METABOLISM OF NATURALLY OCCURRING POLYMERS OF ADENYLIC ACID AND GUANYLIC ACID

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Previous work from this laboratory has shown the presence of an enzyme in Escherichia coli which will specifically polymerise ATP to form polyadenylic acid (Gottesman, Canellakis & Canellakis, 1962). Enzymes with similar properties have been isolated from calf thymus nuclei (Edmonds & Abrams, 1960) and chick embryo (Ventkataraman & Mahler, 1963). In E. coli it was also reported to be associated with ribosomes (August, Ortiz & Hurwitz, 1962) and that the enzymatic activity is lost upon infection with T-even bacteriophages (Ortiz, et al., 1964). Later work indicates that the enzyme can be disassociated from ribosomes under conditions which remove adsorbed proteins (Smith & August, 1966; Hardy and Kurland, 1966). Recently, naturally occurring Poly A has been isolated from rat liver by Hadjivassiliou & Brawerman (1966). The widespread occurrence of the ATP polymerase and the natural existence of the homopolymer has prompted an investigation into its possible biological role.

In this article an isolation procedure for Poly A and Poly G from bulk tissue nucleic acid is described. Cellular subfractions of mouse liver contain such polymers, at least 3S in size, which are metabolically active. The deoxycholate soluble portion of microsomes contains predominantly Poly A while the nuclei and the ribosomes appear to contain Poly A rich and Poly G rich fractions. The synthesis of these polymers appears to be under nuclear control.

Isolation of Poly A (and Poly G) on polystyrene columns. The development of a sensitive method for the selective extraction of Poly A (and Poly G) made possible the investigation into the metabolism of this homopolymer which is

only available in minute amounts in tissue.

The method utilizes the ability of polystyrene to specifically adsorb Poly A (and Poly G) under certain conditions (Polystyrene, Av. M. W. 150,000 is dissolved in formamide, precipitated with water , repeatedly washed with 0.2 M phosphate pH 7.6 and mixed with celite).

TABLE I. POLYSTYRENE COLUMNS. RECOVERY OF ^{3}H POLY A ADDED TO PHENOL EXTRACTS OF MOUSE LIVER HOMOGENATES

	Nucleic acids as per cent of total					
Treatment	Phosphate Fraction			SDS Fraction		
	RNA	DNA	^{3}H Poly A	RNA	DNA	^{3}H Poly A
Control	70	10	0	30	90	100
Nuclease Digestion	95	100	0	5	0	100

^{3}H Poly A is added to a phenol extract (0.1 M Tris, pH 9.0) of a mouse liver homogenate. After dialysis against 0.33 M NaCl (2x), 0.01 M Tris pH 7.6 (2x) the extract is digested with 16 μg DNase and 2 μg RNase S per 10.0 $O.D._{260}$ units, in the presence of 5 x 10^{-3}M Mg for 15 min, 37°. RNase S is inactivated with trypsin (Allende and Richards, 1962), the solution made 0.2 M in phosphate pH 7.6 and applied to the column (0.8 cm^2 x 2 cm). Elution with 0.2 M phosphate (Phosphate Fraction) followed by 0.2% sodium dodecyl sulfate (SDS Fraction). The control sample was applied to the polystyrene column without nuclease digestion.

As shown in Table I, when a nucleic acid extract of mouse liver containing added ^{3}H-Poly A is eluted through the polystyrene columns, all the ^{3}H-Poly A and DNA is held back along with 30% of the tissue RNA; these can be quantitatively eluted with 0.2% sodium dodecyl sulfate (SDS Fraction). After a controlled nuclease treatment (see Legend, Table I), the ^{3}H-Poly A can be quantitatively eluted in the SDS Fraction without contamination by DNA or bulk tissue RNA. Sucrose density gradients and acrylamide gel electrophoresis show that the nuclease treatment resulted in extensive hydrolysis of the nucleic acids without affecting the added ^{3}H-Poly A. Similar results have been obtained with Poly G.

Kinetics of polynucleotide labeling. 1mC of carrier-free ^{32}P, as inorganic phosphate, was administered to each of 8 male mice, previously starved for 16 hours. After administration, animals were sacrificed at various time intervals and the nuclei and microsomes of the livers were isolated. The total RNA of these fractions was extracted, digested with nuclease and eluted through a

polystyrene column as previously described. The SDS fractions were isolated, hydrolysed with alkali and the individual nucleotides analysed for radioactivity. Well over 95% of the radioactivity was accounted for by the adenylic and guanylic acid content of the hydrolysate. The results presented in Table I include the radioactivity of the nucleic acid; the radioactivity in the adenylic and guanylic acids is expressed as A/G ratio.

