STUDIES ON DNA SYNTHESIS IN MURINE MYELOMA: II.* ACTIVATION OF LATENT RNA-DEPENDENT DNA POLYMERASE ACTIVITY IN MEMBRANE FRACTIONS

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SUMMARY: The poly(rA)-directed DNA polymerase activity detected in the presence of 250 mM KCl and 12.5 mM magnesium acetate is latent in a membrane fraction from mouse myeloma. The activity may be activated by several nonionic surfactants and by the cationic surfactant cetyltrimethylammonium bromide. Activation of this RNA-dependent DNA polymerase activity will permit its measurement in various myeloma subcellular fractions and in other tissues.

In a previous study (1) DNA polymerase activity that promoted high levels of poly(dT) synthesis was found in preparations of intracisternal A-type particles (A-particles) isolated from several mouse tumors including myeloma MOPC-104E, RPC-20, and RPC-9, neuroblastoma C-1300 and squamous cell carcinoma D. Certain properties of the enzyme activity and its almost exclusive use of poly(rA) for direction of poly(dT) synthesis appeared to distinguish it from known cellular DNA polymerase activities. The enzyme activity appeared tightly associated with A-particles but its intracellular distribution was not determined.

The aim of the current study was to develop assay conditions for the quantitative measurement of A-particle DNA polymerase activity in various myeloma subcellular fractions. This was possible because conditions for its activity were different from those of other known cellular DNA polymerases and also because the A-particle enzyme was sufficiently active so that small

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amounts of tissue extract (0.2 - 1 μg protein) could be used, thus reducing the effect of other enzymes which might influence DNA polymerase activity. However, as shown here, very little poly(dT) polymerase activity was detected when a mitochondrial:microsomal fraction was assayed even though this fraction was known to contain A-particles and substantial DNA polymerase activity. In order to measure DNA polymerase activity in crude membrane fractions from myeloma MOPC-104E a number of nonionic and two ionic surface active agents (surfactants) were tested for their ability to activate latent poly(dT) polymerase activity.

MATERIALS AND METHODS

Preparation of subcellular fractions and A-particles. Except where indicated, studies were performed with intracisternal A-particles and a membrane fraction isolated from the BALB/c mouse transplantable myeloma (2) MOPC-104E (passage 122-1). The procedure for tissue fractionation and particle isolation has been described (3). In brief, a 20% homogenate was prepared in 50 mM Tris·HCl (pH 7.6 at 25°), 25 mM KCl, 5 mM $MgCl_2$ and 250 mM sucrose (Solution A) and filtered through cheese cloth to remove debris. The homogenate was centrifuged at 700 x g for 10 min; the supernatant was centrifuged at 10,000 x g for 10 min to produce a pellet containing mitochondria and microsomes which is referred to as the membrane fraction. A portion of the membrane fraction was further fractionated to yield intracisternal A-particles as follows: the membrane fraction containing 30-40 mg protein per ml was made to 1.67% Triton X-100 with 0.2 volumes 10% Triton X-100, sheared by repeated expression through a 23-gauge needle, diluted 7 to 10-fold with 0.15 M potassium citrate pH 7.2 and centrifuged at 78,000 x g for 30 min. The pellet, referred to as the Triton X-100 resistant particulate fraction, was collected and

subjected to density equilibrium centrifugation for the purification of A-particles.

Poly(dT) polymerase assay. Unless otherwise indicated, each reaction contained in a final volume of 0.052 ml: 50 mM Tris·HCl buffer, pH 8.3 at 37°, 19% (v/v) glycerol, 346 μg/ml bovine serum albumin, 250 mM potassium chloride, 12.5 mM magnesium acetate, 1 mM dithiothreitol, 0.5 mM ATP, 10 μg/ml nucleoside diphosphokinase (E.C. 2.7.4.6), 460 μg/ml poly(rA)·$(dT)_{14}$ (1:1), 1.6 to 2.6 μg enzyme protein from myeloma MOPC-104E, and 0.5 mM [^{3}H]-methylthymidine 5'-triphosphate (235 to 250 cpm/pmole). Reactions containing all components except nucleotides and nucleoside diphosphokinase were incubated in silicon treated 10 x 75 mm soft glass tubes at 37° for 20 min before incorporations were begun by addition of the remaining components. Reactions were then incubated at 37° for an additional 75 min. Incorporation of [^{3}H]-TMP into cold acid insoluble material was measured as previously described (1). All activity measurements were made in a range of proportionality between enzyme activity and both time of incubation and protein concentration. Synthetic polynucleotides and oligonucleotides were from Miles Laboratories and Collaborative Research, respectively. Radioactive deoxynucleotides were from Schwarz/Mann. The sources and chemical compositions of the surfactants tested are shown in Table I.

