Antigenic Relationships in Mammalian DNA Polymerase

Abstract. *Rabbit antibody was prepared against a high-molecular-weight DNA polymerase purified from the soluble fraction of calf thymus gland. This antibody does not inhibit terminal deoxynucleotidyl transferase isolated from that source, but does inhibit both low-molecular-weight and high-molecular-weight DNA polymerases isolated from cytoplasmic and nuclear fractions of a number of mammalian tissues (mouse L cells, calf thymus, phytohemagglutinin-stimulated human lymphocytes, rat liver, and rabbit bone marrow). The results suggest that (i) no antigenic relationship exists between terminal transferase and DNA polymerase, (ii) common antigenic determinants exist in the DNA polymerases from all mammalian sources, and (iii) multiple forms of DNA polymerase found in mammalian cells are related by having polypeptide sequences or subunits in common.*

An inhibitory antibody has been prepared against calf thymus DNA polymerase (*1*) by injecting a rabbit with a partially purified DNA polymerase. Our original purpose in preparing this antibody was to explore possible immunological relationships between calf thymus DNA polymerase and calf thymus terminal deoxynucleotidyl transferase (*2*), but none was found. Recent work from several laboratories (*3*) has demonstrated that mammalian DNA polymerase exists in several molecular forms and intracellular locations. The activities with which we have been concerned have been described as 3.3*S* and 6*S* to 8*S* DNA polymerases from the cytoplasmic fraction, and a 3.3*S* activity from the nuclear fraction. The 3.3*S* activities from cytoplasm and nuclei appear to be identical in reaction properties and chromatographic behavior (*3*). Mitochondrial DNA polymerase has also been described (*4*). We now report our studies on the possible immunological relations between the various molecular forms of DNA polymerase in mammalian cells and in different kinds of tissue.

The calf thymus DNA polymerase used to prepare the antibody was the 6*S* to 8*S* activity isolated from the soluble fraction of calf thymus gland (*2*). Although we did not observe any antigenic relationship between 6*S* to 8*S* DNA polymerase and terminal transferase, we do find common antigenic determinants among DNA polymerases from several mammalian species.

We now describe some immunological reactions of the forms of DNA polymerase now available. The effect of antibody to calf 6*S* to 8*S* polymerase on terminal transferase isolated from calf thymus gland and DNA polymerase activities isolated from calf thymus gland, mouse L cells, phytohemagglutinin-stimulated human lymphocytes, rabbit bone marrow, rat regenerating liver, rat liver mitochondria, *Escherichia coli* polymerase I, and *E. coli* polymerase II are summarized in Table 1. The data are presented as the antibody dilution that gave approximately 50 percent inhibition at the stated enzyme concentration for the cases showing inhibition. The lowest antibody dilution tested is listed for those enzymes showing no inhibition.

Antigen-antibody reactions were carried out by mixing serial dilutions of antibody with a constant amount of antigen (that is, a fixed enzyme unitage was used) in 0.1*M* NaCl containing 50 m*M* potassium phosphate at *p*H 7.5 at room temperature for 30 minutes and storing the mixtures overnight at 4°C. A portion of each antigen-antibody mixture was then assayed for DNA polymerase activity (*3*). As indicated in Table 1 the antibody is active against its homologous antigen at a 1 : 32 dilution. It is also active at a 1 : 8 dilution against a DNA polymerase that has quite different enzymatic properties, the 3.3*S* polymerase purified from the homologous tissue. A third enzyme from calf thymus gland (*2*), terminal deoxynucleotidyl transferase, which catalyzes random polymerization of deoxynucleoside triphosphates is not inhibited by this antibody. This set of titrations establishes the specificity of the antibody against DNA polymerases and the cross-reaction between molecular species in the homologous tissue. It also indicates that the antibody preparation does not contain enzymes that degrade deoxynucleoside triphosphate substrates or polydeoxynucleotide products.

