0.2 A_{260}) were used in the peptidyl transferase assay. KSP particles were prepared by treatment of 50S subunits with proteinase K, SDS, and one phenol extraction, separated by sucrose gradient centrifugation, and precipitated from the gradient peaks (see Fig. 1B) by 3 volumes of ethanol.

†Average of two independent experiments.

‡The background ethyl acetate-extractable radioactivity apparently corresponds to unidentified methanolysis products of fMet-tRNA (50).

after multiple phenol extractions directly correlated with the combined amount of material in KSP50 and KSP80 peaks.

The RNA compositions of the KSP50 and KSP80 particles were identical and indistinguishable from that of untreated *T. aquaticus* large ribosomal subunits (Fig. 2). The 23S rRNA did not form a distinct band, probably because of partial *in vivo* degradation of the high-molecular-weight rRNA described previously for *T. aquaticus* ribosomes (27). Sequencing of the RNA band whose electrophoretic mobility was similar to that of *Escherichia coli* 5S rRNA verified that it was *T. aquaticus* 5S rRNA. Thus, the KSP50 and KSP80 particles that exhibit peptidyl transferase activity contain both 23S and 5S rRNA in a ratio similar to that of native 50S subunits. The amount of 5S rRNA in the inactive and less compact material from 30S (Fig. 2) and 15S (not shown) peaks was reduced significantly.

Previous results indicated that some ribosomal proteins remain associated with *T. aquaticus* rRNA even after extensive protein extraction (17). In agreement with these findings, when KSP particles were prepared from ³⁵S-labeled *T. aquaticus* ribosomes and fractionated by sucrose gradient sedimentation, radioactive material was found associated with KSP50 and KSP80 peaks (15S and 30S peaks contained very little radioactivity) (Fig. 3A). The specific radioactivity of the gradient peaks, reflecting their relative protein content, was reduced approximately 3-fold in the KSP50 and KSP80 peaks compared with untreated 50S subunits (Fig. 3).

To determine the correlation between the presence of the protein component and peptidyl transferase activity, increas-

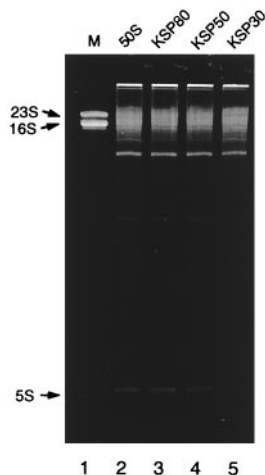


Fig. 2. RNA composition of KSP particles. RNA extracted from the gradient peaks was fractionated on a 6% denaturing polyacrylamide gel. Lanes: 1, marker (rRNA from *E. coli* 70S ribosomes); 2, untreated *T. aquaticus* 50S subunits; 3, KSP80; 4, KSP50; 5, KSP30.

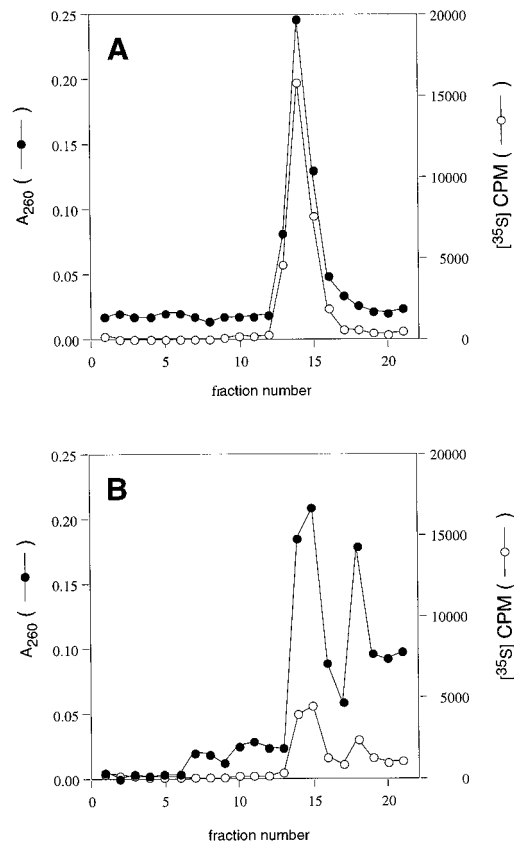


Fig. 3. Sucrose gradient fractionation of ³⁵S-labeled 50S ribosomal subunits (A) and KSP particles (B). Solid circles show optical density (A_{260}), and open circles show amount of [³⁵S] radioactivity in gradient fractions.

