Electrophoresis of Adenovirus-Specified Thymidine Kinase

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Stimulation of thymidine kinase (TK) activity occurs after abortive or productive infection with human adenoviruses. Striking changes in TK electrophoretic profile accompany this stimulation.

Thymidine kinase (TK) activity has been reported to increase in cells infected by certain human adenoviruses in both productive (2, 9, 12) and abortive (2, 12) interaction. As in the case of other oncogenic deoxyribonucleic acid viruses, simian virus 40 and polyoma (5-7, 11), which also induce increases in TK activity, it is not certain whether these increases result from synthesis of viral gene coded enzymes or from alterations (e.g., derepression or stabilization) of host cell enzymes. Evidence against the former hypothesis for polyoma virus is provided by the failure of this virus to induce TK in TK-minus mouse cells (1). The 31 human adenoviruses can now be divided into four major subgroups based on T-antigen sharing (10). Members of each subgroup transform rat or hamster cells, or both, although only the A and B subgroups have induced tumors *in vivo*. We have found that representatives of each T antigen subgroup induce increases in TK activity in both productive and abortive interactions. These increases were accompanied by a striking, consistent alteration in the electrophoretic profile of TK obtained in the abortive interaction in simian cells.

Primary African green monkey kidney (GMK) and human embryonic kidney cells (HEK) were infected with various adenovirus types at multiplicities of 10 to 100 TCID₅₀/ml, based on 21-day tube titrations in primary HEK. Cells were maintained in petri dishes (60 by 15 mm) with Eagles medium supplemented with 10% fetal bovine serum. Incubation was at 37 C in a CO₂-flushed incubator. At 20 to 30 hr postinfection, cells were scraped into the medium by a rubber policeman and were pelleted by low-speed centrifugation. The cells were then washed and homogenized in 0.01 M tris(hydroxymethyl)-aminomethane buffer (pH 8.0), containing 0.001 M dithiothreitol and 5×10^{-5} M thymidine (TdR). Cell debris was removed by low-speed centrif-

ugation, and further clarification was achieved by centrifugation at $100,000 \times g$ for 40 min. The soluble fraction was then assayed for enzyme activity by the procedure of Dulbecco, Hartwell and Vogt (4). Thymidine was separated from its phosphorylated derivatives by chromatography on Whatman no. 3 paper with isopropanol-water-28% NH₃ (425:75:6.5, v/v) as solvent. Enzyme reactions were stopped by the addition of one-third volume of a solution containing TdR, thymidine monophosphate, and ethylenediaminetetraacetate (all 10^{-2} M). Radioactivity was determined in a scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Polyacrylamide gel electrophoresis was carried out in glass cylinders (6 by 1.1 cm), using 7% gels and glycine buffer, pH 9.5. After electrophoresis (5 ma/gel) for 30 min, gels were sliced into 30 segments of equal length. The slices were added immediately to the TK reaction mixture (*see footnotes to Table 1*), and incubation was carried out for 10 to 20 min at 37 C. Activity was then determined as described above.

Table 1 presents the results of experiments utilizing 14 adenoviruses on both HEK (productive) and GMK (abortive) cultures. As shown in Table 1, viruses derived from all of the four T-antigen subgroups induced significant increases in TK activity in both cell systems. It would seem reasonable to conclude that all of the 31 human adenoviruses are capable of inducing increases in TK activity.

TK activity in uninfected primary and continuous GMK (BSC-1, CV-1, Vero) cultures was found to resolve as a single major peak (90 to 95% of recoverable activity) and as one to two minor peaks in polyacrylamide gels (Fig. 1).

In an effort to detect virus-specified TK, clarified supernatant fluids from infected GMK or BSC-1 cultures representing all four adenovirus subgroups were examined by this technique.

TABLE 1. Adenovirus-induced stimulation of thymidine kinase (TK) activity in African green monkey kidney (AGMK) and human embryo kidney (HEK) cells

T-Antigen subgroup	Virus types tested	Enzyme activity ^a	
		Primary GMK	HEK
A	12	2,600	5,340
	18	1,290	4,230
	31	1,900	6,650
B	7	1,670	3,710
	11	940	3,110
	14	1,590	3,200
C	1	8,260	5,940
	2	9,380	9,900
	5	9,950	8,580
D	9	3,000	2,210 ^b
	10	3,270	3,850
	13	1,090	2,060
Uninfected		765	1,950

^a Expressed as pmoles of thymidine phosphorylated per milligram of protein during 20 min at 37°C. Reaction mixtures contained 250 pmoles of ³H-TdR (1.9 c/mmmole), 7.5 μ moles of adenosine triphosphate, 7.5 μ moles of MgCl₂, and 50 μ moles of tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 8.0) in 0.15 ml (14).

^b In repeat experiments, TK activity was several times the control value.

In each case, the same striking alteration in pattern was observed (i.e., increase of TK activity in the cathodal region of the gel). In most instances, a clear diminution in the major component characteristic of uninfected cells (peak A) was also observed. These effects were most clearly seen with adenovirus 2-infected cells, which showed the greatest increases in TK activity (Fig. 1), but patterns obtained with the other adenoviruses were qualitatively superimposable on those illustrated.

The areas under the two characteristic activity peaks were determined with a planimeter, and the ratio of these areas is shown in Table 2. As is also obvious from Fig. 1, there is an extremely large absolute and relative increase in the cathodal peak (B), and we conclude that this peak contains the induced TK activity. Since this activity is superimposable on a low but reproducible normal cell activity, and for each virus the same qualitative changes were obtained, we tentatively suggest that adenovirus specified TK activity results from nonspecific stabilization of one of two (or several) alternate molecular forms

FIG. 1. Electrophoretic analysis of thymidine kinase from uninfected (●) or adenovirus 2-infected (■) BSC-1 cells. Conditions of electrophoresis and enzyme determination are described in the text and in the footnote to Table 1. Qualitatively, the same patterns were obtained for all uninfected primary and continuous green monkey cells; therefore, peak A is considered the normal cell major component. Based on similar findings for all adenovirus-infected cells, peak B is considered to represent the adenovirus-induced activity.

TABLE 2. Changes in electrophoretic profile of thymidine kinase (TK) in adenovirus-African green monkey kidney (AGMK) cells

Cells	Ratio of TK activity (peak A/peak B) ^a
AGMK, uninfected	10.0
AGMK, Ad 2	0.7
AGMK, Ad 12	3.0
AGMK, Ad 9	1.0
BSC, uninfected	20.0
BSC, Ad 2	0.12
BSC, Ad 12	0.8
BSC, Ad 9	0.9

^a As shown in Fig. 1, two main areas of TK activity were resolved by polyacrylamide gel electrophoresis. The area under each peak was determined by using a Planimeter (Gelman Instrument Co., Ann Arbor, Mich.).

of this enzyme. Actual conversion of the major normal cell TK component into virus-specified TK is suggested by the data of Fig. 1. A similar hypothesis, with more rigorous data, has been suggested for the new valyl transfer ribonucleic acid synthetase which appears in *Escherichia coli* infected with T4 bacteriophage (3).

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