RESULTS AND DISCUSSION

Results of an experiment designed to measure poly(rA)·$(dT)_{14}$-dependent poly(dT) polymerase activity in a mitochondrial-microsomal membrane fraction, in a Triton X-100-resistant particulate fraction and in purified A-particles are shown in Table II. Each fraction was derived from the same amount of myeloma homogenate. It may be seen that in the absence of surfactant the membrane fraction was relatively inactive in promoting [^{3}H]-TMP incorpo-

TABLE I

Surfactants Tested

Category and common name	Source	Compound
A)Nonionic		
Tween 80	Schwarz/Mann	polyoxyethylene(n)sorbitan monooleate
Tween 20	Schwarz/Mann	polyoxyethylene(n)sorbitan monolaurate
Neodol 25-9	Shell Chem. Co.	polyoxyethylene(9)alcohol-(12-15 carbon)
Neodol 25-12	Shell Chem. Co.	polyoxyethylene(12)alcohol-(12-15 carbon)
Sterox SL	Monsanto Co.	polyoxyethylene(12)alcohol-(14-15 carbon)
Triton X-100	Packard Inst. Co.	polyoxyethylene(9-10)octaphenol
Triton X-102	Rohm & Haas Co.	polyoxyethylene(12-13)octaphenol
Nonidet P40	Shell Chem. Co.	polyoxyethylene(9)octaphenol
Triton N-101	Rohm & Haas Co.	polyoxyethylene(9-10)nonylphenol
B)Anionic		
DOC	Schwarz/Mann	sodium deoxycholate
C)Cationic		
CETAB	Eastman Chem. Co.	cethyltrimethylammonium bromide

ration. In contrast, the Triton X-100-resistant particulate fraction and the isolated A-particles possessed higher levels of activity. The addition of 1% Tween 80 to reactions containing the membrane fraction resulted in a level of activity in the range of those observed in the two A-particle preparations. Isolated A-particles were equally active in the presence and absence of 1% Tween 80.

These results indicated that poly(dT) polymerase in crude tissue fractions was latent and could be activated by surfactant. Thus, a number of surfactants

TABLE II

DNA Polymerase Activity in Subcellular Fractions From Myeloma MOPC-104E

Enzyme preparation	Modification	
	Minus Tween 80	Plus 1% Tween 80
	pmoles TMP incorp/min x 10^2	
None	.8	.5
Membrane fraction	1.2	7.4
Triton X-100 resistant particulate fraction	3.6	8.1
Intracisternal A-type particle	5.6	5.2

were tested over a range of concentrations for their ability to activate membrane fraction activity. The results are shown in Fig. 1. It may be seen that all of the surfactants tested were effective in activating poly(dT) polymerase activity, however, the concentrations required for optimal activity varied. The maximum activity attained was similar except for Nonidet P40 and sodium deoxycholate.

The activity of isolated A-particles did not require the presence in reaction mixtures of surfactants Tween 80 or Nonidet P40 - see Table II and ref. 1. This indicated that enzyme in particulate form was capable of activity and suggested that the activation in crude membrane fractions did not involve enzyme solubilization. In order to investigate this possibility, the sedimentation properties of poly(dT) polymerase activity in a Triton X-100 treated membrane fraction were determined. A membrane fraction was treated with 1.7% Triton X-100,