ing amounts of protein were removed from ³⁵S-labeled *T. aquaticus* 50S subunits by SDS/proteinase K treatment and multiple phenol extractions followed by peptidyl transferase assay. As can be seen from Fig. 4, removal of about two-thirds of the proteins present in *T. aquaticus* large ribosomal subunits

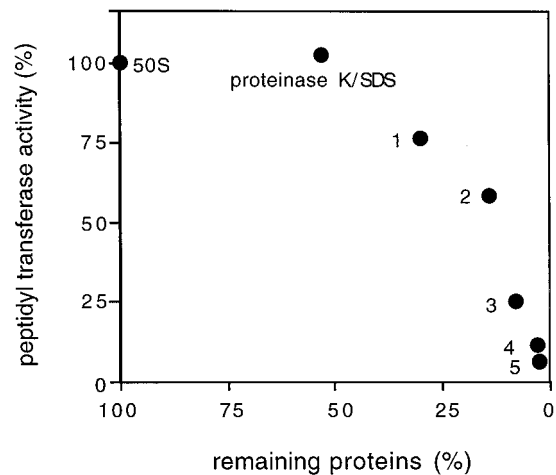


Fig. 4. Peptidyl transferase activity of partially deproteinized large ribosomal subunits. ³⁵S-labeled *T. aquaticus* 50S subunits were treated with proteinase K in the presence of SDS (proteinase K/SDS) followed by one, two, three, four, or five phenol extractions. Peptidyl transferase activity of the material precipitated from the aqueous phase with ethanol (y axis) was plotted against the amount of remaining proteins (x axis). Activity of the untreated 50S subunits (50S) was taken as 100%.

(estimated by the amount of ^{35}S -labeled-protein-associated radioactivity) does not significantly affect the ability of the particles to catalyze the peptidyl transferase reaction, whereas removal of the remaining proteins appears to correlate with progressive loss of activity. Thus, multiple phenol extractions converted compact and active KSP50 and KSP80 particles, which contained proteins, into unfolded, inactive, protein-free material sedimenting at 15S and 30S (Figs. 1 and 3). Treatment of 50S subunits with 6 M LiCl or with guanidine thiocyanate, which led to complete protein removal, was always accompanied by complete loss of activity (data not shown).

When the proteins from KSP50 and KSP80 particles were separated by two-dimensional gel electrophoresis, nine protein spots could be seen (Fig. 5A). Since both the protein (Fig. 5B and C) and RNA (Fig. 2) compositions of KSP50 and KSP80 particles were indistinguishable, it is likely that KSP80 particles correspond to KSP50 dimers. The electrophoretic mobilities of proteins from KSP50 or KSP80 particles coincided precisely with the subset of those from the untreated large subunits, demonstrated by comigration of radiolabeled proteins from KSP50 particles with unlabeled proteins from 50S subunits. Eight of the particle-associated proteins were present in substantial amounts as judged by the intensity of staining of protein spots on the gel. The ninth protein (spot X, Fig. 5) was either completely missing in some experiments or its amount was reduced significantly in particles compared with the large ribosomal subunits.

The major proteins in the KSP particles were identified by transferal to poly(vinylidene difluoride) membrane and microsequencing. Comparison with known ribosomal protein sequences either from *Thermus thermophilus* or other bacteria identified the eight major proteins as L2, L3, L13, L15, L17, L18, L21, and L22 (Fig. 6). Given the presence of 5S rRNA in functionally active KSP particles, it was surprising to find only L18 out of three 5S rRNA-binding proteins (homologous to *E. coli* proteins L5, L18, and L25) identified in genus *Thermus* (28–30). Though we cannot exclude loss of some proteins during purification, it is possible that the missing proteins are less critical for retaining 5S rRNA in functional subunits than was found previously for mesophilic ribosomes (see ref. 31 for review).

