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Proteolysis of Ribosomal Protein S1 from *Escherichia coli* and *Thermus thermophilus* Leads to Formation of Two Different Fragments

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Abstract—As a result of limited tryptic proteolysis of S1 ribosomal protein (molecular mass 60 kD) from *Thermus thermophilus*, 25 N-terminal amino acid residues and 71 C-terminal amino acid residues are split off and a stable high-molecular-weight fragment with molecular mass of 49 kD is formed that retains RNA-binding properties and is capable of interacting with 30S ribosomal subunit. Earlier, application of a similar procedure for the formation of a fragment of S1 protein from *Escherichia coli* resulted in splitting of 171 N-terminal amino acid residues with the formation of a 41.3 kD fragment that possesses RNA-binding properties only. Thus, in spite of high homology between *E. coli* and *T. thermophilus* proteins, the proteolysis leads to the formation of two different fragments, which points, in our opinion, to the fact of significant differences between their structures.

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Key words: ribosome, S1 ribosomal protein, limited proteolysis, *Thermus thermophilus*

Ribosomal S1 protein was found recently as a component of the *Thermus thermophilus* ribosome [1]. This protein is homologous in its amino acid sequence to S1 protein of *Escherichia coli*, and they have similar molecular masses (~60 kD) and length of 536 amino acid residues. It is known that S1 protein of *E. coli* is a polyfunctional protein. It interacts with mRNA, plays an important role in initiation and successful translation of most mRNAs *in vivo* [2], and participates in the elongation process as well [3]. S1 protein was found to interact with unstructured mRNA region enriched with pyrimidines, ~10 nucleotides in length, located upstream from the Shine–Dalgarno sequence (SD) [4, 5]. Like some other ribosomal proteins, S1 protein is an autogenic repressor of its own synthesis [6]. S1 protein interacts with an mRNA-like region of tmRNA, thus participating in the *trans*-translation process [7]. In this case, S1 protein seems to exhibit helicase activity [8] and induces conformational changes in tmRNA via its unfolding [9], thus enabling establishment of proper reading frame [10, 11]. This possibility is feasible because *E. coli* S1 protein is necessary for translation initiation of mRNA devoid of a Shine–Dalgarno sequence [12].

The *E. coli* S1 protein executes a number of nonribosomal functions. In particular, it is a component of Qβ-replicase as one of its four subunits, and it is necessary for the replication of (+)-chain of Qβ RNA [13, 14]. It interacts with bacteriophage T4 ribonuclease *reg*B and enhances its activity tenfold [15]. S1 protein was shown to be a poly(A)-binding protein, which was co-purified with ribonuclease E (RNase E) and polynucleotide phosphorylase (PNPase), components of the degradosome [16].

The *E. coli* S1 protein is a ribosomal protein with unique primary structure. Its amino acid sequence contains six homologous RNA-binding repeats (R) ~70 amino acids in length, separated by 15-20 amino acid residues [17, 18]. This repeat was first found in *E. coli* S1 protein and named RNA-binding S1-motif or S1-domain [17]. The structure of S1 protein from *E. coli* can be functionally divided into two regions: N-terminal including two S1-repeats (R1, R2) and participating in interaction with ribosome, and C-terminal containing four S1-repeats (R3-R6) and directly interacting with mRNA [17]. One copy of the RNA-binding S1-motif can be found in many RNA-binding proteins, such as PNPase [19], bacterial and chloroplast translation initiation factor IF1, eukaryotic translation initiation factor eIF2a [18], RNase E [20], and transcription factor NusA [21].

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Proteins containing 9-12 copies of this motif are found in yeast, *Caenorhabditis elegans*, and human [21, 22]. The structure of RNA-binding S1-domain of *E. coli* PNPase has been determined by NMR [21]. It consists of a five-stranded antiparallel β-barrel. However, the 3-D structure of ribosomal S1 protein is still unknown. According to early physicochemical studies, *E. coli* S1 protein is not compactly folded in solution [17, 23-26], and efforts to obtain crystals from this protein have not been successful.

