PARTIAL SEPARATION OF THE DNA NUCLEOTIDYLTRANSFERASES OF THE SHOPE RABBIT FIBROMA[1]

Lucy M. S. Chang[2] and M. E. Hodes

Cancer Research, Departments of
Medicine and Biochemistry,
Indiana University Medical Center,
Indianapolis, Indiana 46202

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Rabbit kidney cells maintained in culture after infection with Shope fibroma virus and fibromas removed from rabbits contain two DNA nucleotidyltransferase activities (1). These differ in several ways, including stability to heat, Mg^{++} and pH optima and the state (native or denatured) of the DNA required for most efficient priming of the reaction. Two activities, differing in their sensitivities to anti-Shope fibroma virus γ-globulin, are separable from fibroma by chromatography on phosphocellulose and DEAE-cellulose. The DNA nucleotidyltransferase sensitive to anti-Shope fibroma virus γ-globulin can be separated into fractions that differ in requiring DNA of different physical states for most efficient priming of the reaction.

MATERIALS AND METHODS

The preparation of Shope fibroma DNA and enzyme extracts and the DNA

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[2] Predoctoral trainee, National Cancer Institute training grant CA-5066.

The following abbreviation is used: PhM, potassium phosphate buffer containing 10 mM 2-mercaptoethanol, pH 7.2, unless stated otherwise.

nucleotidyltransferase assays were described previously (1). Protein concentration was determined with the biuret reaction (2) or by measurement of absorbance at 280 mμ.

Whatman cellulose phosphate PII, medium fibrous powder, 7.4 meq/g and DEAE-cellulose (Brown, Lot no. 1656, 0.87 meq/g) were prepared according to Yoneda and Bollum (3). Sephadex G-100 was swollen in 0.1 M potassium phosphate (pH 7.2) for 2 days, placed in a column (2.3 x 18 cm) and washed with buffer until free of material absorbing at 280 mμ.

The anti-Shope fibroma virus γ-globulin has been described before (4).

RESULTS

Differential Absorption of DNA Nucleotidyltransferase Activities on Phosphocellulose and DEAE-cellulose

The 105,000 x g supernatant from 70 g of fibroma was adjusted to pH 6.5 with 1% acetic acid and held for 30 min. The precipitate after centrifugation at 36,000 x g for 10 min was discarded. Seven grams (dry weight) of phosphocellulose were added as a wet paste in 0.05 M PhM (pH 6.5) to the supernatant, the suspension was mixed thoroughly for 10-15 min and poured onto a coarse fritted glass funnel. From 40 to 60% of the DNA nucleotidyltransferase activity was not retained regardless of the amount of phosphocellulose added. The phosphocellulose was transferred from the funnel to a column and washed with approximately 1 liter of 0.05 M PhM, followed by 1 liter of 0.1 M PhM, and eluted in one step with 0.2 M PhM.

The effluent from the phosphocellulose and 200 ml of 0.05 M PhM (pH 6.5) wash were combined and dialyzed against 3 changes of 40 volumes each of 0.01 M PhM. The dialysate was treated with 10 g (dry weight) DEAE-cellulose previously treated with buffer, and the suspension poured onto a coarse fritted glass funnel. The DEAE-cellulose funnel was washed with approximately 1 liter of 0.01 M buffer and eluted in one step with 0.2 M PhM. The separation is summarized in Table 1.

Table I

Differential absorption of DNA nucleotidyltransferase activities on phosphocellulose and DEAE-cellulose

Fraction	DNA[1]	Sp.Ac.[2]	Total units[3]	Total protein (mg)	Recovery %
Crude	n	0.52	1340	2600	100
	d	0.55	1430		100
Phosphocellulose pool	n	4.06	223	55	16.6
	d	17.4	964		66.8
DEAE-cellulose pool	n	0.97	172	178	12.8
	d	5.61	1000		70.0
		Total recovery			DNA (native) 29.4%
					DNA (denatured) 136.8%

[1] DNA primer, n = native DNA; d = heat-denatured DNA
[2] Specific activity = mμmoles dTMP-2-^{14}C incorporated per mg protein per hour of incubation at 37° C
[3] One unit = 1 mμmole of dTMP per hour

Inhibition by anti-Shope fibroma virus γ-globulin

The γ-globulin was prepared at a concentration of 20 mg protein/ml (4). Normal rabbit or sheep γ-globulin at the same concentration served as control The enzyme preparations (phosphocellulose and DEAE-cellulose pools) were incubated for 45 min at 35° at a 3:2 ratio with 0.1 M phosphate buffer (pH 7.2), sheep γ-globulin, rabbit γ-globulin or anti-Shope fibroma virus γ-globulin. The preparations were then assayed as previously described. The results (Table II) show marked inhibition of the phosphocellulose pool and no effect on the DEAE-cellulose pool.

