REGULATION OF A SERINE TRANSFER RNA OF BACILLUS SUBTILIS

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It was shown recently that the valyl-transfer RNA pattern of Bacillus subtilis was altered during the initial stages of sporulation (Kaneko and Doi, 1966a). During this investigation the effect of media on the various transfer RNAs was also investigated, since there was a possibility that the alteration of transfer RNA patterns could be effected simply by a change in the environment of the cell. In this paper results are reported which illustrate that the serine transfer RNA pattern is altered when cells are grown in media in which the growth rates differ; 15 other aminoacyl-transfer RNAs including valyl-transfer RNA did not show any alterations under these conditions.

MATERIAL AND METHODS

B. subtilis W23 cells were used as the source of transfer RNA and aminoacyl-transfer RNA synthetase. The cells were grown in SCM medium (Doi and Igarashi, 1964) and in Penassay broth (Difco) at 37° C. The growth rates of the cells in Penassay and SCM media were 1.5 and 1.2 (doublings of optical density at 660 mμ per hour), respectively. Cells were harvested in log phase at a density of 1-2 x 10^8 cells per ml. The tRNA was extracted from cells as described by von Ehrenstein and Lipmann (1961). The synthetase and methylated albumin kieselguhr (MAK) column

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were prepared as described by Sueoka and Yamane (1962). Details of these methods and the preparation of aminoacyl-transfer RNA with B. subtilis cell extracts were described previously (Kaneko and Doi, 1966a). The serine accepting capacities of the tRNA preparations from Penassay and SCM grown cells were 64 and 86 μμmoles per A^{260} unit, respectively.

RESULTS AND DISCUSSION

The aminoacyl-transfer RNA of cells grown in Penassay and SCM media were compared by double isotope labeling with C^{14}- and H^3-labeled amino acids and by co-eluation from a MAK column. All the amino acids except proline, asparagine, glutamine and cysteine were examined. Of the sixteen amino acids tested the elution patterns for fifteen were identical whether the cells were grown in either Penassay or SCM medium. However, in the case of seryl-transfer RNA, the profile obtained from Penassay grown cells had three elution peaks, whereas the cells grown in SCM medium had only two major peaks and possibly a small third peak. This is illustrated in Fig. 1. The appearance of more than one transfer RNA peak for an amino acid has been interpreted in most cases as evidence for multiple transfer RNAs for the particular amino acid. In the case of B. subtilis valyl-transfer RNA, T_1 RNase digestion followed by DEAE-cellulose chromatography gave two discrete valyl-oligonucleotide peaks (Kaneko and Doi, 1966b). For seryl-transfer RNA only one large peak has been obtained after T_1 RNase digestion and elution from a DEAE-cellulose column, indicating either the lack of resolution of seryl-oligonucleotides or the presence of only one species of seryl-transfer RNA. This point has been partially resolved by differential oxidation of the seryl-transfer RNAs with I_2 and is discussed below.

The following tests were made on the transfer RNA to see whether the alteration could be an artifact of preparation or elution, or a non-

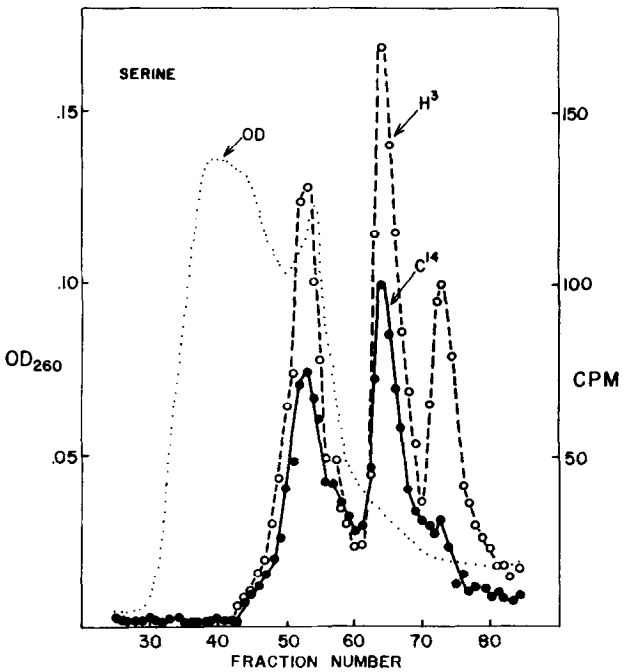

Figure 1. MAK column chromatography of seryl-H^3-transfer RNA and seryl-C^{14}-transfer RNA from B. subtilis cells grown in Penassay and SCM media respectively. The open circles represent seryl-H^3-transfer RNA and the closed circles represent seryl-C^{14}-transfer RNA.

physiological alteration of the transfer RNA:

1. Longer treatment (pH 8.8, 35 C, 3 hr) of transfer RNA before charging with labeled amino acid was performed to test whether differential stability of the seryl-transfer RNA was involved. No differences from the results in Fig. 1 were observed.

2. The transfer RNA was heated at 60 C for 5 min in the presence of 0.001 M EDTA-0.01 M Tris-HCl, pH 8.0 or 0.01 M Tris-HCl-0.001 M EDTA-0.02 M $MgCl_2$, pH 8.0, and then charged with serine. When this was done, the three peaks of seryl-tRNA were obtained under both conditions suggesting that the third peak was not due to the alteration of the secondary structure of tRNA (Lindahl, et al., 1966; Gartland and Sueoka, 1966). Similar treatment of tRNA from SCM grown cells still revealed only two major peaks and a small shoulder or third peak.

3. Recently it has been shown that thiopyrimidine exist in B. subtilis tRNA (Goehler et al., 1966). Upon mild oxidation with iodine (Carbon et al., 1965), it was shown that the second and third peaks were not able to accept serine. However, upon reactivation by thiosulfate treatment, both peaks reappeared suggesting that there were indeed three separate peaks of seryl-transfer RNA.

4. Three serine transfer RNAs were obtained from both log and stationary phase cells grown in Penassay medium. In the stationary phase, the amount of tRNA in the third peak was relatively increased. When B. subtilis W23 was grown in Penassay medium, sporulation did not occur readily.

There are at least two possible explanations for these results. During growth in a relatively poorer medium the third seryl-transfer RNA peak may appear to be lacking, because some modification of the transfer RNA has occurred which either prevents the tRNA from being charged or at least reduces the amount of tRNA which can be charged with serine. The other possibility is that there is a differential synthesis of the serine transfer RNA when the cells are grown in the two different media.

The present results with valyl-transfer RNAs suggest that the alteration of valyl-transfer RNAs during sporulation (Kaneko and Doi, 1966a) may not be the direct result of a microenvironmental change.

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