This is a reason to search for stable fragments of S1 protein. One such fragment with molecular mass of 41.3 kD (386 amino acid residues) was obtained by limited tryptic hydrolysis of *E. coli* S1 protein [27]. It has been demonstrated that this fragment represents a truncated form of the protein from its N-terminus by two S1-domains (by 171 amino acid residues). However, according to data of small-angle X-ray scattering, like the full-length protein this fragment has no compact conformation [17]. Other fragments of *E. coli* S1 protein were obtained using biochemical methods, and functions of some of them were determined. N-Terminal fragment (R1, R2) of the protein was able to compete with the whole S1 protein when they interact with ribosomal 30S-subunit, but it does not interact with RNA [28]. The protein fragment truncated from the C-terminus by two S1-repeats (R1-R4) interacts with the subunit, participates in translation of synthetic mRNA (for instance, poly(U)), but is not effective in translation of natural mRNAs [29]. Also, a natural mutant S1 protein is known (M1-S1) devoid of the last C-terminal repeat (R1-R5) [30]. This protein fulfills all general functions of S1 protein, but its activity in natural mRNA translation is decreased by 25%. The sixth S1-repeat (R6) of *E. coli* S1 protein participates in autogenic regulation of its own synthesis [31]. Several works appeared during recent years in which various fragments of *E. coli* S1 protein obtained by the methods of genetic engineering were studied. The third S1-repeat (R3) of S1 protein is of principal importance for the interaction with mRNA and tmRNA [32]. This repeat is important for the interaction of S1 protein with *reg*B ribonuclease [33]. Thus, data has accumulated on various functions fulfilled by fragments and distinct S1-domains in cellular processes.

The primary structure of *T. thermophilus* S1 protein, like that of *E. coli* S1 protein, contains RNA-binding S1-repeats. According to our data, *T. thermophilus* S1 protein contains six S1-repeats [1]. However, other data exists suggesting that the amino acid sequence of *T. thermophilus* S1 protein contains five S1-repeats, because the last (sixth) repeat (amino acid residues 466-536) is degenerate (UniProtKB/Swiss-Prot: Q83YV9_THETH). The S1 protein from *T. thermophilus* was shown recently to conform to a compact globular conformation under particular ionic conditions, possesses developed tertiary structure, and contains at least three thermodynamic domains [34, 35]. However, attempts to obtain its crystals were not successful, like for *E. coli* S1 protein. This may be associated with slow spontaneous hydrolysis of *T. thermophilus* S1 protein, which was detected earlier in S1 protein from *E. coli* [17]. Recently, substantial progress has been achieved in structural studies of thermophilic proteins. They are more stable in a solution and more easily form crystals. We have obtained a stable fragment of 49 kD from *T. thermophilus* S1 protein using limited hydrolysis. It is formed as a result of cleavage of 25 amino acid residues from the N-terminus and 71 amino acid residues from the C-terminus. The truncated form of the protein retains five S1-domains (R1-R5). The fragment retains RNA-binding properties and is capable of interacting with ribosomal 30S-subunit. Thus, in spite of high homology of S1 proteins from *E. coli* and *T. thermophilus*, the limited hydrolysis of these proteins leads to the formation of totally different fragments. This is evidence for substantial differences in their structures.

MATERIALS AND METHODS

Materials. All chemicals and enzymes were purchased from Sigma (USA) and Serva (Germany), DEAE-Sepharose from Pharmacia Biotech (Sweden), nitrocellulose membrane from GE Healthcare (USA), and [α-^{32}P]UTP (400 MBq/ml, 148 TBq/mol) from Izotop (Russia).

Protein isolation and purification. Recombinant *T. thermophilus* S1 protein was isolated from the *E. coli* overproducing strain BL21(DE3)pET21d-tthS1 and purified as described earlier [1]. The protein quality was analyzed by standard SDS-PAGE in 12% polyacrylamide gel [36]. The protein sample was stored as a sulfate pellet (2 M) at 4°C. Protein concentration was determined by Bradford's standard protocol [37].

Before starting the experiments, the protein–sulfate pellet was dissolved in buffer containing 20 mM Tris-HCl, pH 8.0, and 1 mM EDTA and dialyzed against appropriate buffers at room temperature overnight.

Limited proteolysis of S1 protein from *T. thermophilus* and production of the fragment in analytical and preparative amounts. Freshly prepared trypsin solutions (TPCK-treated enzyme from bovine pancreas (Serva)) were used in the study. For the methods requiring only small amounts of protein sample (analysis by mass-spectrometry and N-terminal amino acid sequence determination) the reaction of limited proteolysis of S1 protein with trypsin was carried out in a buffer containing 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 0.01% NaN_3 at 0°C and protein/enzyme ratio of 100 : 1 (w/w). The volume of reaction mixture was 50-100 μl, and protein mass was 20-30 μg. The proteolysis reaction was stopped by the transfer of an aliquot from total reaction mixture after 1, 3, 10, 30, and 60 min into the sample buffer containing SDS and by heating at 100°C for 5 min. The products formed

after proteolysis of S1 protein from *T. thermophilus* were analyzed using a standard SDS-PAGE protocol according to the Laemmli method [36].