N-terminal sequence analysis and electrophoretic mobilities of the KSP particle-associated proteins showed that, in spite of extensive protease digestion of the large ribosomal subunit, the

<u>L2</u>	Taq	AVKKFKP *:** **	<u>L17</u>	Taq	MRHLKSGRKLNRHSS *** **
	Bsu	AIKKYKPTS...		Eco	MRHRKSGRQLNRNSS...
<u>L3</u>	Taq	MKGILGTVKVG ***** **	<u>L18</u>	Taq	ARLTAYERRK *****
	Tth	MKGILGTVKVG...		Tth	MARLTAYERRK...
<u>L13</u>	Taq	MKTYVPEKETE ***:* **	<u>L21</u>	Taq	MFALVKTGGK * **;*****
	Hin	MKTFVAKPET...		Bsu	MYALIKTGGK...
<u>L15</u>	Taq	MKLSDLKPNP *****;***	<u>L22</u>	Taq	MEAKAIARYV *****
	Tth	MKLSDLRPNP		Tth	MEAKAIARYV...

FIG. 6. N-terminal amino acid sequences of proteins from KSP50 particles and comparison with eubacterial ribosomal proteins. Bsu, *Bacillus subtilis*; Eco, *Escherichia coli*; Hin, *Hemophilus influenzae*; Taq, *Thermus aquaticus*; Tth, *Thermus thermophilus*.

remaining proteins were remarkably protease-resistant. Similarly, additional protease treatment after isolation of KSP particles caused only slight protein degradation (Fig. 7, lane 2). However, even brief treatment of the particles with RNase A led to a dramatic increase in the susceptibility of the proteins to digestion by proteinase K (Fig. 7, lane 3). Thus, directly or indirectly, rRNA protects proteins in the KSP particles (and in 50S ribosomal subunits) from protease degradation.

Interestingly, small ribosomal subunits were much more sensitive to protein extraction than large subunits. Incubation with 0.5% SDS, even in the absence of protease digestion and phenol extraction, removed most of the 30S subunit proteins, while only a limited number of 50S subunit proteins dissociated under these conditions (Fig. 8). This observation suggests a fundamental difference in structural organization between the large and small ribosomal subunits.

DISCUSSION

These studies show that, in spite of extensive protease digestion and vigorous extraction of *T. aquaticus* 50S ribosomal subunits with detergent and phenol, functionally active particles cosedimenting with untreated 50S subunits are obtained. The macromolecular composition of these particles was analyzed to

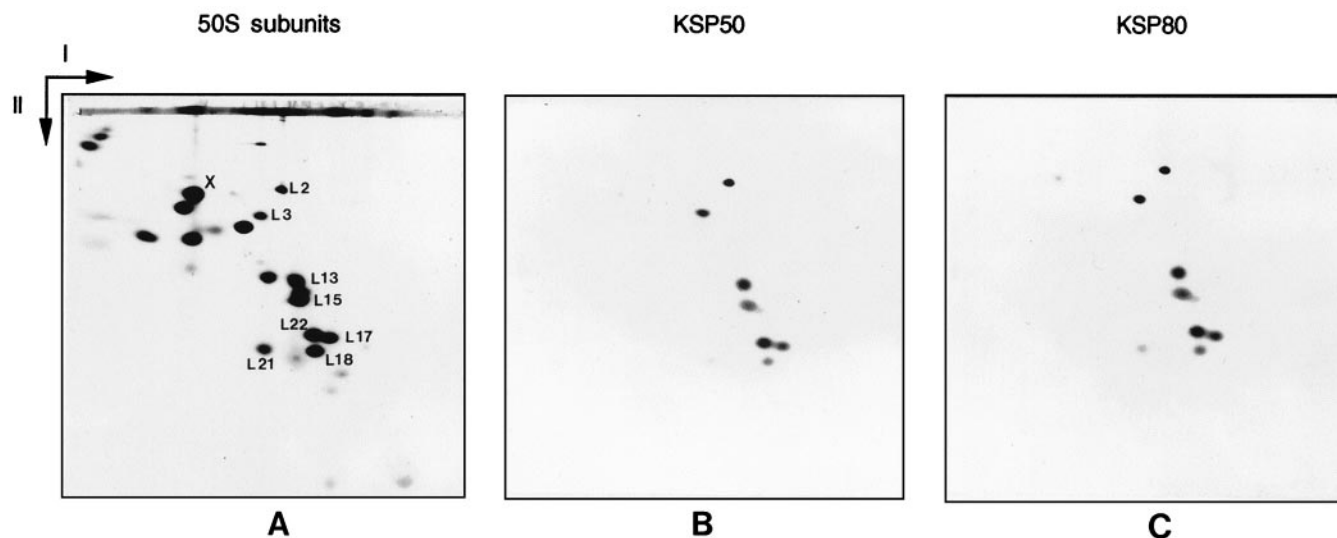


FIG. 5. Two-dimensional electrophoresis of *T. aquaticus* ribosomal proteins from 50S subunits (A), KSP50 (B), and KSP80 (C) particles. Proteins present in the KSP particles are marked on total 50S subunit protein gel (A). Correspondence between KSP and 50S subunit proteins was determined by coelectrophoresis of ^{35}S -labeled proteins from KSP50 particles with unlabeled proteins from 50S subunits followed by Coomassie staining of the gel and autoradiography.

