CONFORMATIONAL DIFFERENCES BETWEEN s-RNA AND AMINOACYL s-RNA

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Current interest in the mechanism of protein biosynthesis has been directed to the study of the role of s-RNA,* m-RNA and ribosomes in the biosynthetic process. According to present concepts, aminoacyl s-RNA binds to the messenger-polysome complex, and after transferring its amino acid to the growing polypeptide chain, dissociates from the template. To satisfy the requirement of the binding of the anticodon on s-RNA to the codon on the template, a conformation dependent parameter may be involved. To search for a possible conformational difference between s-RNA and aminoacyl s-RNA, the technique of optical rotatory dispersion has now been employed.

Optical rotatory dispersion measurements have been widely used in the past for the study of the helix-coil transition in polypeptides and proteins (Urnes and Doty, 1961), and have been extended recently to the study of polynucleotides and s-RNA (Fasman et al., 1964; Holcomb and Tinoco, 1965; Lamborg et al., 1965). In an earlier publication (Sarin and Zamecnik, 1965), we reported on the application of ORD to the study of structural patterns of the components of the amino acid acceptor and transfer systems. We now wish to report a conformational

*Abbreviations: Tris, tris(hydroxymethyl)aminomethane; aminoacyl s-RNA, s-RNA charged with 21 amino acids; s-RNA, this polynucleotide stripped of all amino acids; m-RNA, messenger RNA; A, adenine: C, cytosine; G, guanine; U, uracil; and ORD, optical rotatory dispersion.

difference between s-RNA and aminoacyl s-RNA. It remains to be determined whether such may be related to the apparently greater degree of binding to the m-RNA-ribosome complex, of aminoacyl s-RNA compared to unesterified s-RNA.

E. coli s-RNA (General Biochemicals, Chagrin Falls, Ohio) was stripped of all amino acids by incubation at 37° in the presence of Tris-acetate (1.8 M, pH 8) (Sarin and Zamecnik, 1964). After incubation, the reaction mixture was cooled in ice, and s-RNA was precipitated by adding 4 vols. of absolute ethanol. The excess Tris was removed by this extraction. The precipitated s-RNA was removed by centrifugation, dissolved in a small volume of water, and dialyzed against distilled water. Water was changed every 2 hrs., and after the first three changes the sample was dialyzed against distilled water for 48 hrs. and finally lyophilyzed. The stripped s-RNA was then charged with 21 amino acids, following conditions similar to the ones already reported (Sarin and Zamecnik, 1965). One marker ^{14}C amino acid (^{14}C Phe) was added to check the labeling of s-RNA. After incubation, the aminoacyl s-RNA was freed of protein material by extraction with chloroform:isoamyl alcohol (3:1, v/v) 2 vols. (Scott, unpublished procedure). The mixture was well shaken, and the separated precipitate was removed by centrifugation. The extraction with chloroform:isoamyl alcohol was repeated until there was no precipitate at the interface. The aqueous phase containing the aminoacyl s-RNA was made 0.1 M with respect to sodium chloride and dialyzed against 0.1 M sodium chloride (aqueous) for 2 hrs. and finally dialyzed against distilled water in the cold overnight. The dialyzed sample was then lyophilyzed, and the sample was checked for the absence of ATP.

Optical rotatory dispersion was measured from 320 mμ to 230 mμ with a Cary 60 spectropolarimeter using conditions similar to the ones already reported (Lamborg et al., 1965). ORD measurements of s-RNA and aminoacyl s-RNA were determined at a concentration of 0.4 mg/ml

in 0.2 M sodium citrate buffer (pH 7). Concentration of s-RNA samples was determined by inorganic phosphate estimation. ORD measurements of s-RNA and aminoacyl s-RNA were also carried out as a function of pH, in order to gain insight into the similarity or differences in their stabilities to protonating environments.

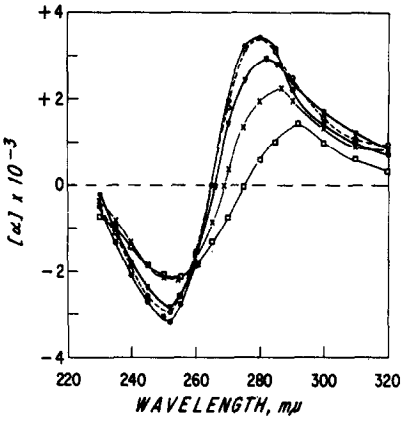

Fig. 1. Optical rotatory dispersion of s-RNA in 0.2 M sodium citrate buffer. pH 7, (●——●); pH 5, (O---O); pH 4, (▼——▼); pH 3.5, (✕——✕); pH 3, (□——□).