To produce the stable protein fragment in preparative amounts, the reaction of limited tryptic hydrolysis was carried out in buffer containing 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 0.01% NaN_3 at 0°C for 60 min with the protein/enzyme ratio of 400 : 1 (w/w). The hydrolysis reaction was stopped by trypsin inhibitor (aprotinin from bovine lung; Sigma) at the enzyme/inhibitor molar ratio of 1 : 3. The volume of reaction mixture was 200-500 μl, and the mass of S1 protein was 0.5-3.0 mg.

The stable protein fragment was purified by ion-exchange chromatography on DEAE-Sepharose according to the method described earlier [1] with some modifications. The column volume was 10 ml, and the rate of protein loading was 100 ml/h. The sorbent-bound protein fragment was eluted with 2 × 100 ml of NaCl linear gradient (0-200 mM) in buffer containing 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 0.01% NaN_3. The elution rate was 100 ml/h, the volume of fractions 5 ml. The fractions absorbing at 280 nm were analyzed by SDS-PAGE [36]. The fractions containing the protein fragment were pooled and precipitated with 2.5 M ammonium sulfate. Protein concentration was determined taking the absorbance coefficient calculated by the software Vector NTI v.6.0.0.0 (InfoMax Inc.).

Mass-spectrometry of *T. thermophilus* S1 protein fragments. Proteolytic protein fragments were separated by SDS electrophoresis in 10 or 12% polyacrylamide gel. The gels were stained with Coomassie G-250, the stained bands were cut off the gel, and full tryptic digestion was carried out according to a standard protocol. Mass-spectroscopy was carried out using a Reflex III MALDI-TOF mass spectrometer (Bruker, USA) (in the Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences, Moscow). The software MASCOT (http://www.matrixscience.com) was used to search for the full-length sequence and alignment of the obtained fragments with this sequence.

Determination of N-terminal amino acid sequence of the *T. thermophilus* S1 protein fragment. The N-terminal amino acid sequences of proteins were determined by the Edman method on an Applied Biosystems automated sequencer (model 447) with 120A HPLC-system (in the Institute for Bioorganic Chemistry of the Russian Academy of Sciences, Moscow). Electrotransfer of the fragment onto an Immobilon-P^{sq} polyvinylidene fluoride membrane [38] in buffer containing 10 mM Na_3BO_3 (pH 11.0) and 0.02% (v/v) β-mercaptoethanol was carried out before the sequence determination.

Examination of RNA-binding properties of the *T. thermophilus* S1 protein and its fragment. An RNA fragment 115 nucleotides in length was produced by *in vitro* transcription using T7 RNA-polymerase by the method described earlier [39] with some modifications. The plasmid pBluescript II SK(−) linearized by *Ecl*136II restrictase was used as a template. [α-^{32}P]UTP and non-labeled UTP at 10-fold decreased concentration were added to the transcription mixture to obtain radiolabeled RNA.

The formation of RNA–protein complex was followed by the method of retardation of mobility of RNA–protein complexes in polyacrylamide gel (gel-shift assay or electrophoretic mobility shift assay) described in [40] with some modifications. The α-^{32}P-labeled RNA (0.4 pmol, 5000 cpm) was incubated with various amounts of protein or its fragment (from 0.4 to 6.4 pmol) for 15 min at 30°C in 10 μl of buffer containing 20 mM Hepes-KOH (pH 7.6) and 100 mM KCl. Glycerol was added to the samples to the final concentration of 10%. RNA–protein complexes were separated from free RNA by non-denaturing electrophoresis in 5% polyacrylamide gel prepared in TBE buffer (89 mM Tris-borate, pH 8.2, containing 2 mM EDTA). The gels were dried after the electrophoresis, and visualization and data processing was conducted using the Cyclone®StoragePhosphorSystem (Packard Instrument Company Inc., USA) and OptiQuant software (version 03.00).

Dissociation constants of RNA–protein complexes were determined by the method of sorption on nitrocellulose filters [41] with some modifications. The reaction mixture (total volume 50 μl) contained 0.4 pmol of α-^{32}P-labeled RNA fragment and from 0.4 to 12.8 pmol of S1 protein or its fragment in buffer containing 20 mM Hepes-KOH (pH 7.6) and 100 mM KCl. After incubation, the reaction mixture was filtered through a nitrocellulose membrane (GE Healthcare) wetted with the same buffer. The sorption on membrane was evaluated using a phosphoimager as described above. Nonspecific RNA sorption measured in absence of protein in the mixture was 1-2%. Saturation extent at maximum protein concentration (maximum amount of radioactive membrane-bound RNA in experiment) was taken as 100%. Apparent dissociation constant was determined as protein concentration necessary for 50% saturation.