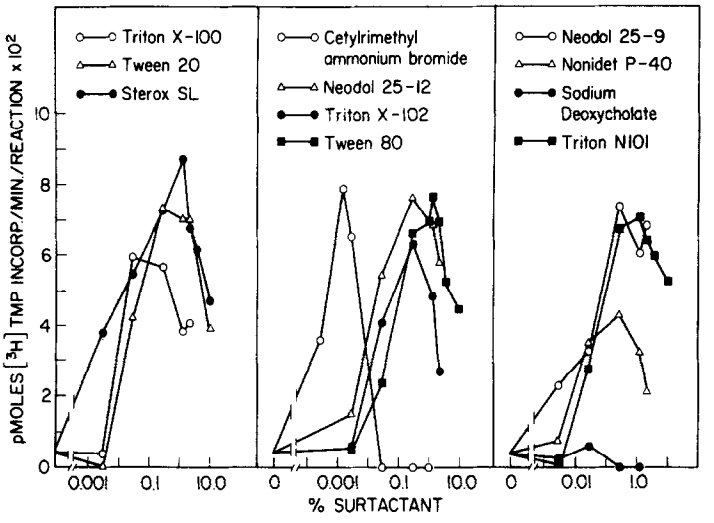

Fig. 1. Effect of surfactants upon DNA polymerase activity of a membrane fraction from myeloma MOPC-104E. Surfactants were present in reaction mixture at the final concentration indicated. Each reaction contained 2.6 μg fraction protein in a final value of 0.052 ml. Values obtained in reactions without membrane fraction were subtracted and were equal to less than 0.01 pmoles/min except in the cases of CETAB and DOC where the values were as high as 0.063 and 0.027 pmoles/min, respectively.

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sheared by expression through a 23 gauge needle, adjusted to 135 mM potassium citrate (to dissociate 80S ribosomes) and analyzed using a 9-45% linear sucrose gradient formed over a layer of 68% sucrose. The results are shown in Fig. 2. Essentially all of the DNA polymerase activity recovered from the gradient was at the interface between the 45 and 68% sucrose solutions. No activity or inhibitor of activity was found in regions of the gradient corresponding to lower sucrose concentrations. Thus, like the A-particle itself, the poly(dT) polymerase activity was sedimentable through 45% sucrose after Triton X-100 treatment. Activation was not accompanied by solubilization of the enzyme.

The results indicate that use of a surface active agent such as 1% Tween 80 is essential for the measurement of A-particle DNA polymerase activity in crude membrane fractions. Levels of the enzyme activity in various

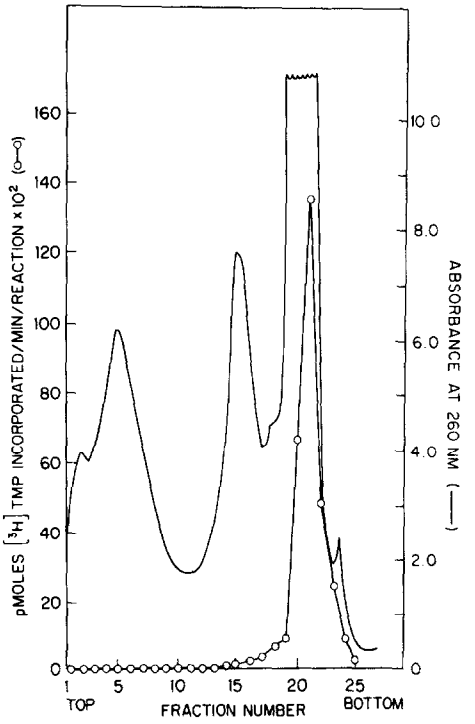

Fig. 2. Density equilibrium centrifugation of a myeloma MOPC-104E (passage 128-2) membrane fraction after treatment with Triton X-100. Membrane fraction was suspended in Solution A and made to 1.4% Triton X-100 in a final volume of 2 ml containing 78 mg protein. Triton X-100 used in this experiment was from New England Nuclear Corp. The suspension was then dispersed by expression through a 23 gauge needle, adjusted to 135 mM potassium citrate, pH 7.2 and 6.9 mg membrane protein was layered over a 3.8 ml 9-45% linear sucrose gradient that had been formed over a layer of 0.8 ml 68% sucrose. All sucrose solutions contained 50 mM Tris·HCl pH 7.4 at 25°, plus 100 mM potassium chloride. Centrifugation was at 300,000 x g for 3 hrs at 4°. DNA polymerase activity was determined using 1 to 5 µl of each 200 µl fraction as described in Methods. Each reaction contained 1% Tween 80. 237 pmoles TMP incorp/min of DNA polymerase activity was applied to the gradient and 125 pmoles TMP incorp/min was recovered in gradient fractions.

tissues and its subcellular distribution in myeloma cells may now be assessed.

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