TABLE II. DISTRIBUTION OF RADIOACTIVITY IN THE SDS FRACTIONS OF LIVER NUCLEI AND MICROSOMES

	Nuclei		Microsomes	
Time Hours	Per cent of total RNA	A/G ratio	Per cent of total RNA	A/G ratio
1	3.5	2.0	12	6.0
2	2.2	1.6	10	5.0
4	3.7	1.4	7	4.2
9	3.8	1.2	5	1.4

These results indicate that the SDS fraction of the nuclear RNA is metabolically active, that it is synthesized in concert with the rest of nuclear RNA (since it remains a constant proportion of the total) and that it is composed of two fractions. One fraction is A-rich and more rapidly labelled than the other which is G-rich as is evidenced by the decrease in the A/G ratio (the A/G ratio of the total nuclear RNA remained constant at 0.6 during these time intervals). On the other hand, the SDS Fraction derived from the microsomes falls with time as a per cent of the total microsomal RNA from 12 to 5 per cent. This correlates with a correspondingly dramatic drop in A/G ratios and indicates a different rate of turnover of the adenylic acid and guanylic acid-rich polymers. In order to determine the localization of the various components, microsomes were treated with deoxycholate to provide free ribosomes and a deoxycholate-soluble portion of the microsomes. The SDS fractions of these two preparations were isolated and analysed (Table III).

The results presented in Table III emphasize that the Poly-A is associated at all times after ^{32}P administration with the deoxycholate soluble fraction of the membranes microsomes. In contrast, although in the early times after ^{32}P administration the ribosomes accumulate polyadenylic acid, this subsequently

TABLE III. DISTRIBUTION OF RADIOACTIVITY IN THE SDS FRACTIONS OF LIVER RIBOSOMES AND OF THE DEOXYCHOLATE SOLUBLE FRACTION

	Ribosomes		Deoxycholate Soluble	
Time Hours	Per cent of total RNA	A/G ratio	Per cent of total RNA	A/G ratio
2	13.0	2.8	17	4.3
4	6.5	1.6	18	8.4
8	6.0	0.6	15	6.0

TABLE IV. THE EFFECT OF ACTINOMYCIN D ON THE LABELING OF THE NUCLEIC ACIDS OF NUCLEI, RIBOSOMES AND THE DEOXYCHOLATE SOLUBLE FRACTIONS OF MOUSE LIVER.

		% Inhibition by Act. D		SDS Fraction as A/G Ratio		
Time Hours	Act. D	Total RNA	SDS Fraction	% of total RNA	Total RNA	SDS Fraction
			NUCLEI			
2	--	--	--	6	0.5	1.2
	+	55	54	8	0.3	1.0
4	--	--	--	5	0.6	1.1
	+	55	30	8	0.7	0.7
8	--	--	--	6	0.6	0.8
	+	40	--	10	0.7	0.9
			RIBOSOMES			
2	--	--	--	13	--	2.8
	+	79	78	15	--	4.5
4	--	--	--	6	0.6	1.6
	+	96	87	20	1.4	4.0
8	--	--	--	6	0.6	0.6
	+	94	94	7	0.8	1.1
			DEOXYCHOLATE SOLUBLE			
2	--	--	--	17	0.8	4.3
	+	53	20	16	1.3	3.2
4	--	--	--	18	1.2	8.4
	+	76	37	35	1.5	10.0
8	--	--	--	15	1.2	6.0
	+	40	33	17	1.1	6.0

Actinomycin D was administered to mice at a level of 1.5 mg per Kg. Two hours later ^{32}P carrier free inorganic phosphate was administered and at various subsequent time intervals, the distribution of radioactivity in the RNA of the various cellular subfractions was determined as previously.

becomes masked by a polymer rich in guanylic acid.

In order to establish whether the Poly-A is synthesized independently by

the ATP polymerase referred to in the introduction or by a DNA dependent reaction, mice were treated with Actinomycin D according to the Legend in Table IV.

From the A/G ratios in Table IV, it is seen that treatment with Actinomycin D does not significantly alter the A/G ratios of the nuclear total RNA or of that of the SDS fraction, nor is the customary fall in the A/G ratio of the SDS fraction modified by the administration of Actinomycin D. In addition, the degree of inhibition of the SDS fraction by Actinomycin D treatment seems to be proportional to the degree of inhibition of the total RNA of the nuclear and ribosomal RNA. Both of these results argue for a nuclear synthesis of Poly-A. An independent synthesis of Poly-A in the deoxycholate soluble fraction of the microsomes cannot be excluded.

Characteristics of the SDS Fractions. The SDS Fractions derived from the various cellular components are non-dialyzable, acid insoluble and when centrifuged in a 5-20% sucrose gradient, they sediment close to 4S RNA. This value has to be tempered by the possibility of degradation during isolation as well as the aggregation that may occur especially with guanylic acid polymers.

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