Mechanism of peptide-bond formation by ribosome revisited

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A sensation in X-ray crystallography as well as ribosomology was created when the crystallographic analyses of ribosomes were reported by three different laboratories1–3 in 1999. Microcrystals of 30S and 50S ribosomes were, however, obtained and studied by Yonath and co-workers4 earlier. Detailed information about 30S ribosomes was reported later by the same group5. The group at New Haven headed by Moore and Steitz6, subsequently reported the atomic structure of the large subunit from Haloorcarula marismortui at 2.4 Å resolution. The study of the complex containing three RNA and more than 50 proteins in the two subunits (30S and 50S ribosomes) revealed the positions of practically all the proteins and three-dimensional orientation of RNAs in the protein-synthesizing machinery. It was suspected for a long time that the peptide-bond formation, following the assembly of the amino acids in proper sequence was effected by ribosomal RNAs rather than proteins7,8. Therefore, it was encouraging to observe from the crystallographic data that the site of peptide-bond formation in the cavity, formed due to the association of the two subunits where aminoacyl tRNAs are assembled, is composed of RNA alone and no protein was found in the vicinity. The mechanism of peptide-bond formation appeared to be solved by one of the laboratories9 by studying the crystal structure of 50S ribosomes complexed with Yarus inhibitor10, which is an analogue (tetrahedral carbon replaced with phosphorus) of tetrahedral intermediate expected to be produced prior to the formation of the peptide bond. A particular base of 23S RNA (A2460 of H. marismortui which is equivalent to A2451 of E. coli) was found to be nearest to the tetrahedral carbon (P in this case), and assumed to act as acid/base catalyst as histidine in the serine protease. It was suggested that the peptide-bond formation takes place by a mechanism which is opposite to that of the proteolytic breakdown. However, the problem is the pKₐ of adenine (N1 or N3 atom), which is in the acidic range. pKₐ of the particular adenine at the catalytic centre was found to be 7.6 by dimethyl sulphate modification11. It was assumed that the neutral pKₐ was the effect of the nearby base G2452 (G2349 of E. coli), and due to the environment surrounding the adenine base. Details of this mechanism have been presented earlier11. Thus the problem of peptide-bond formation finally appeared to be solved after a period of about three decades.

However, soon after the mechanism was proposed it faced criticisms from several laboratories, basically on two grounds. Substitution of the particular adenine base (A2451) with other bases did not drastically affect the peptidyl tRNA trans-ferase activity13,14. The other point of controversy is the method of determination of the pKₐ of adenosine by dimethyl sulphate treatment15. The change in pKₐ of adenine was observed only in inactive ribosomal preparation13. Further, G2457 (E. coli), a base proposed to be critical for neutral pKₐ of adenine was found to be inessential. Another concern was the relevance of understanding the mechanism of peptide-bond formation from the crystal structure16. This was refuted by the fact that even 50S ribosomes in crystalline state were capable of peptide-bond formation17, and the rate measurements are not reliable under any circumstances18. It was also argued on the same ground that the base-substitution data19,4 may also not be reliable. Katunin et al.19, who developed a rapid method of determination of peptide-bond formation, observed that the substitution of A2451 with uracil has strong inhibitory effect on peptide-bond formation. However, Moore and Steitz’s group has stated that “the chemical reactivity data that appeared to support the concept that A2460 (Ad2451) acts as a general acid/base catalyst do not speak of its role in protein synthesis17,18. This was due to several objections raised by Muth et al.20. First, A2451 in ribosomes from H. marismortui displays an inverted pH profile and second, in yeast C2455 rather than A2451 is modified in a pH-dependent manner. Third, it appears that the base A2451 is not accessible for pH-dependent dimethyl sulphate modification without structural rearrangement. This is also supported by the observation of Bayfield et al.15 which showed that the base is modified in an inactive but not in an active preparation of ribosome. Therefore the group headed by Moore and Steitz21,22 re-examined the crystal structure of 50S ribosomes substituting the Yarus inhibitor with analogues of aminoacyl tRNA and peptidyl tRNA individually, and superimposed the two structures to derive the most probable structure of 50S ribosomes at the time of peptide-bond formation. There was an indication that the Yarus inhibitor must be distorting the physiological structure at the site of peptide-bond formation. Further, Muth et al.23 hinted at the contribution to overall catalysis of general acid/base and/or conformational catalysis involving a group at the active site. Yonath (pers. commun.) is of the opinion that the conformational change of ribosome takes place prior to the peptide-bond formation and that the change is pH-dependent and may also depend on a protein. It may be mentioned in this connection that a similar suggestion (conformational change) was made as early as in 1985 (ref. 21), which was supported by cryoelectron microscopic studies in Frank’s laboratory24. This was also observed by various laboratories from their own studies (references cited in ref. 22).

About three years ago, a model was proposed for peptide-bond formation in which 2’ OH group of peptidyl tRNA is involved in forming a six-member cyclic intermediate which spontaneously results in formation of the peptide bond as shown by quantum chemical calculations (Figure 1)25. For the cyclic intermediate to be formed, the configuration of carbon of the cyclic intermediate has to be preferably in S-configuration from the direction of the nucleophilic attack and the peptide bond that is formed is expected to be in trans-configuration (as shown in Figure 1), as present in the natural peptide. Moore (pers. commun.) indicated that 2’ OH group is unlikely to be involved in peptide-bond formation, but now his group has observed that the crucial adenine base A2451 may form a second hydrogen bond with 2’ OH of peptidyl tRNA besides the hydrogen bond with the 2’ OH group of A2450 (A2451)17. In our model the NH₂ group is expected to form a hydrogen bond with...
the 2′ OH group of terminal adenosine of peptidyl tRNA. We argued on the basis of earlier work and quantum chemical data that no additional catalyst is necessary for peptidyl-bond formation, but peptidyl tRNA itself plays the catalytic role. 26 The strongest evidence in favor of our model is the earlier demonstration in several laboratories27,28 that the reduction of the 2′ OH group of terminal adenosine of peptidyl tRNA leads to complete loss of peptidyl transferase activity. Thus, the pH-dependent conformational change of 50S ribosomes may be crucial for the final step of peptidyl-bond formation. X-ray crystallographic data may be of some help in solving the puzzle, and the ribosome as a whole may act as a proper catalyst, simply by properly positioning the activated substrates.


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