Further tests for cross-reaction were then carried out with 3.3*S* and 6*S* to 8*S*

Table 1. Immunological relationships in mammalian DNA polymerases. The antibody to calf thymus 6*S* to 8*S* DNA polymerase was partially purified immunoglobulin G (IgG) from a rabbit immunized with calf thymus 6*S* to 8*S* DNA polymerase. The IgG was obtained by ammonium sulfate fractionation followed by chromatography on diethylaminoethyl (DEAE)-cellulose. The final protein concentration of this antibody preparation was 40 mg/ml. The rabbit antiserum to *Escherichia coli* polymerase I (*10*) was inactivated for 60 minutes at 70°C. When the antibody to calf thymus 6*S* to 8*S* DNA polymerase was used, the control titration was carried out with purified rabbit γ-globulin; when the antiserum *E. coli* polymerase I was used, the control titration was carried out with normal rabbit serum. Normal rabbit γ-globulin or normal rabbit serum does not inhibit the DNA polymerase activity, but both have some stimulating effect. The antigens used were partially purified enzyme preparations except for DNA polymerases from phytohemagglutin (PHA)-stimulated human lymphocytes, which were obtained by separation of the nuclear and soluble polymerases. An enzyme unit (E.U.) is that amount that catalyzes the incorporation of 1 nmole of nucleotide in 1 hour. In all cases except that of mitochondrial DNA polymerase at least 90 percent inhibition of the enzyme activity can be obtained at lower antibody dilutions. Mitochondrial DNA polymerase was 80 percent inhibited at the lowest dilution.

Antigen	Inhibition	Activity (E.U.)	Antibody dilution	Inhibition (%)
Antibody to calf thymus 6S to 8S DNA polymerase				
Calf thymus, 6*S* to 8*S*	+	3.60	1/32	48
Calf thymus, 3.3*S*	+	3.00	1/8	45
Calf thymus terminal transferase	−	7.50	2/3	0
Rabbit bone marrow, 3.3*S*	+	1.48	1/16	59
Rat regenerating liver, 6*S* to 8*S*	+	0.48	1/64	37
Rat regenerating liver, 3.3*S*	+	0.08	1/32	72
PHA human lymphocyte, 3.3*S* + 6*S* to 8*S*	+	0.43	1/3	86
PHA human lymphocyte, 3.3*S*	+	0.33	1/8	70
Mouse L cells, 6*S* to 8*S*	+	0.83	1/64	66
Mouse L cells, 3.3*S*	+	0.34	1/16	36
Rat liver mitochondria	+	0.06	1/16	63
E. coli polymerase I	−	0.57	1/3	0
E. coli polymerase II	−	0.80	2/3	0
Antiserum to E. coli *polymerase I*				
E. coli polymerase I	+	0.57	1/96	88
Calf thymus 6*S* to 8*S*	−	2.40	2/3	0

DNA polymerases from other species. Table 1 indicates that the antibody is active at a 1 : 16 dilution against the 3.3*S* DNA polymerase purified from rabbit bone marrow. This is a rather striking result since the antibody was produced against calf thymus protein in a rabbit, that is, the animal produced an antibody that will react quite specifically with one of its own intracellular proteins. Other entries in Table 1 demonstrate that both forms of cellular DNA polymerase found in rat liver, mouse L cells, phytohemagglutinin-stimulated human lymphocytes (*5*), and rat liver mitochondrial DNA polymerase are inhibited by rabbit antibody to calf thymus 6*S* to 8*S* DNA polymerase. The last four entries confirm the specificity of this antibody in not inhibiting *E. coli* DNA polymerase I, and the converse, that rabbit antiserum to *E. coli* polymerase I serum does not inhibit calf thymus 6*S* to 8*S* DNA polymerase. Others (*6*) have demonstrated that *E. coli* polymerase II is not inhibited by antiserum to *E. coli* polymerase I, Table 1 shows that antibody to calf thymus 6*S* to 8*S* does not inhibit *E. coli* polymerase II. This confirms the specificity of the cross-reactivity of antibody to calf DNA polymerase with mammalian DNA polymerases.

The titrations of antibody to calf 6*S* to 8*S* polymerase have also been carried out with fixed amounts of antibody and variable amounts of antigen (6*S* to 8*S* calf thymus polymerase). Antigen-antibody precipitates were removed by centrifugation, and the supernatant fractions were assayed for DNA polymerase activity. The results confirmed the validity of fixed antigen-variable antibody titrations and also indicated that a precipitating antibody was present.

The final question to be answered is whether the antibody preparation contains two different populations of antibodies, one active against 6*S* to 8*S* polymerase and a second active against the 3.3*S* enzyme, or whether the same population of antibody molecules is active against both enzymes. To test these alternatives we mixed the antibody preparation with calf thymus 6*S* to 8*S* polymerase, and the mixture was centrifuged. The supernatant was then tested for activity against rabbit bone marrow and calf thymus 3.3*S* polymerase. The supernatants were depleted of antibody to 3.3*S* polymerase in proportion to the depletion of the antibody to calf 6*S* to 8*S* polymerase. We conclude that one antibody population is reacting with all forms of polymerase present.

