INHIBITORY EFFECTS OF PUROMYCIN AND FLUOROPHENYLALANINE ON INDUCTION OF THYMIDINE KINASE BY VACCINIA INFECTED L-CELLS*

Saul Kit, D.R. Dubbs, and L.J. Piekarski**

Division of Biochemical Virology
Baylor University College of Medicine, Houston, Texas

Received March 25, 1963

The induction of thymidine kinase in vaccinia infected mouse fibroblast cells (Strain L-M) can be grossly inhibited by pretreating cells grown in phenylalanine deficient medium with p-fluorophenylalanine (FPA) and this inhibition can be prevented by phenylalanine (Kit, Dubbs, and Piekarski, 1962 b; Kit, Piekarski, and Dubbs, 1963). The interpretation of the foregoing experiments is not without ambiguity, however. The FPA effect could be ascribed to: (a) the inhibition of de novo synthesis of thymidine kinase; (b) the incorporation into cell proteins of FPA in place of phenylalanine, yielding abnormal proteins low in enzymatic activity (Baker, Johnson, and Fox, 1958; Cohen and Munier, 1959; Cowie et al., 1959; Creaser, 1955; Halvarson and Spiegelman, 1952; Levintow et al., 1962; Townsley, 1962; and Wecker and Schonne, 1961); and (c) the inhibition of the synthesis of an enzyme needed to "uncoat" vaccinia and render the virus-DNA available for template action (Joklik, 1962).

This study rules out indirect effects of FPA mediated via the uncoating mechanism. In addition, experiments are described in which puromycin was

* Aided by grants from the Leukemia Society Inc., the National Cancer Institute (CA-06829-02, CA-06656-01), the National Science Foundation (GB-620), and the American Medical Association (ERF-71). The experiments were carried out with the expert technical assistance of David Cullop, Barbara Davis, and Sandra Waldman.

** Rockefeller Foundation Postdoctoral Fellow.

used to inhibit protein synthesis. The mechanism of action of puromycin is quite different from that of FPA. Puromycin is thought to inhibit protein synthesis by causing the premature release from the ribosomes of incomplete polypeptide chains (Allen and Zamecnik, 1962; Allfrey et al., 1960; Creaser, 1955; Morris et al., 1960; Nathans and Lipmann, 1961; Rabinovitz and Fisher, 1962; Takeda et al., 1960; and Yarmolinsky and De La Haba, 1959).

As shown in Table 1, 5×10^{-5}M to 10^{-4}M puromycin inhibits the incorporation of DL-tryptophane-2-C^{14} into cell protein by 62 to 91 per cent. This is in contrast to FPA, which, under the conditions employed, only weakly inhibits the incorporation of tryptophane-2-C^{14} into cell protein.

Table 1

INHIBITION BY p-FLUOROPHENYLALANINE (FPA) AND PUROMYCIN OF INCORPORATION OF DL-TRYPTOPHANE-2-C^{14} FOR ONE HOUR INTO THE PROTEINS OF TWO-DAY-OLD L-M CELLS

Expt.	Addition	Concentration mM	Counts per minute per mg protein
1	Control	0	320
	FPA	1.1	214
	FPA plus L-phenylalanine	1.1 1.8	318
2	Control	0	77
	Puromycin	0.05	29
	Puromycin	0.10	7

Expt. 1: L-M cells were incubated for 4 hours in phenylalanine deficient suspension B medium (lacking phenylalanine, lactalbumin hydrolysate, and calf serum), with or without FPA before adding DL-tryptophane-2-C^{14}.

Expt. 2: L-M cells were incubated for 4 hours in normal suspension B medium with or without puromycin before adding DL-tryptophane-2-C^{14}.

Figure 1 shows that in a phenylalanine deficient medium, 200 μg/ml FPA (1.1×10^{-3}M) prevents the induction of thymidine kinase in vaccinia infected

L-M cells. If 300 μg/ml L-phenylalanine (1.8×10^{-3}M) is introduced into the medium at 3 hours PI, the FPA inhibition is reversed and thymidine kinase activity rapidly increases. On the other hand, when FPA is added to the medium 3 hours after the cell suspensions are inoculated with vaccinia, the increase in thymidine kinase activity is halted. If at 6 hours PI, L-phenylalanine is supplied, the induction of thymidine kinase commences once again.

