A HELICAL MODEL FOR THE POLYSOME IN SOLUTION[1]

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Electron micrographs of air-dried polysome preparations disclose two types of configuration, an extended open one and a compact cluster, depending on the type of staining used to prepare the sample (Warner et al., 1962). On the other hand, electron micrographs of tissue sections disclose polysomes disposed in linear series, loops, double rows, and helices (Palade 1955, Behnke 1963, Waddington and Perry 1963).

It was thought that hydrodynamic methods could be used to distinguish between these possible configurations for polysomes in solution by utilizing the dependence of the frictional coefficient on molecular weight. Preliminary results of these studies are given below, and will be reported in more detail elsewhere.

The sedimentation rates of the various polysomes of rat liver were determined at infinite dilution in the analytical ultracentrifuge. These values at infinite dilution were then used to calibrate sucrose density gradient centrifuge runs. The polysomes were given a short incubation with C^{14} amino acid. Following this, separation of the larger polysomes was accomplished by linear sucrose density gradient centrifugation. Because of the low concentrations used in the gradients, the sedimentation coefficients would be expected to be close to their values at infinite dilution.

The final values from both methods have been listed in Table I. They were found to obey the relationship $s^{\circ}_{20,w} = kM^{0.58}$. The exponent of M is slightly higher than 0.50, the theoretical limit for a random coil (Kirkwood and Riseman 1948). According to both the Kirkwood-Riseman (1948) and the Debye-Bueche (1948) theories for a random coil, as the chain length becomes smaller the exponent

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of M approaches a number less than 0.3. The polysome in solution, having a degree of polymerization of from 1 to 12 and an exponent of 0.58, cannot be a random coil, and must represent a compact configuration. This excludes the open configuration seen in electron micrographs of air-dried sections.

TABLE I. Sedimentation coefficients of rat liver polysomes

No. of ribosome units	$s^{\circ}_{20,w}$	
	Analytical ultracentrifuge runs	Sucrose gradient runs
1	83 ± 3 S	85 ± 1 S
-	105 ± 2 S	
2	123 ± 4 S	122 ± 3 S
3	154 ± 4 S	156 ± 5 S
4	183 ± 5 S	180 ± 5 S
5	211 ± 10 S	211 ± 4 S
6		237 ± 3 S
7		257 ± 6 S
8		(282)S
9		299 ± 5 S
10		316 ± 6 S
11		
12		(353)S

Values in parentheses were only observed once. Analytical values are averages of three preparations, each of which was extrapolated to infinite dilution at 5 to 9 different concentrations. Runs were made at 50,740 rpm at temperatures between 3 and 8°C using the schlieren optical system.

Linear sucrose density gradients and conditions of protein synthesis were as described previously (Cammarano et al. 1963). The polysomes were given a 3- to 5-minute incubation under these conditions. The sucrose gradient results are averages of 7 runs.

Frictional ratios were determined from the sedimentation rates listed in Table I by the formula

$$\frac{f}{fo} = \frac{83n^{2/3}}{s^{\circ}_{20,w}}$$

assuming that the n = 1 polysome is a sphere with unit frictional ratio (Gierer 1963). If we separate the frictional ratio into f/fo = (f/fe) · (fe/fo), where the first factor involves hydration and the second asymmetry (Oncley 1941), then the frictional ratio determined above is that caused by asymmetry alone, assuming that all the polysomes have the same hydration as the ribosome. This assumption may not be true for the polysome, as will be discussed later. The axial ratios of the equivalent ellipsoid determined from the frictional ratio has been plotted vs. the number of ribosome units (n) in Figure 1.

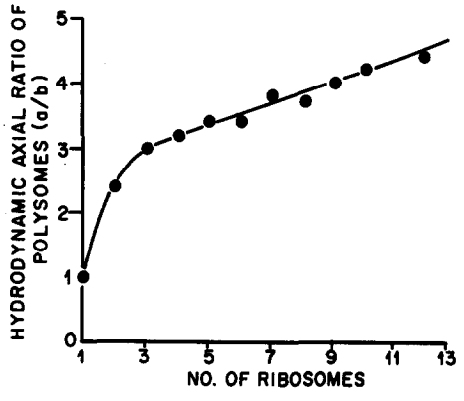

FIG. 1. Experimental axial ratio of polysomes vs. number of ribosome units.

These values were obtained by extrapolation from the tables in Svedberg and Pederson (1940). The axial ratio is seen to increase rapidly for the first three polysomes and then to level off at a constant slope. The axial ratio for n = 2 is exactly that to be expected of two 230-Å spheres rigidly held 100 Å apart. The axial ratio for n = 3 falls short of a linear model. Using the same ribosome and spacing dimensions, one finds that the third ribosome would have to go in at an angle of approximately 90°, if we can consider the trimer to have an effective width of one ribosome. If the fourth also goes in at 90° angle, one forms either a square or the start of a helix-like structure. The slope of the line from n = 3 to n = 12 can be used to determine the pitch of an assumed helix by the formula

$$\tan (90° - \Phi) \simeq \frac{d (a/b) (2D + L)}{dn (L + D)}$$

where Φ is the pitch of the helix, L is the spacing between the ribosomes, and D is the diameter of the ribosome. Using the previous dimensions, one obtains a pitch of 73°.

Behnke (1963) and Waddington and Perry (1963) have observed polysome helices with a pitch of 60 to 70° in thin-section electron micrographs of embryonic tissue. Using 230 Å as the ribosome diameter, 100 Å as the spacing between ribosomes, 90° as the interribosomal angle, and 70° as the helix pitch, one obtains a helical structure very similar to that seen in their electron micrographs.

If the assumption that the polysome contains the same hydration as the ribosome is not true (see Hall and Slater 1959; Shulman 1953 on fibrinogen, a beadlike molecule with a large amount of water of hydration) then a gain in hydration as the polysome increases in size would lead to a configuration more compact than the above helix. This configuration would be more nearly like the cluster observed in air-dried electron micrographs; i.e., a compact configuration with a very slow increase in axial ratio at the longer polysomes.

In conclusion, if there is no change in hydration going from the ribosome to the polysome structure, then the polysome is in a configuration intermediate between the open configuration and the cluster. In this case, the polysome helices observed in embryonic tissue show the best fit to the hydrodynamic data. But if there is a large gain in hydration as the polysome is formed, which is possible, then the cluster fits the data best.

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