Study of the interaction between *T. thermophilus* S1 protein and its fragment with 70S ribosome devoid of S1 protein. 70S ribosomes from *T. thermophilus* were prepared by the method described earlier [42]. S1 protein was removed from 70S ribosome by affinity chromatography on poly(U)-Sepharose as described earlier [43] with some modifications. The column with sorbent (1 ml in volume) was preliminarily equilibrated with buffer of high ionic strength containing 20 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 1 M NH_4Cl, and 2 mM β-mercaptoethanol. 70S ribosomes were dialyzed against the same buffer overnight at room temperature and loaded on the column (sample volume 300-500 μl, concentration 20 mg/ml). The ribosomes were eluted from the column with the same buffer after 20 min incubation at room temperature. The procedure was done twice. The absence

of S1 protein was monitored by electrophoresis in 12% polyacrylamide gel with SDS [36]. 70S ribosomes devoid of S1 protein (70S(–S1)) were dialyzed overnight at room temperature against dissociating buffer containing 20 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 400 mM NaCl, and 2 mM β-mercaptoethanol [44]. Then they were activated for 40 min at 70°C, and the S1 protein or its fragment preliminarily transferred into the same buffer were added at the molar ratio 70S(–S1)/S1(fragment) of 1 : 2. The mixture was incubated for 10 min at the same temperature. Ribosomal subunits were separated by centrifugation in sucrose gradient (10-30%) prepared in the same buffer according to the protocol described elsewhere [42]. The loading of ribosomal preparation was calculated as 80-100 μg of ribosomes per ml of sucrose gradient. Centrifugation was carried out in an SW-27 rotor for 19 h at 65,000*g* and 4°C. Then the content of centrifuge tubes was divided into fractions, and absorption of fractions was measured at 260 nm. The peak fractions containing 30S and 50S subunits were pooled separately and analyzed by Laemmli electrophoresis.

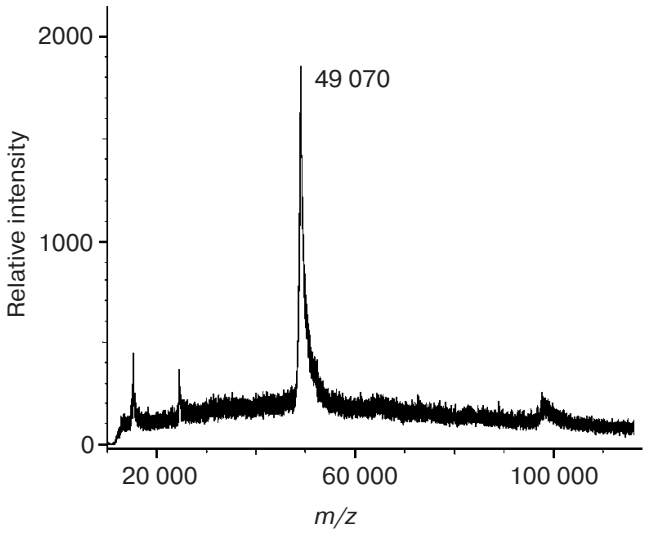

Fig. 2. MALDI-TOF-mass spectrum of the stable fragment of S1 protein from *T. thermophilus*.

RESULTS AND DISCUSSION

Production of the stable fragment of *T. thermophilus* S1 protein. Various protein/enzyme ratios such as 400 : 1, 250 : 1, 100 : 1, and 50 : 1 (w/w) were used when conducting the limited proteolysis of the protein with trypsin. The ratio of 100 : 1 (Fig. 1) was used for obtaining small amounts of stable *T. thermophilus* S1 protein fragment (total reaction volume 50-100 μl, protein concentration 0.3-0.4 mg/ml). Electrophoretic analysis of limited tryptic hydrolysis of the *T. thermophilus* S1 protein (protein/enzyme ratio 100 : 1) demonstrated that the formation of the stable high-molecular-weight product is of stepwise nature. The high-molecular-weight fragment is formed less than in 1 min (lane *2*, upper band), which is gradually subjected to further hydrolysis (decreasing intensity of the upper band on the lanes *2-6*). This high-molecular-weight protein fragment is virtually completely transformed in 60 min into a stable high-molecular-weight fragment (increasing intensity of the lower band on lanes *2-6*), which is not subjected to further hydrolysis at least for 8 h. Both high-molecular-weight fragments are present 60 min after beginning of the reaction at protein/enzyme ratios of 400 : 1 and 250 : 1. However, the stable fragment is subject to hydrolysis at the protein/enzyme ratio of 50 : 1 (electrophoretic data are not shown).

