A NON-FUNCTIONAL ARGININE BIOSYNTHETIC PATHWAY IN POLYOMA INFECTED-MOUSE EMBRYO CELLS

A. L. Winters,[1] R. A. Consigli,[2] and Q. R. Rogers

Division of Biology, Subdivision of Molecular Biology and Genetics
Kansas State University, Manhattan, Kansas and
Department of Physiological Sciences, School of Veterinary Medicine
University of California, Davis, California

Received April 24, 1972

The normal pathway for the biosynthesis of arginine was non-functional in polyoma infected-mouse cells. During viral replication, equimolar concentration of citrulline substituted for arginine but equimolar or ten times equimolar concentration of ornithine did not replace arginine. Purified polyoma virus obtained from infected cultures that were allowed to incorporate citrulline-^{14}C revealed upon amino acid analysis that the label was found exclusively as arginine-^{14}C. When the experiment was repeated using ornithine-^{14}C the label was found as proline-^{14}C and glutamate-^{14}C and not arginine. Both normal and polyoma infected-mouse embryo cells possessed an active ornithine carbamoyltransferase. The inability of ornithine to serve as an arginine precursor appears to be due to the fact that exogenous ornithine is utilized by ornithine-δ-transaminase to synthesize proline and glutamate and not by ornithine carbamoyltransferase to synthesize citrulline.

Successful infection of tissue cultures by herpes virus (1-4), adenovirus (5), SV_{40} (6) and polyoma virus (7) requires arginine in the maintenance medium. It has been suggested (5,7,8) that arginine deprivation effected the inhibition of an arginine-rich structural protein of the virus. However, the reason(s) for the strict arginine requirement of virus infected tissue cultures have not been demonstrated. Thus, an investigation of arginine

---

[1]Recipient of an NIH Predoctoral Fellowship: 5-F_1-GM-30,342. His present address is: Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

[2]Recipient of USPHS Career Development Award: CA12056. This work was supported by a grant from the USPHS: CA07139.

biosynthesis in polyoma virus-infected mouse embryo cultures was performed to gain a fuller understanding of the arginine requirement.

MATERIALS AND METHODS

Primary mouse embryo cell cultures were grown and maintained in Eagle's (MEM) medium containing 5% fetal calf serum and supplemented with antibiotics: penicillin, 200 units/ml; streptomycin, 0.2 mg/ml; and kanamycin, 0.01 mg/ml. Experiments were initiated 72 hr after the cultures appeared confluent. After adsorption of virus (100 p.f.u./cell) for 3 hr, the cultures were maintained in Eagle's medium (with or without arginine) containing 5% dialyzed fetal calf serum supplemented on occasion with citrulline, or ornithine.

Polyoma virus used for amino acid analysis was purified from infected cultures that were maintained in complete Eagle's medium supplemented with 1 μc/ml medium of DL-ornithine-5-^{14}C (sp. act. 8 mc/mmole, Schwarz/Mann) or L-citrulline-ureido-^{14}C (sp. act. 5 mc/mmole, Schwarz/Mann) for five days. Polyoma virus was purified and quantitated as previously described (9). The purified virus from the second cesium chloride density gradient was hydrolyzed with 6N HCl (nitrogen atmosphere) at 121° for 19 hr. After removal of the HCl, the sample was analyzed in a modified Beckman 120 B amino acid analyzer using a three step gradient buffer system. Radioactivity of individual amino acids was determined by passing the effluent from the ion exchange column through a 2.0 ml flow cell packed with crystalline anthracene. The cell was monitored in a Packard scintillation spectrometer which was connected to a rate meter and recorder. The rate meter recorder was set to the same speed as the amino acid analyzer recorder, thus facilitating identification of each radioactive peak. The factor for quantitation of radioactivity was determined using standard citrulline-^{14}C and arginine-^{14}C.

Ornithine carbamoyltransferase (OTC; EC No. 2.1.3.3) activity was determined in normal and polyoma infected cultures. Cell free lysates were prepared by suspending the harvested cells in Tris-acetate buffer (pH 8.0, 10 μmoles) and homogenizing the cells in a Sorvall Omnimixer for 0.5 min

at maximum rheostat setting. The homogenate was centrifuged 1500 x g for 10 min and the supernatant assayed for OTC activity. The 2.0 ml assay mixture containing 1.0 ml of lysate; 25 μmoles L-ornithine; 15 μmoles carbamyl phosphate; 0.1 μmoles $MgCl_2$ was incubated at 37° for 60 min. The reaction was terminated by addition of cold TCA (5% final) and the supernatant fluid assayed for citrulline (10).