As can be seen from Fig. 1 the first change in the amplitude of Cotton effects is observed at pH 5, when the amplitude is found to be 90% of the amplitude at pH 7, suggesting thereby a partial disruption of the ordered structure. No change is observed when the pH is changed from 5 to 4.5, but on lowering the pH to 4 a further disruption occurs, presumably due to the protonation of the C residues. The amplitude of the Cotton effects at pH 4 is 84% that of the amplitude at pH 7. Further lowering of the pH to 3.5 brings about a considerable change, and the amplitude becomes 57% of the amplitude at pH 7. This change in amplitude is presumably due to the protonation of the A residues with a concomitant disruption of the A-U hydrogen bonded structures. Only a relatively small change, from 57% to 49%, is observed when the pH is changed from 3.5 to 3. Thus at pH 3 when most of the C and A residues

would have been protonated, the amplitude of the Cotton effects is found to be 50% of the amplitude at pH 7. In addition, a marked shift to longer wavelength of the peak and the crossover point occurs when the pH is lowered from 4 to 3 through 3.5. It is of interest to note that this disruption of the secondary structure with lowering in pH is non-linear, suggesting the presence of regions of different ordered structures which seem to shield the availability of the nitrogenous bases to the protonating environments. The inaccessibility of the bases to protonation may be due to the burying of the bases in hydrophobic regions or in strongly hydrogen bonded environments. The non-linearity in the opening of the s-RNA structure could also be due to the engulfing of some bases in strong G-C hydrogen bonded regions which are likely to be disrupted only at low pH. Thus it seems plausible to suggest that s-RNA structure is composed of short, ordered regions rather than a single, completely hydrogen bonded helical structure. This suggestion agrees with the primary structural determination of alanine s-RNA by Holley et al. (1965).

When s-RNA is charged with 21 amino acids (aminoacyl s-RNA), an alteration in the secondary structure seems to occur, as shown in Fig. 2. Thus at pH 7 the amplitude of Cotton effects of aminoacyl s-RNA is 82% of that of s-RNA. A noticeable change in amplitude with respect to lowering of pH occurs at pH 4.5. A large decrease in amplitude occurs at pH 3.5 and 3, which is parallel to the change observed with s-RNA. A non-linear decrease in amplitude with change in pH of aminoacyl s-RNA is also observed, again suggesting the presence of differently shielded nitrogenous bases.

In order to answer the question as to whether the observed difference in amplitude might be simply a reflection of a change in specific absorption at 260 mμ, a sample of aminoacyl s-RNA was incubated at 37° in the presence of Tris-acetate (2 M, pH 8). Fifty μl aliquots

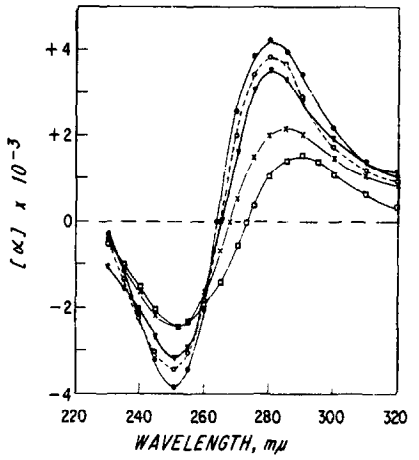

Fig. 2. Optical rotatory dispersion of aminoacyl s-RNA in 0.2 M sodium citrate buffer. pH 7, (●——●); pH 4.5, (O---O); pH 4, (▼——▼); pH 3.5, (X——X); pH 3, (□——□).

containing 40-50 μg s-RNA were removed at 10 min. intervals and diluted to 1 ml with 0.14 M sodium chloride. The absorbance at 260 mμ was then read in a Zeiss ultraviolet spectrophotometer. No detectable hypochromic shift was observed over a period of 90 min. incubation. All the radioactivity due to ^{14}C amino acid was lost during this period. Both types of sample were subjected to the same procedure of incubation, chloroform:isoamyl alcohol extraction, and dialysis during preparation of the aminoacyl s-RNA. The differences in the ORD of aminoacyl s-RNA and s-RNA were also found when a sample of aminoacyl s-RNA was stripped, dialyzed and the pH readjusted to pH 7.

It becomes apparent from the above observations that s-RNA charged with 21 amino acids has a different conformation (less helical) compared to s-RNA stripped of all amino acids. This difference in the conformation of aminoacyl s-RNA may be important in the preferential binding of aminoacyl s-RNA to the template in protein biosynthesis.

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