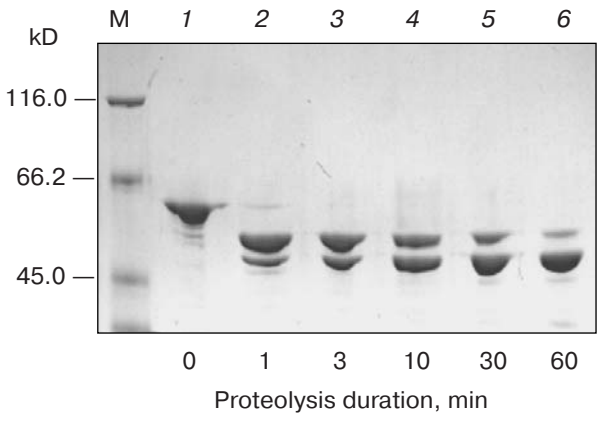

Fig. 1. Electrophoretic analysis of products obtained from limited tryptic hydrolysis of the *T. thermophilus* S1 protein in 10% polyacrylamide gel with SDS. The protein/trypsin ratio was 100 : 1. The protein amount in samples is ~5 μg. M, molecular mass markers; lanes: *1*) S1 protein from *T. thermophilus* before addition of trypsin; *2-6*) samples after 1, 3, 10, 30, and 60 min incubation with trypsin, respectively.

Identification of stable proteolytic fragment of *T. thermophilus* S1 protein. The stable fragment of the *T. thermophilus* S1 protein and products of its exhaustive tryptic digestion were subjected to mass-spectrometric analysis, and the molecular mass of the fragment and approximate cleavage sites of the protein were determined. In accordance with these data, the molecular mass of the stable *T. thermophilus* S1 protein fragment is 49,070 daltons (Fig. 2) and localized within the full-length protein (Fig. 3).

N-Terminal amino acid analysis of the stable protein fragment demonstrated that the sample contains a mixture of peptides. The predominant portion (~80%) starts from the LEKRV sequence, and other portions from RVRPG and VRPGQ. The ambiguity of the trypsin attack site is due to the close vicinity of lysine and arginine residues in this region of the primary structure of *T. thermophilus* S1 protein. The data obtained by mass-spectrometry and N-terminal amino acid analysis suggest that

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                                   ↓  ↓↓
  1 MEDKATQTPE QTFSMEAALQ ETEARLEKRV RPGQILTGKV VLVGSEGVAV
 51 DIGAKTEGII PFNQLTTKPL SEEELRNLLS PGDEVKVQVL RVDPETGQIL
101 LSRKKIEAQE KWDRIQELYE KGEPVTVTIK ERVKGGVVAE LDGVQGFMPA
151 SQLDLRRVPN LDEFVGQQVL AKIIEFHRRK GRVILSRRAV LEEEQKKARE
201 AFLKSLEPGQ VVEGTVVEVT DFGVFVNLGP VDGLVHRSEI TWGRFNHPRE
251 VIQKGQKVKA RVLSVDPEKE RVNLSIKALI PDPWTTVAEK YPVGTRVRGK
301 VVGLTQFGAF VEVEPGLEGL IHISELSWTK RPKHPSEVVK EGDEVEAVVL
351 RLDPEERRLS LGLKQTQPDP WQQLTEKYPP GTVLKGKVTG VTDFGVFVEI
401 EPGIEGLVHV SELDHKRVEN PAALFKKGDE MEVVVLNIDP VEQRVSLSRK
451 RLLPPPLPQE EERPRRARSG KERARRKGAP RREDRREYEY GAVAEYNLYD
501 ASSVPTTTAT VKLGDLYGDL LASLGLEEEA EEKSRG
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Fig. 3. Amino acid sequence of ribosomal S1 protein from *T. thermophilus*. Peptides identified by mass-spectrometric analysis after exhaustive tryptic hydrolysis of the protein fragment are given in bold. Arrows show the protein cleavage sites determined by N-terminal amino acid analysis.

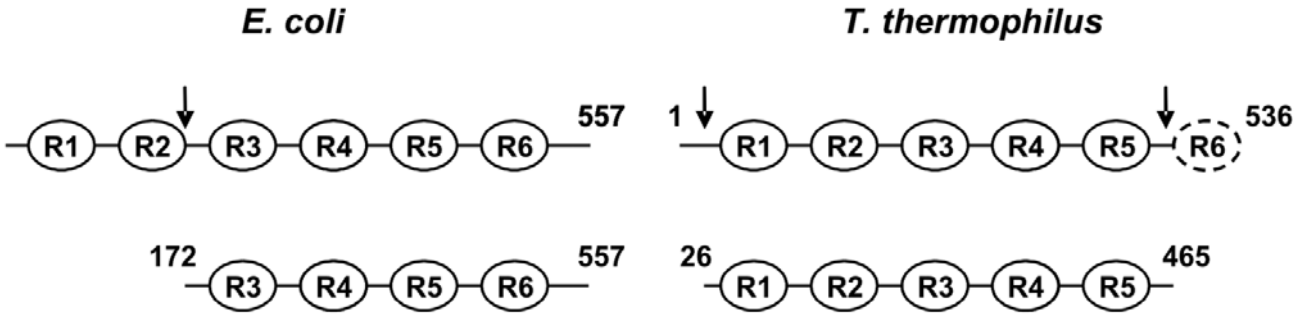

Fig. 4. Schematic representation of products formed by limited tryptic proteolysis of S1 ribosomal proteins from *E. coli* and *T. thermophilus*. R, homologous repeats (S1-domains). Arrows show the sites of protein cleavage. The broken line encircles the sixth degenerate S1-domain.