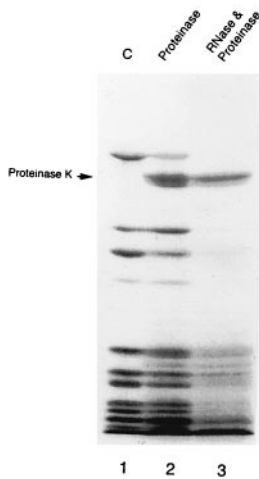


FIG. 7. Effect of RNase treatment on accessibility of proteins in KSP50 particles to proteinase K. SDS-gel electrophoresis of proteins obtained from KSP particles (lane 1); KSP particles treated with proteinase K (lane 2); and KSP particles preincubated with RNase before proteinase K treatment (lane 3).

further define the minimum number of components required to sustain peptidyl transferase activity. Our findings can be summarized as follows. (i) Functionally active particles contain 23S rRNA and 5S rRNA in addition to near-stoichiometric levels of ribosomal proteins L2, L3, L13, and L22, and reduced levels of proteins L15, L18, and L21. (ii) Removal of these remaining proteins is correlated with unfolding of the particles and concomitant loss of peptidyl transferase activity. (iii) Susceptibility of the remaining proteins to protease digestion is increased dramatically by treatment with ribonuclease.

Macromolecules Required for Peptidyl Transferase. Early salt extraction and reconstitution experiments, designed to identify the essential components of peptidyl transferase, have shown that all but 23S rRNA and three proteins—L2, L3, and L4—are dispensable for catalytic activity (4–7, 32). Of these, protein L4 was notably absent from the active KSP particles characterized here. Although the mobility of spot X (Fig. 5) is potentially compatible with that of L4, it was found only in very low amounts or was absent in some preparations. We conclude that only 23S rRNA and ribosomal proteins L2 and L3 remain

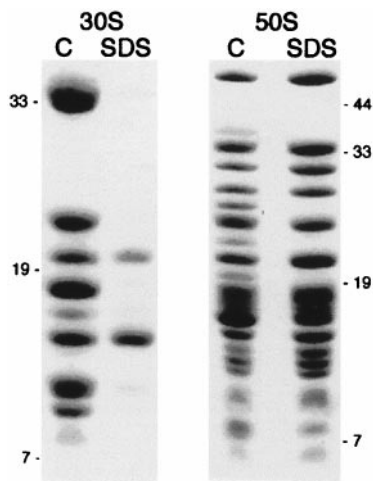


FIG. 8. Extraction of *T. aquaticus* 30S and 50S ribosomal subunits with SDS. Ribosomal subunits were incubated in the absence (C) or in the presence (SDS) of 0.5% SDS as described in *Materials and Methods*, purified by sucrose gradient centrifugation, and analyzed by SDS/urea gel electrophoresis. Positions of molecular weight markers are indicated.

on the list of potential macromolecular components required for catalysis of peptide bond formation.

Biochemical and genetic data have implicated 23S rRNA as a component of the peptidyl transferase center. Affinity crosslinking and RNA footprinting demonstrated that several nucleotides in domain V (and perhaps domain IV) of 23S rRNA interact with the acceptor ends of the peptidyl- and aminoacyl tRNAs as well as with antibiotic inhibitors of the peptidyl transferase reaction (33–35). Mutational analysis (see refs. 14, 36, and 37 for review) and modification–interference studies (38) suggested the importance of several conserved positions in domain V for tRNA binding and catalysis of peptide bond formation. Furthermore, *in vitro* genetics experiments have shown that formation of a base pair between C74 of peptidyl-tRNA and G2252 of 23S rRNA is critical for the peptidyl transferase function (39). Thus, it is clear that rRNA plays a central role in the function of the catalytic center of the ribosome.