RESULTS AND DISCUSSION

In viral replication equimolar concentration of citrulline substituted for arginine whereas equimolar or ten times equimolar concentrations of ornithine did not replace arginine (Fig. 1). Since both citrulline and ornithine are constituents in the arginine biosynthetic pathway, it was of interest to allow infected cells to separately incorporate citrulline-^{14}C

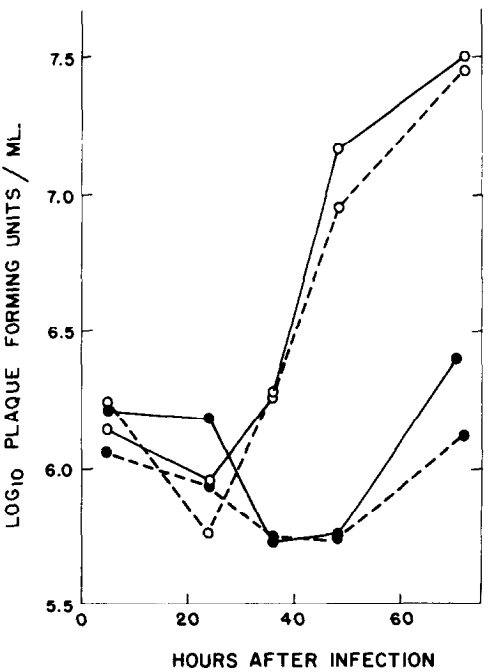

Fig. 1. Growth of polyoma virus in Eagle's medium. 0———0, complete medium; ●———●, medium without arginine; 0------0, medium with 0.6 mM L-citrulline substituted for arginine; ●------●, medium with 0.6 mM or 6.0 mM L-ornithine substituted for arginine.

and ornithine-^{14}C and to analyze the purified polyoma progeny to determine if the label was ultimately incorporated as ^{14}C labeled arginine. Amino acid analysis of these purified viral preparations revealed that radioactive

Table 1. Amino acid analysis[a] of purified polyoma virus.

Amino acid	Moles per 100 Moles		% total radioactivity
	range	average	
aspartic acid	8.4-10.9	10.0	
threonine	9.5-10.4	9.9	
serine	5.2-8.3	6.5	
glutamic acid	7.7-9.6	8.7	7.1[c]
proline	8.5-10.6	9.6	74.4[c]
glycine	7.9-10.9	9.3	
alanine	2.6-5.1	4.1	
valine	8.9-9.2	9.0	
cystine		trace	
methionine		trace	
isoleucine	4.3-4.9	4.7	
leucine	8.6-9.4	8.9	
tyrosine		trace	
phenylalanine	2.2-2.8	2.4	
lysine	8.5-10.4	9.7	
histidine	1.5-2.9	2.1	
ammonia	not calculated		
arginine	4.7-5.7	5.1	100[b]
tryptophan	not determined		

[a] average of three amino acid analyses

[b] virus was grown in L-citrulline-ureido-^{14}C labeled medium

[c] virus was grown in DL-ornithine-5-^{14}C labeled medium. The lack of 100% recovery of ^{14}C input counts was probably due to $^{14}CO_2$ which was not determined.

incorporation from the citrulline-^{14}C labeled cultures was found associated exclusively with the arginine peak of the analysis (Table 1). However, amino acid analysis of the ornithine-^{14}C labeled purified viral preparation demonstrated that the ^{14}C label was not associated with arginine, but primarily with proline (74.4%) and glutamic acid (7.1%). The amino acid analysis of polyoma virus did not reveal any unusual amino acids. In general, the analyses obtained were similar to analyses reported by Murikami et al., (11) and Kass (12), except for the consistently low tyrosine content. The low tyrosine content may be characteristic of the polyoma virus strain used in this investigation.

The above data (Fig. 1, Table 1) raised the possibility that OTC which catalyzes the synthesis of citrulline from ornithine and carbamyl phosphate may be lacking in the polyoma infected-mouse embryo cells. Normal and polyoma infected-mouse embryo cells were then assayed for OTC activity over