25 N-terminal and 70-71 C-terminal amino acids (cleavage sites R_{25}-L_{26} from N-terminus and R_{465}-R_{466} from C-terminus) (Fig. 4) are cleaved from the *T. thermophilus* S1 protein under its trypsinolysis. The calculated molecular mass value of the stable fragment having regard to the data of N-terminal amino acid analysis is 49,275 daltons, which coincides, within the accuracy of the analysis with that determined by mass-spectrometry. This fragment is named 49-kD fragment.

As evident from the mass-spectrometry data, the hydrolysis of full-length protein starts from the C-terminus of the molecule, and ~60 amino acids are cleaved (data not shown) in the case of unstable high-molecular-weight fragment of the *T. thermophilus* S1 protein (Fig. 1). The molecular mass of the unstable fragment is ~53 kD.

Study of RNA-binding properties of *T. thermophilus* S1 protein and its fragment. It is known that S1 protein from *E. coli* is not strictly specific to RNA sequence [17]. Therefore, an RNA fragment of arbitrary sequence was used for the study of RNA-binding properties of *T. thermophilus* S1 protein and its fragment. An RNA fragment 115 nucleotides in length was produced by *in vitro* transcription using RNA-polymerase T7 from pBluescript II SK(–) plasmid linearized at the site *Ecl*136II. The apparent dissociation constants for *T. thermophilus* S1 protein and its stable fragment were determined by RNA–protein complex sorption on nitrocellulose filters. The titration curve of ^{32}P-labeled RNA with the protein (fragment) is shown in Fig. 5. The calculated dissociation constant for RNA–protein complexes for the whole S1 protein (K_d = $0.9 \cdot 10^{-9}$ M) is not significantly different from that for its fragment ($K_d = 1.3 \cdot 10^{-9}$ M). This suggests that S1 protein being shortened from N- and C-termini does not alter substantially its RNA-binding properties.

The formation of RNA–protein (fragment) complex was monitored by the gel shift method. With this aim, RNA was titrated with various amounts of S1 protein or its fragment. The non-denaturing electrophoretic analysis in 5% polyacrylamide gel demonstrated that both S1 protein and its fragment interact with RNA (Fig. 6). As is evident from the data, the number of RNA-associated protein molecules increases while RNA is titrated with

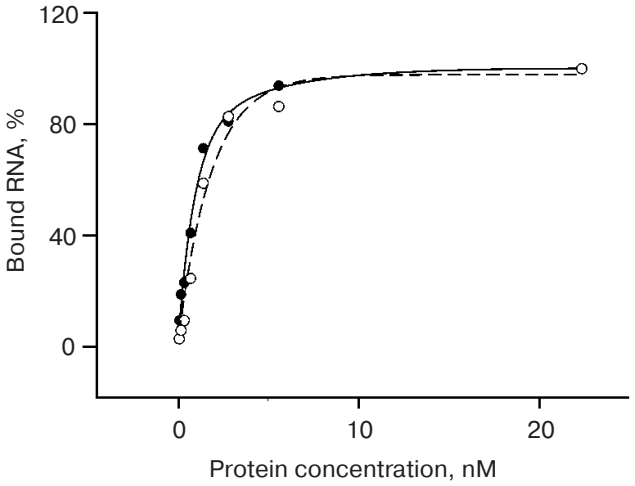

Fig. 5. Titration of ^{32}P-labeled RNA with the protein (solid curve, closed circles) and fragment (dashed line, open circles). Molar RNA/protein (fragment) ratio varies from 1 : 1 to 1 : 32.