Table II

Inhibition of DNA nucleotidyltransferase activities

from Shope fibroma extract by anti-Shope fibroma virus gamma globulin

Enzyme	Primer DNA	0.1 M phosphate	Sheep gamma globulin	rabbit gamma globulin	anti-SFV γ-globulin	% activity
Phosphocellulose pool	native	x				100
			x			107
				x		149
					x	43
	denatured	x				100
			x			106
				x		67
					x	8
DEAE-cellulose pool	native	x				100
			x			104
				x		104
					x	99
	denatured	x				100
			x			100
				x		64
					x	71

Each determination is obtained by an average of two independent assays. For details, refer to methods and materials and text.

Phosphocellulose and Sephadex Column Chromatography

The supernatant from 50 g of Shope fibroma was treated with 8 g phosphocellulose as described in the previous section. The material adsorbed on phosphocellulose was eluted in steps with 0.1 M, 0.15 M and 0.2 M PhM. Fractions of 4 ml were collected. The partial separation of the activities is shown in Fig. 1.

The active fractions were combined into 2 pools, concentrated on carbowax, and dialyzed against 2 changes of 0.1 M PhM. The volumes of the pools after dialysis were 23 and 27 ml. Seven ml of each pool were loaded on a Sephadex G-100 column (2.3 x 18 cm). The column had been equilibrated with 0.1 M PhM. The DNA nucleotidyltransferase activities from both pools were eluted with the void volume (Fig. 2). This separation is summarized in Table III.

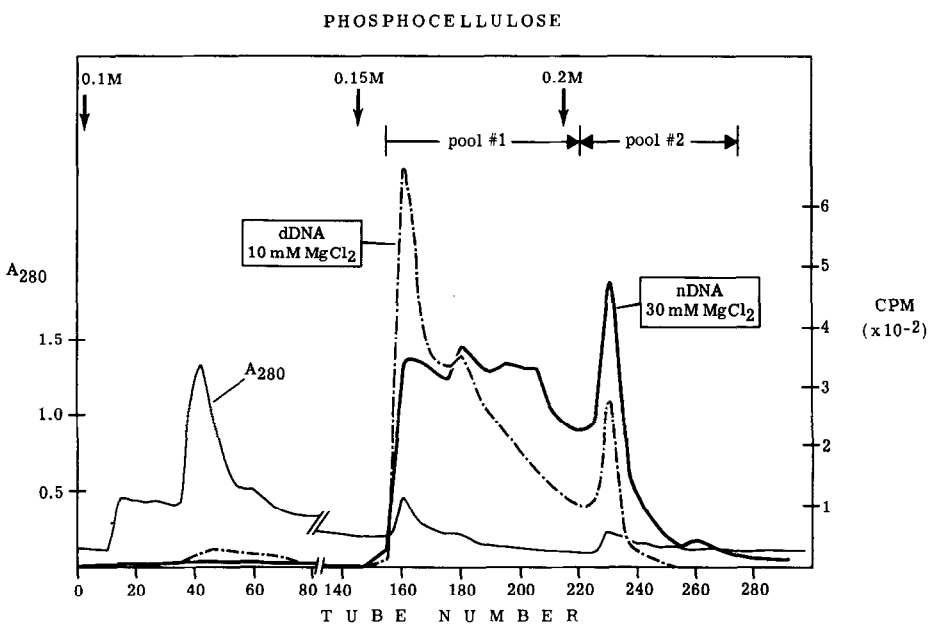

Figure I

Fractionation of Shope fibroma extract on phosphocellulose. The DNA nucleotidyltransferase activities were separated by stepwise elution with PhM. For enzyme assay, 0.05 ml of each fraction was used. Heavy solid line: Native DNA primed activity. Broken line: Denatured DNA primed activity. Light solid line: Absorption at 280 mμ.

Table III

Separation of DNA nucleotidyltransferases of Shope fibroma extract

Fraction	Primer DNA	Sp.Ac.	Total units	Total protein (mg)	Recovery (%)
Crude	n	0.29	1048	3610	100
	d	0.20	722		100
1st phospho-cellulose pool	n	16.9	208	12.3	20
	d	27.6	339		47
2nd phospho-cellulose pool	n	27.6	152	5.5	14.5
	d	13.9	76		10.5
G-100 on 1st phosphocellulose pool*	n	22	60	2.7	5.7
	d	29.5	80		11.1
G-100 on 2nd phosphocellulose pool*	n	31.0	38.8	1.25	3.7
	d	16.0	20.0		2.6

* Only part of 1st phosphocellulose or 2nd phosphocellulose pool was loaded onto the Sephadex G-100 column. Refer to the text for details.

DISCUSSION

The state of the DNA primer for the DNA nucleotidyltransferase of normal tissue has been the subject of much research. The preparations from mammalian sources of highest specific activity are primed by single-stranded DNA (2), whereas bacterial enzyme may be primed by either native or heat-denatured DNA (5). Following viral infection, new DNA nucleotidyltransferases may appear. When Shope fibroma virus is the infecting agent, one of the induced transferase activities differs markedly from the enzyme of normal tissues in several ways (1).

