ON RNA COMPOSITION AND GROWTH RATES OF A STREPTOCOCCUS AND DERIVED STABLE L-FORM

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Stable L-forms derived from group A streptococci permanently lack the ability to synthesize a rigid bacterial cell wall, require a high osmotic environment for growth and display a decreased growth rate from that of the parent bacterial form. Prior studies from these laboratories have shown that a stable (i.e., non-reverting to the bacterial form) logarithmically growing L-form, although slower by 1/3 to 1/2 in its growth rate is (a) capable of balanced growth, (b) contains an appreciably lower total cellular ribonucleic acid content and (c) displays no alterations in its deoxyribonucleic acid and ribonucleic acid molar base ratios from that of the streptococcus from which it was obtained (Panos, 1965).

It has been established that the ribonucleic acid (RNA) content per cell varies as a function of the growth rate (Kjeldgaard and Kurland, 1963; Rosset et al. 1964). There is no information available, however, concerning the status of soluble (S) and ribosomal (R)-RNA following conversion of a bacterial cell to an L-form. It, therefore, became of interest to examine the RNA composition of a streptococcus to note whether the decreased growth rate observed following cell wall removal (i.e., L-form) is accompanied by a shift in distribution of (S) and (R)-RNA or is the result of the increased osmotic environment necessary for growth of the resulting osmotically fragile L-form.

The non-pathogenic group A type 12 beta hemolytic streptococcus and its stable L-form used previously were again used (Panos, 1962). The medium and growth conditions for each of these organisms was described by Panos and

Barkulis (1959). Mid logarithmic cells were employed throughout. All cells were broken with glass beads in an International Centrifuge shaker head (Shockman et al. 1957) and extraction of (S) and (R)-RNA performed essentially as described elsewhere (Kennell and Magasanik, 1962) using 0.01 M Tris - 0.005 M Mg^{++} buffer, pH 7.4. For the osmotically fragile L-form and bacterial control cells (streptococcus grown in L-form medium), a "modified" buffer containing 3% NaCl was employed. RNA was quantitatively determined by the orcinol reaction. Protein was estimated by the method of Lowry. Amounts of RNA and protein are expressed as % dry weight of total cellular material. The data presented are averages of at least two different experiments performed in duplicate.

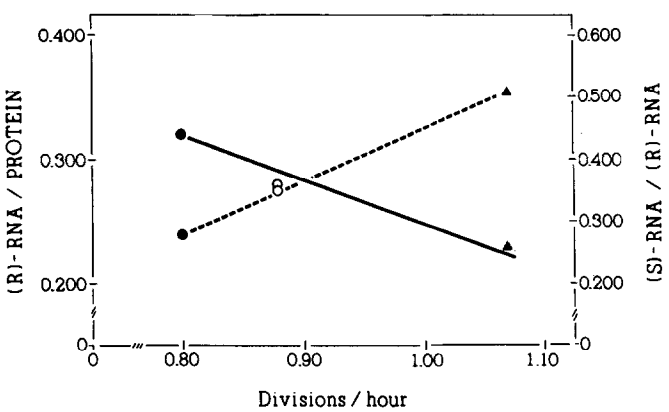

Figure. RNA composition and protein relationship of logarithmic cells at various growth rates. (——) = (S)-RNA/(R)-RNA ratio; (---) = (R)-RNA/Protein ratio. ● = L-form; ○ = Streptococcus grown in L-form medium; ▲ = Streptococcus.

The results of these studies are presented in the figure and table. It is apparent that the (R)-RNA to protein ratio changes upon conversion of the parent coccus to its L-form. These findings mimic those reported by Kjeldgaard and Kurland (1963) for Salmonella typhimurium grown in a variety of media, from nutritionally complex to chemically defined. As with S. typhimurium, these studies revealed that as the growth rate (k) increased, from k = 0.80 to 1.07, the (S)-RNA to (R)-RNA ratio decreased. Since the (S)-RNA stayed relatively

TABLE

Cellular Composition*

Organism	k**	Total RNA	(R)-RNA	(S)-RNA	(R)-RNA/(S)-RNA	(P)	(P)/(R)-RNA X k
Streptococcus	1.07	22.85	18.17	4.68	3.9:1	51.3	3.0
Streptococcus***	0.88	18.68	13.72	4.96	2.8:1	49.1	3.3
L-form	0.80	13.24	9.19	4.05	2.3:1	38.4	3.2

(R) = Ribosomal
(S) = Soluble
(P) = Protein

* = % dry weight of total cellular material.
** = growth rate; divisions per hour.
*** = grown in L-form medium.

constant in all three organisms, the (R)-RNA content remained a function of the growth rate during conversion of the coccus to an L-form. The rate of protein (P) synthesis per unit weight of (R)-RNA in all organisms [(P)/(R)-RNA X k] was constant. The difference here, however, is that the decreased growth rate of the L-form is seemingly related, in part, to its permanent inability to synthesize the rigid bacterial cell wall structure and is not the result of an obvious external nutritional alteration; as was the case with S. typhimurium (Kjeldgaard and Kurland, 1963).

As a control, initial growth of the parent coccus in the high osmotic environment of the L-form (k = 0.88, medium with 3% NaCl) resulted in a decrease in total and (R)-RNA cellular content as compared with that of the streptococcus grown in the usual manner (k = 1.07, medium without 3% NaCl). The (R)-RNA to (S)-RNA ratio of the coccus, grown in the presence of NaCl, was closer to that of the derived L-form than to similar cells from a "salt free" medium. However, the total and (R)-RNA content of the L-form was found to be significantly lower (by more than 30%) than that of the coccus grown under L-form conditions.

These results suggest that the decreased growth rate observed following conversion of a streptococcus to a stable L-form (i.e., permanent loss of cell wall synthesizing ability) is accompanied by a shift downward in (R)-RNA content and is not a manifestation of a less efficient ribosomal protein synthesizing system in the resulting L-form. Further, the characteristic slower growth of the coccal L-form can not be solely ascribed to its absolute need for a high osmotic growth environment for structural maintenance and cellular perpetuation; as is apparent from comparable data of streptococci grown in L-form medium.

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