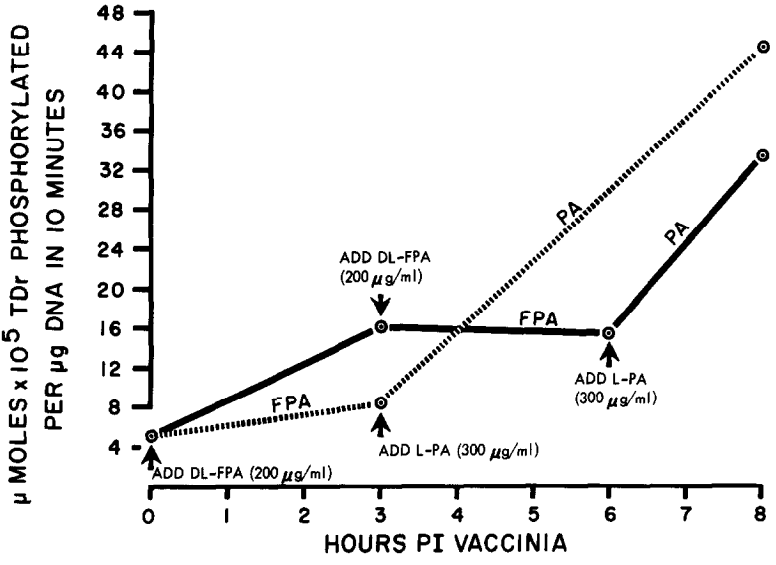

Figure 1: Effect of DL-p-fluorophenylalanine (FPA) on the induction of thymidine kinase by vaccinia infected L-M cells suspended in an L-phenylalanine (L-PA) deficient medium (See Table 1). The input multiplicity was 5 ID_{50} of virus per cell. The experimental procedures and thymidine kinase assays were performed as described previously (Kit, Piekarski, and Dubbs, 1963; Kit and Dubbs, 1963).

The L-M (TK^-) subline of L-cells, which is completely devoid of thymidine kinase activity (Dubbs and Kit, 1963; Kit, Dubbs, Piekarski, and Hsu, 1963), was employed for the experiments with puromycin. Thymidine kinase is rapidly induced following infection of L-M (TK^-) cells either by vaccinia (Figure 2) or herpes simplex virus (Kit and Dubbs, 1963). Puromycin (5×10^{-5}M), when added to the medium at the time of vaccinia infection, markedly depresses thy-

midine kinase induction. Moreover, the addition of puromycin at 2.5 hours PI almost completely prevents the normally observed increase of thymidine kinase activity between 2.5 and 5.5 hours PI (Figure 2).

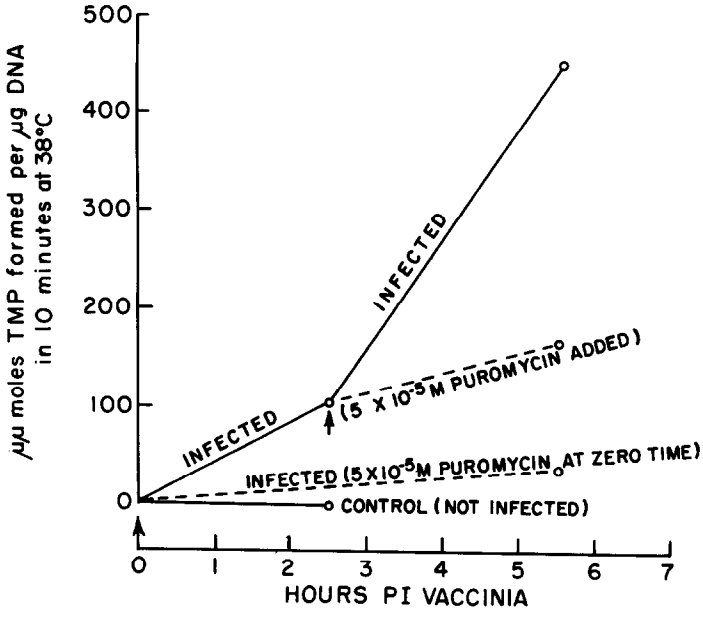

Figure 2: Effect of puromycin on the induction of thymidine kinase activity by vaccinia infected L-M (TK^-) cells.