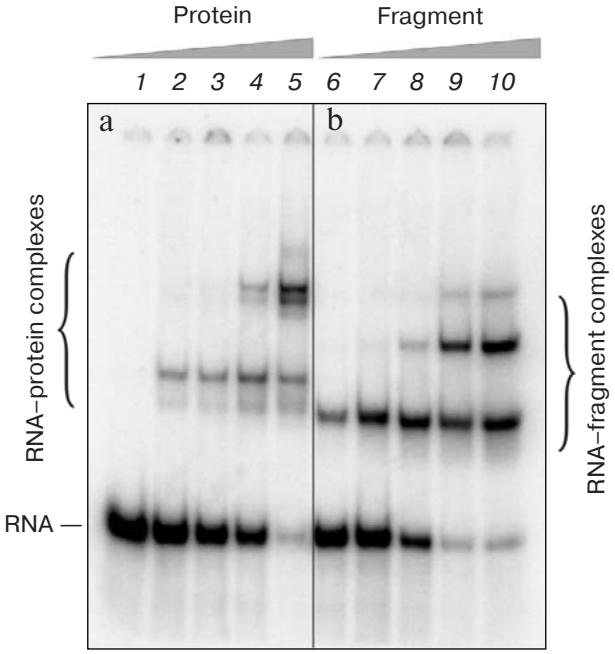

Fig. 6. Interaction between *T. thermophilus* S1 protein and its 49-kD fragment with ^{32}P-labeled RNA (5% polyacrylamide gel, non-denaturing conditions). Lane *1*: free ^{32}P-labeled RNA. a) ^{32}P-labeled RNA at various RNA/protein ratios: 1 : 1 (lane *2*), 1 : 2 (lane *3*), 1 : 4 (lane *4*), and 1 : 8 (lane *5*). b) ^{32}P-labeled RNA at various RNA/fragment ratios: 1 : 1 (lane *6*), 1 : 2 (lane *7*), 1 : 4 (lane *8*), 1 : 8 (lane *9*), and 1 : 16 (lane *10*).

the protein (fragment), and virtually complete RNA association with the protein occurs at the molar ratio of the reaction components of 1 : 8 (lanes *5* and *9*). Two models of the association are possible. In the first, the RNA–protein interactions play the main role, and with increase in the protein (fragment) concentration the number of protein (fragment) molecules directly interacting with RNA increases too. This model corresponds to the data of studies [45-47], from which it is evident that more than one S1 protein molecule interacts with various RNA molecules. Moreover, a positive cooperativity of binding of S1 protein and its fragments with synthetic templates, tmRNA, and various model mRNAs is marked in a number of studies [9, 32, 45].

In the second model, the main role is played by protein–protein interactions. In this model, the binding of the first protein (fragment) molecule with RNA stimulates further increase in the RNA–protein complex mass, due to association of protein (fragment) molecules with each other. The second model seems preferable, because a tendency to association was revealed recently in S1 protein from *T. thermophilus* by small-angle X-ray scattering [35]. The protein maintains its compact conformation when it comprises associates. The tendency of the protein to association can be of functional value, because it was noted that up to two protein molecules can bind with non-activated (not heated at 37°C) ribosomal 30S subunit under its titration with S1 protein [25]. This suggests that the association of S1 protein molecules is realized through their S1-domains, because S1 domain is present in many proteins interacting or associating with it.

Study of interaction between *T. thermophilus* S1 protein or its fragment and 70S ribosome devoid of S1 protein. To obtain the stable fragment of S1 protein from *T. ther-*

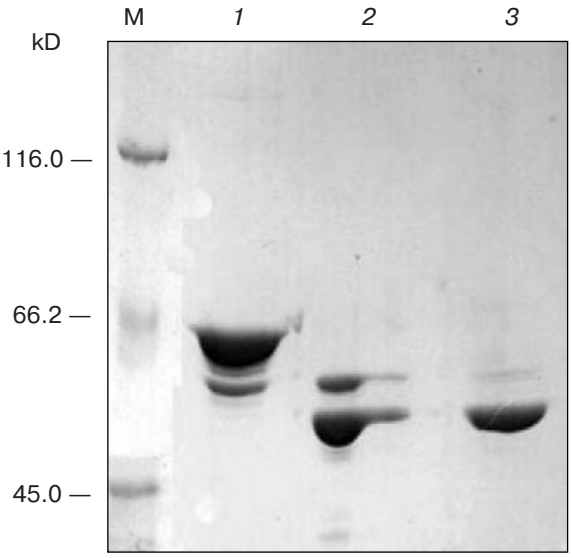

Fig. 7. Electrophoretic analysis in 10% polyacrylamide gel with SDS of 49-kD fragment after ion-exchange chromatography on DEAE-Sepharose. M, molecular mass markers. Lanes: *1*) S1 protein from *T. thermophilus*; *2*) 49-kD protein fragment before the chromatography (protein/trypsin ratio is 400 : 1, proteolysis duration 60 min, aprotinin inhibitor at enzyme/inhibitor molar ratio of 1 : 3); *3*) 49-kD fragment after chromatography.