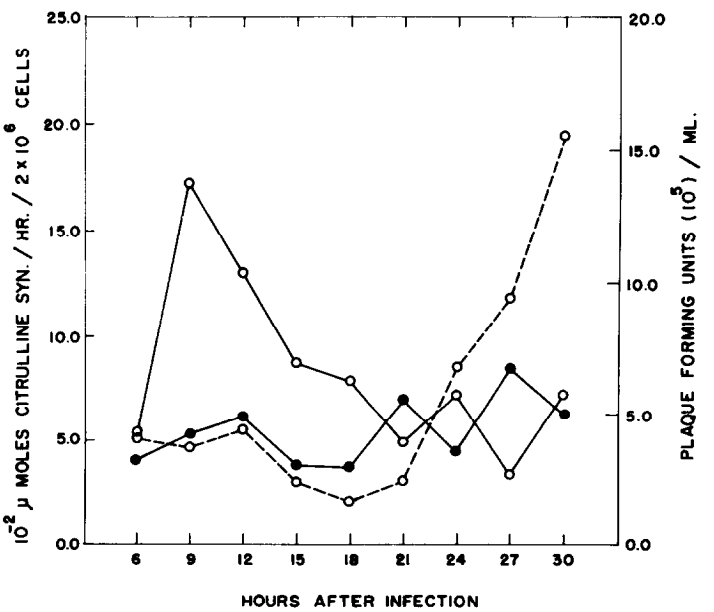

Fig. 2. Ornithine carbamoyltransferase activity in normal and polyoma infected-mouse embryo cells. 0———0 infected; ●———● normal; and 0------0 p.f.u.

a 30 hr multiplication cycle. It was found that both normal and polyoma infected cells possessed OTC activity (Fig. 2). The three-fold increase in enzyme activity observed 9 hr post infection can not be explained at this time.

The reason for the failure to synthesize arginine in this system appears to be at the intracellular substrate (ornithine) level. The initial enzyme OTC appeared non-functional since exogenous ornithine was not used to synthesize citrulline but instead was utilized to synthesize proline and glutamic acid via the ornithine δ transaminase (OTA) pathway. It was demonstrated in Neurospora that the OTA pathway handled exogenous ornithine and endogenous ornithine was channeled to OTC for arginine biosynthesis (13, 14). Apparently, polyoma infected-mouse cells lack an intracellular source of ornithine since the removal of arginine from maintenance medium produced total suppression of infectious progeny (7). An alternate possibility which might result in a non-functional pathway may be due to a lack of carbamyl phosphate synthetase required for channeling carbamyl phosphate into arginine biosynthesis. This may be a reality since it was demonstrated in eucaryotic cells that two carbamyl phosphate synthetases existed, one for pyrimidine biosynthesis and one for arginine biosynthesis (15 - 21).

It is concluded from the data presented that the arginine biosynthetic pathway is non-functional in polyoma infected-mouse embryo cells, thus producing a stringent requirement for exogenous arginine.

References

1. Tankersley, R. W. J. Bacteriol., 87, 609 (1964).
2. Becker, Y., Olshevsky, U., and Levitt, J. J. gen. Virol. 1, 471 (1967).
3. Inglis, V. B. J. gen. Virol. 3, 9 (1968).
4. Spring, S. B., Roizman, B. and Spear, P. G. Virology 38, 710 (1969).
5. Rouse, H. C., and Schlesinger, R. W. Virology 33, 513 (1967).
6. Goldblum, N., Ravid, Z., and Becker, Y. J. gen. Virol. 3, 143 (1968).
7. Winters, A. L., and Consigli, R. A. J. gen. Virol. 10, 53 (1971).

8. Russell, W. C., and Becker, Y. Virology 35, 18 (1968).

9. Consigli, R. A., Minocha, H. C., and Abo-Ahmed, H. J. Bacteriol. 92, 789 (1966).

10. Koritz, S. B., and Cohen, P. O. J. Biol. Chem. 209, 145 (1954).

11. Murakami, W. F., Fine, R., Harrington, M. R., and Ben Sassen, Z. J. Mol. Biol. 36, 153 (1968).

12. Kass, S. J. J. Virol. 5, 381 (1970).

13. Davis, R. H. J. Bacteriol. 96, 389 (1968).

14. Davis, R. H., and Mora, J. J. Bacteriol. 96, 383 (1968).

15. Lacroute, F., Pierard, A., Grenson, M., and Wiame, J. M. J. gen. Microbiol. 40, 127 (1965).

16. Hirsch, M. L. C. R. Acad. Sci. (Paris) 267, 1473 (1968).

17. Lan, S. J., Sallach, H. J., and Cohen, P. P. Biochemistry 8, 3673 (1969).

18. Nakanishi, S., Ito, K., and Tatibana, M., Biochem. Biophys. Res. Commun. 33, 774 (1968).

19. Hager, S. E., and Jones, M. E. J. Biol. Chem. 242, 5674 (1967).

20. Williams, L. G., and Davis, R. H. J. Bacteriol. 103, 335 (1970).

21. Williams, L. G., Bernhardt, S. A., and Davis, R. H. J. Biol. Chem. 246, 973 (1971).