In control experiments, it was demonstrated that, when incubated with the enzyme in vitro, FPA (2.7×10^{-4}M to 8.7×10^{-4}M) and puromycin (2×10^{-5}M to 10^{-4}M) have no significant effects on thymidine kinase activity.

The cessation of thymidine kinase induction after the addition of FPA or puromycin, respectively, at 3 or 2.5 hours PI, cannot be ascribed to interference with the vaccinia "uncoating" process. Joklik (1962) has shown that uncoating is substantially complete by 2.5 to 3 hours PI. Further, "uncoating enzyme", formed during a two hour interval after cellular infection with a first virus in the absence of FPA, was effective in uncoating a second superinfecting virus, even in the presence of FPA. Moreover, Figure 2 clearly shows that

appreciable enzyme induction can take place by 2.5 hours PI, confirming the fact that uncoating has occurred.

Although a reversing agent equivalent to L-phenylalanine was not available, the interpretation of the puromycin experiments is more straightforward than that of the FPA experiments, in that the formation of abnormal proteins need not be invoked. Also, the use of L-M (TK^-) cells provides a zero background against which to measure the enzyme induction. The experiments reported here with FPA and with puromycin in conjunction with earlier studies contraindicating the activation of "latent" enzymes following virus infection (Kit, Dubbs, and Piekarski, 1962 a; Kit, Piekarski, and Dubbs, 1963) strongly support the thesis that enzyme induction involves the de novo synthesis of thymidine kinase.

REFERENCES

Allen, D. W. and Zamecnik, P.C., Biochim. et Biophys. Acta 55, 865 (1962).

Allfrey, V. G., Hopkins, J. W., Frenster, J. H. and Mirsky, A. E., Ann. of the N. Y. Acad. of Sc. 88, 722 (1960).

Baker, R. S., Johnson, J. E. and Fox, S. W., Biochim. et Biophys. Acta 28, 318 (1958).

Cohen, G. N. and Munier, R., Biochim. et Biophys. Acta 31, 347 (1959).

Cowie, D. B., Cohen, G. N., Bolton, E. T. and De Robichon-Szulmajster, H., Biochim. et Biophys. Acta 34, 39 (1959).

Creaser, E. H., J. of Gen. Microbiol. 12, 288 (1955).

Dubbs, D. R. and Kit, S., Exptl. Cell Res. (1963), in press.

Halvarson, H. O. and Spiegelman, S., J. of Bact. 64, 207 (1952).

Joklik, W. K., Cold Spring Harbor Symposia on Quantitative Biology 27, 199 (1962).

Kit, S. and Dubbs, D. R., Biochem. Biophys. Res. Comm. (1963), in press.

Kit, S., Dubbs, D. R. and Piekarski, L. J., Biochem. Biophys. Res. Comm. 8, 72 (1962 a).

Kit, S., Dubbs, D. R. and Piekarski, L. J., Science 138, 992 (1962 b).

Kit, S., Piekarski, L. J. and Dubbs, D. R., J. of Mol. Biol. 6, 22 (1963).

Kit, S., Dubbs, D. R., Piekarski, L. J. and Hsu, T. C., Exptl. Cell Res. (1963), in press.

Levintow, L., Thoren, M. M., Darnell, J. E. and Hooper, J. L., Virology 16, 220 (1962).

Morris, A., Favelukes, S., Arlinhaus, R. and Schweet, R., Biochem. and Biophys. Res. Comm. 7, 326 (1962).

Nathans, D. and Lipmann, F., Proc. of the Natl. Acad. of Sc., U.S. 47, 497 (1961).

Rabinovitz, M. and Fisher, J. M., J. of Biol. Chem. 237, 477 (1962).

Takeda, Y., Hayashi, S., Nakagawa, H. and Suzuki, F., J. of Biochem. 48, 169 (1960).

Townsley, P. M., Canadian J. of Biochem. and Physiol. 40, 999 (1962).

Wecker, E., and Schonne, E., Proc. of the Natl. Acad. of Sc., U.S. 47, 278 (1961).

Yarmolinsky, M. B. and De La Haba, G. L., Proc. of the Natl. Acad. of Sc., U.S. 45, 1721 (1959).