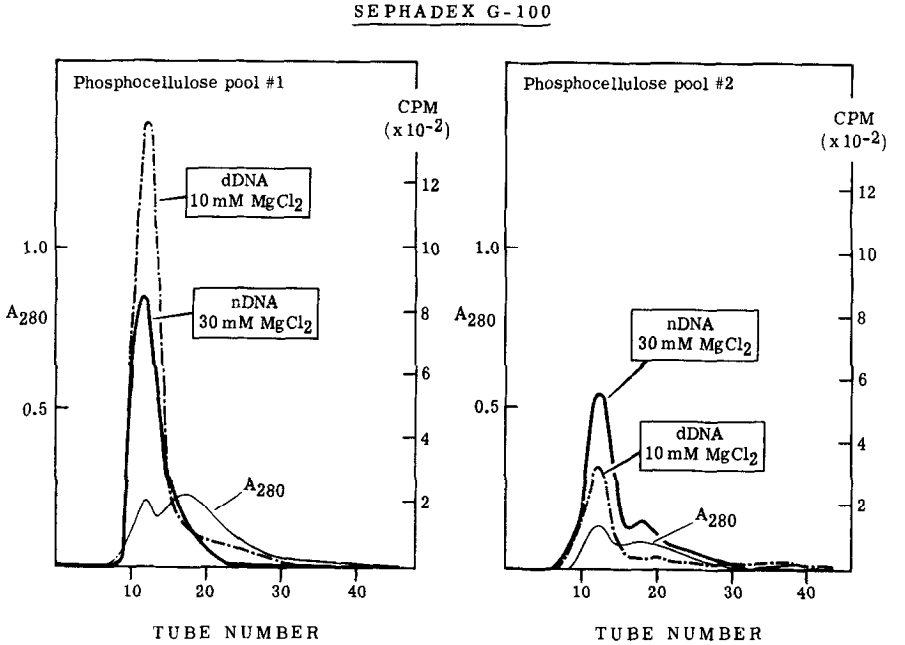

Figure 2

Gel filtration of the DNA nucleotidyltransferase pools from the phosphocellulose column. Part of each phosphocellulose pool was loaded on a Sephadex G-100 column (2.3 x 18 cm) and eluted with 0.1 M PhM. For enzyme assay, 0.05 ml of each fraction was used. Heavy solid line: Native DNA primed activity. Broken line: Denatured DNA primed activity. Light solid line: Absorption at 280 mμ.

It is now possible to separate the activities in part. One of the enzyme is retained by phosphocellulose at pH 6.5, whereas the second enzyme passes through this ion exchanger but is retained by DEAE-cellulose. That the nucleotidyltransferase activities are distinct is evident from the experiments with anti-Shope fibroma virus γ-globulin. The enzyme that adsorbs to phosphocellulose is strongly inhibited by anti-viral γ-globulin whereas the enzyme that adsorbs to DEAE-cellulose is not sensitive to the immune globulin. We may assume with Salzman and Sebring (6) that after virus infection, antibodies to all viral products will appear, whereas an untreated animal will have little or no antibodies to its own normal tissues. The anti-Shope

γ-globulin thus contains antibodies to the new virus induced enzyme, but not to the normal (induced) enzyme.

It should be noted that in both the DEAE-cellulose and phosphocellulose pools, the denatured-DNA primed activity predominated. This property of the partially purified enzyme preparations may be due to the removal of a nuclease, present in the crude extract, which activates the priming ability of native DNA. The problem of interconversion of activities primed preferentially by one or the other forms of DNA has been discussed elsewhere (1).

The enzyme adsorbed on phosphocellulose can be further separated according to preference for the physical state of the primer (Fig. 1), and the differences noted persisted through separation on Sephadex (Fig. 2). The inhibition of the phosphocellulose pools by antibody indicates that the 2 peaks of Fig. 1 may be different forms of the same enzyme. Evidence (unpublished) has been obtained suggesting that DNA nucleotidyltransferase (particularly the activity that prefers denatured DNA as primer) is a multi-component system. Extensive fractionation on ion exchanger results in separation of the components and a drastic loss in activity.

REFERENCES

1. L.M.S. Chang and M. E. Hodes, *Virology* 32:258 (1967).
2. A. C. Cornall, C. J. Bardawill and M. M. David, *J. Biol. Chem.* 177:751 (1949).
3. M. Yoneda and F. J. Bollum, *J. Biol. Chem.* 240:3385 (1965).
4. M. E. Hodes and L.M.S. Chang, *Virology* 34:134 (1968)
5. T. Okazaki and A. Kornberg, *J. Biol. Chem.* 239:259 (1964).
6. N. P. Salzman and E. D. Sebring, *J. Virol.* 1:16 (1967).
7. C. C. Richardson, C. L. Schildkraut, H. V. Aposhian, A. Kornberg, W. Badmer and J. Lederberg, in *Symposium on Informational Macromolecules*, ed. by H. Vogel, Acad. Press, New York, (1963) p. 13.