Potential involvement of ribosomal proteins in this catalytic function cannot be excluded. Omission of either L2 or L3 during reconstitution of 50S ribosomal subunits dramatically reduces the peptidyl transferase activity of the assembled particles (6). Crosslinking and footprinting results revealed that L2 and L3 bind, respectively, to segments of domains IV and VI of 23S rRNA that flank domain V (40–42). Proximity of both L2 and L3 to the peptidyl transferase center has been demonstrated by crosslinking of L2 to the 3' end of the P site-bound tRNA (43–45) and of L3 to a photolabile oligonucleotide probe annealed to a functionally important segment of domain V (46). Thus, present information concerning the locations of proteins L2 and L3 in the ribosome remains compatible with the possibility that they play essential roles in the peptidyl transferase function.

The available experimental data on the nature of ribosomal peptidyl transferase are compatible with at least two possibilities: (i) peptidyl transferase is a ribozyme, whose functional conformation is stabilized by ribosomal proteins; and (ii) peptidyl transferase is a ribonucleoprotein enzyme, where, for example, binding of substrates is facilitated by RNA and the chemical step of catalysis depends on ribosomal protein(s).

Implications for Structural Organization of the 50S Subunit. Large subunits from both *E. coli* and *T. aquaticus* are dramatically more resistant to extraction with SDS than 30S subunits (ref. 17 and Fig. 8), suggesting a fundamentally different organization of protein and RNA in large and small ribosomal subunits. A further clue comes from the observation that, while proteins in KSP particles are remarkably resistant to protease digestion, RNase treatment renders them susceptible to proteolysis (Fig. 7). These observations, as well as the resistance of the KSP proteins to multiple rounds of phenol extraction, are consistent with a model in which a core of extraction-resistant proteins is enclosed by a 23S rRNA cage (8). Existence of an RNA/protein core in the *E. coli* large ribosomal subunit was proposed earlier by Kuhlbrandt and Garrett based on results of controlled trypsin and nuclease digestion (47). Of six proteins assigned to the *E. coli* 50S subunit core, five (with the exception of L24) correspond to proteins found in *T. aquaticus* KSP particles. Particles obtained by partial reconstitution or disassembly of *E. coli* 50S subunits, whose protein compositions were similar to that of KSP particles, were found to retain the compact size and shape of 50S subunits (48). The protein core could contribute to stabilization of the three-dimensional organization of 23S rRNA, helping to bring together different RNA functional elements dispersed throughout the 23S rRNA structure. Such a model would explain why removal of the core proteins coincides with unfolding of the particles and loss of peptidyl transferase activity. Also compatible with this model is the observation that most of the proteins found in KSP particles

are primary-binding proteins that are involved in early steps of *in vitro* assembly of the large subunit (49).

The studies reported here support the previous finding that peptidyl transferase activity of *T. aquaticus* ribosomes is remarkably resistant to vigorous protein extraction. The protein composition of the KSP particles is significantly higher than the levels of proteins previously measured after extraction of *T. aquaticus* 70S ribosomes (17). Part of this discrepancy can be attributed to the fact that the small subunit proteins, which contribute about 40% of the total 70S proteins, are completely removed under the extraction conditions (Fig. 8; unpublished data). Second, *T. aquaticus* 70S ribosomes contain significant amounts of absorbed nonribosomal proteins whose removal tends to result in overestimation of extraction efficiency; these are removed largely during isolation of 50S subunits in sucrose gradient. Third, the approximately 2-fold drop in specific activity observed after the sucrose gradient isolation of KSP particles (Table 1) may lead to an underestimate of the original activity of the extracted particles.

Association of a limited number of ribosomal proteins with rRNA in the active particles is fully compatible with the possibility that peptidyl transfer is catalyzed by RNA. Indeed, of the proteins found in the KSP particles, only L2 and L3 remain under consideration as direct participants in catalysis of peptidyl transferase reaction. Recently, Nitta *et al.* (15, 16) have reported that 23S rRNA or its individual domains can promote formation of *N*-acetyl-diphenylalanine from *N*-Ac-Phe-tRNA and Phe-tRNA. Though a very low rate of enhancement relative to a nonspecific background reaction and unusual reaction conditions leave room for alternative interpretations, these findings might provide the first direct evidence for participation of 23S rRNA in the catalytic step of peptide bond formation. Nevertheless, we emphasize that, though experimental data continue to be consistent with the notion that RNA is responsible for catalysis of peptide bond formation, conclusive evidence has yet to be found.

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