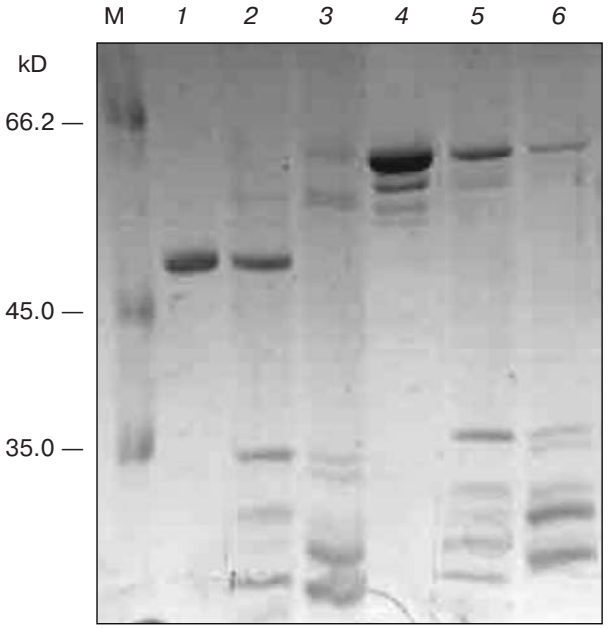

Fig. 8. Electrophoretic analysis in 12% polyacrylamide gel with SDS of the interaction of the *T. thermophilus* S1 protein and its fragment with ribosomal subunits obtained by separation of 70S(–S1)-ribosomes in sucrose gradient. The protein or fragment were added to ribosomes before their separation at the 70S(–S1)/S1(fragment) molar ratio of 1 : 2. M, molecular mass markers. Lanes: *1*) 49-kD fragment; *2*, *3*) 30S- and 50S-subunits with the fragment, respectively; *4*) S1 protein; *5*, *6*) 30S- and 50S-subunits with S1 protein, respectively.

mophilus in preparative amounts (reaction mixture volume of 200-300 μl, protein concentration 2.5-6.0 mg/ml), the protein/enzyme ratio used was 400 : 1. Figure 7 shows the data of electrophoretic analysis of 49-kD protein fragment after its purification on DEAE-Sepharose. The activated 70S(–S1)-ribosomes (70°C, 40 min) were incubated with S1 protein or its fragment at 1 : 2 molar ratio in the dissociating buffer (70°C, 10 min). Subunits were separated by centrifugation in a sucrose gradient (10-30%) prepared in the same buffer. This procedure enables simultaneous segregation of ribosomes into subunits and separation of unbound protein or its fragment. Following fractioning of the sucrose gradient and combining of peak fractions absorbing at 260 nm, the latter were subjected to electrophoretic analysis [36]. As evident from the data in Fig. 8, the protein fragment, like the full-length S1 protein, interacts with ribosomal 30S subunit (lanes *2* and *5*) and does not interact with 50S subunit (lane *3*). S1 protein is capable of partial interaction with 50S subunit (lane *5*), as noted earlier [25].

Comparison of some parameters of the stable *T. thermophilus* and *E. coli* S1 protein fragments. We have shown in the present work that the stable fragment with molecular mass of 49.1 (±0.1) kD and ~439 (±1) amino acid residues (residues 26-465) in length is formed as a result of limited proteolysis with trypsin of ribosomal protein S1 from *T. thermophilus*. When the proteolysis occurs, 25 amino acid residues are cleft from the N-terminus of the molecule, and 71 residues from its C-terminus. The analysis of Swiss-Prot database for the *T. thermophilus* S1 protein has demonstrated that the integrity of the first N-terminal S1-domain (residues 33-104) is not affected, and the last degenerate S1-domain is removed from the C-terminus of the molecule (residues 466-536). Thus, the truncated form of S1 protein from *T. thermophilus* as a result of limited proteolysis with trypsin is formed, containing five S1-domains, like the full-length protein (Fig. 4).

The fragment obtained in our work from *T. thermophilus* protein retains the capacity for interaction with both RNA and ribosomal 30S subunit, like the full-length protein. It drastically differs from the fragment of S1 protein from *E. coli* also produced by limited proteolysis with trypsin [27]. The molecular mass of the fragment from *E. coli* is 41.3 kD, and its length is 386 amino acid residues (residues 172-557). Removal of 171 residues from the N-terminus has no effect on RNA-binding function of this fragment, but leads to inability to interact with ribosomal 30S subunit [17]. Hence, two absolutely different stable fragments are formed as a result of limited proteolysis with trypsin of two homologous S1 proteins from *T. thermophilus* and *E. coli*. This is an indication of difference between the structural organizations of these two proteins. The stability of 49-kD fragment of S1 protein from *T. thermophilus* and its ability to interact with RNA and ribosomal 30S subunit are favorable conditions for its further utilization in structural and functional studies.

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