Antiserums have been prepared against *E. coli* DNA polymerase I (*7*), T_2 DNA polymerase (*7*), Shope fibroma virus (*8*), and herpesvirus (*9*). In each instance the antiserum has been reported to be specific for its specific antigen (DNA polymerase) and no cross-reactions have been reported. The results that we have presented would not have been predicted on the basis of these earlier immunological studies. In retrospect it does seem reasonable that enzymes responsible for chromosome replication might be rather invariant in evolutionary time, particularly in determinant regions related to catalytic activity.

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References and Notes

1. M. Yoneda and F. J. Bollum, *J. Biol. Chem.* **240**, 3385 (1965).
2. L. M. S. Chang and F. J. Bollum, *ibid.* **246**, 909 (1971).
3. ———, *ibid.*, p. 5835; A. Weissbach, A. Schlabach, B. Fridlender, A. Bolden, *Nature* **231**, 167 (1971); E. F. Baril, O. E. Brown, M. D. Jenkins, J. Laszlo, *Biochemistry* **10**, 1981 (1971); J. T. Bellair, *Biochem. Biophys. Acta* **161**, 119 (1968); N. B. Furlong, *Texas Rep. Biol. Med.* **29**, 75 (1971); R. Howk and and T. Y. Wang, *Arch. Biochem. Biophys.* **133**, 238 (1969).
4. R. R. Meyer and M. V. Simpson, *Proc. Nat. Acad. Sci. U.S.* **61**, 130 (1968).
5. L. M. S. Chang and F. S. Bollum, unpublished results.
6. R. E. Moses and C. C. Richardson, *Biochem. Biophys. Res. Commun.* **41**, 1557 (1970); T. Kornberg and M. L. Gefter, *Proc. Nat. Acad. Sci. U.S.* **68**, 761 (1971).
7. H. V. Aposhian and A. Kornberg, *J. Biol. Chem.* **237**, 519 (1962).
8. L. M. S. Chang and M. E. Hodes, *Biochem. Biophys. Res. Commun.* **31**, 545 (1962).
9. H. M. Keir, H. Subak-Sharpe, W. I. H. Shedden, D. H. Watson, P. Wildy, *Virology* **30**, 154 (1966).
10. The antiserum was provided by Drs. L. Bertsch, and A. Kornberg. The *E. coli* polymerase I was obtained from Worthington Biochemical Corp. The *E. coli* polymerase II was a gift from Dr. C. C. Richardson (*6*); the DNA polymerase from rat liver mitochondria was fraction II (*4*).
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Carcinoembryonic Antigen Present in Human Colonic Neoplasms Serially Propagated in Hamsters

Abstract. *Carcinoembryonic antigen, as measured by radioimmunoassay, is present in two different human colonic tumors that have been serially transplanted and maintained in the cheek pouches of unconditioned, adult golden hamsters. This finding shows that a human tumor-associated antigen can be produced in an animal host.*

Gold and Freedman have described (*1*) an antigenic material specific for embryonic digestive tissue and entodermally derived neoplasms of the gastrointestinal tract. This material, named carcinoembryonic antigen (CEA), has been used in the search for a practical means of cancer diagnosis and prognostication (*2*, *3*). One major limitation in the use of this antigen for sensitizing animals, in diagnostic tests, and in the study of its chemical nature and biogenesis has been the difficulty of procuring it from metastatic gastrointestinal cancers and the variability of its activity. The availability of almost unlimited supplies of two human, mucin-producing, colonic tumors (GW-39 and GW-77) growing in the cheek pouches and the hind leg musculature of unconditioned, adult golden hamsters (*4*, *5*) has prompted us to determine whether these implanted tumors might be a good source of consistently large quantities of CEA with relatively uniform activity. The presence of CEA in xenografted tumors would be an indication that such tumor-associated antigens are

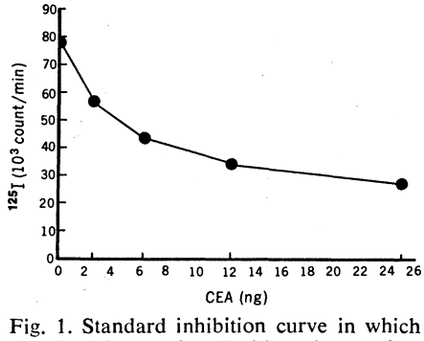

Fig. 1. Standard inhibition curve in which [^{125}I]CEA is complexed with antiserum free after incubation with known quantities of CEA.