THE EFFECT OF EXOGENOUS DEOXYRIBONUCLEOSIDES ON THYMIDINE INCORPORATION IN T4-INFECTED CELLS

Robert J. MELAMEDE and Susan S. WALLACE
New York Medical College, Department of Microbiology, Valhalla, New York 10595, USA

Received 21 December 1977

1. Introduction

Wild-type *Escherichia coli* do not efficiently incorporate exogenously added thymine or thymidine unless deoxyribosyl donors are also supplied [1–4]. Thymidine incorporation can also be increased in these cells by inhibiting thymidine phosphorylase thus preventing thymidine breakdown [3]. In contrast, T4-infected cells readily incorporate exogenous and thymidine, apparently due to an intracellular accumulation of deoxyribosyl donors [5]. The latter are probably breakdown products of host DNA. Catabolism of deoxyribose-1-P is inhibited after T4 infection compared to uninfected cells, or cells infected with T-odd phages [6]. It seems clear, since about 30% of newly synthesized T-even phase DNA nucleotide is derived from host chromosome breakdown products [7], that salvage pathways for nucleotide biosynthesis are of importance in this system.

During the course of our studies on the patterns of DNA synthesis in the T4 non-lethal recombination deficient mutants [8] we observed that the apparent T4 DNA synthetic rate was critically dependent upon the concentration of label supplied to measure it. Also, deoxyadenosine, sometimes used in phage experiments to 'stimulate' uptake of label was found to inhibit incorporation under certain circumstances. Variations in experimental conditions have in many cases led to conflicting interpretations of T4 DNA synthetic rates. Specifically, in the case of the T4*x* and *y* mutants, one group reports T4*x* to synthesize DNA at the same rate as wild-type T4 [9], while others [10,11] claim T4*x* and *y* to be 'DNA arrest' mutants. In this communication evidence is presented that thymidine incorporation in T4-infected cells is affected by exogenously-added deoxyribonucleosides in at least two separate ways. First, when low concentrations of labeled thymidine are used, incorporation is inhibited by exogenous pyrimidine deoxyribonucleosides and deoxyadenosine. Secondly, when high concentrations of labeled thymidine are supplied early in infection, incorporation of this label late in infection is stimulated by exogenous deoxyadenosine.

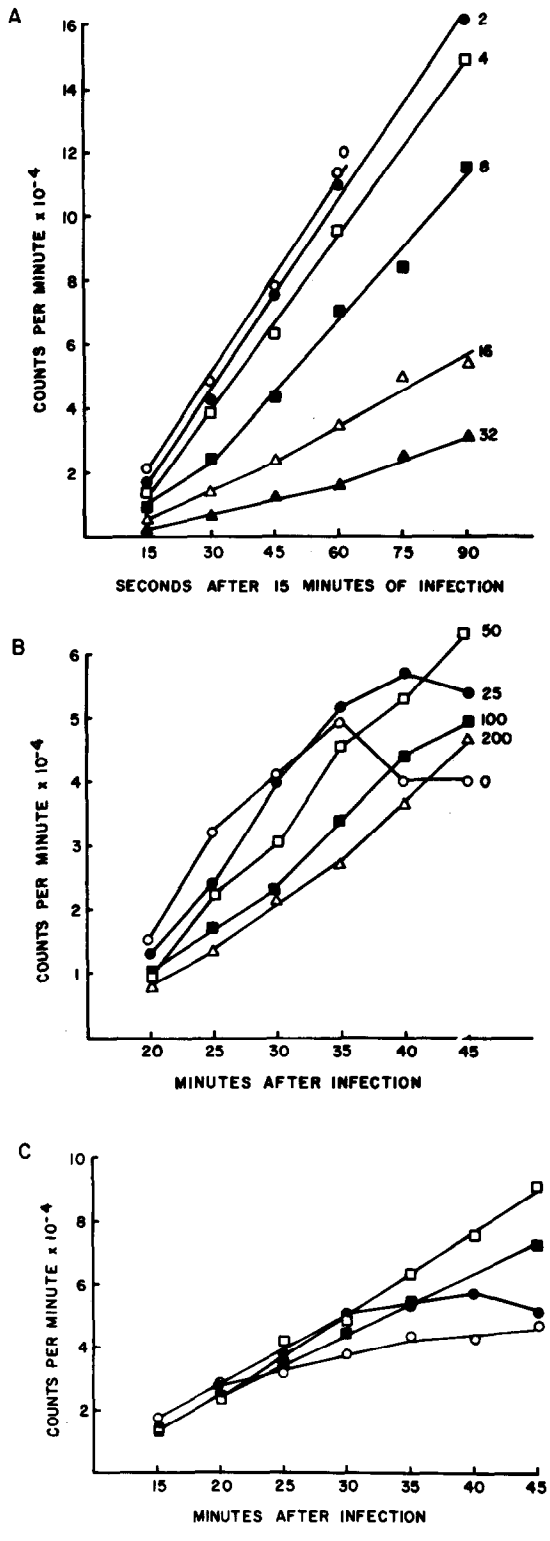
2. Materials and methods

The preparation of T4-infected cells and incorporation of [^{3}H]thymidine and [^{3}H]thymidine into the acid-soluble fraction has been detailed [9]. [^{3}H]-Deoxyadenosine was obtained from International Chemical and Nuclear, and incorporation was measured as described for [^{3}H]thymidine [8]. Unlabeled deoxyribonucleosides were purchased from P&L Biochemicals. In these experiments, 0 time corresponds to the addition of phage to the bacteria. All incubations were performed at 37°C.

3. Results and discussion

In fig.1A it can be seen that incorporation of [^{3}H]thymidine at conc. 0.19 μg/ml can be inhibited by low concentrations (4–32 μg/ml) of exogenously-added deoxyadenosine. In this experiment, incorporation into the acid insoluble fraction was measured at 15 s intervals after 15 min T4 infection. Higher concentrations (50–200 μg/ml) of deoxyadenosine are

 Elsevier/North-Holland Biomedical Press



needed to inhibit the incorporation of 15 μg [^{3}H]-thymidine/ml as shown in fig.1B. This experiment is similar to the one described for fig.1A except that incorporation was measured at 5 min intervals after 15 min T4 infection. Note that at the time when incorporation ceases at 20 min after the addition of label in the absence of deoxyadenosine, the presence of exogenous deoxyadenosine allows for continued incorporation. Figure 1C depicts the long-term incorporation of 25 μg [^{3}H]thymidine/ml in the absence or presence of deoxyadenosine. In one case, tritium-labeled precursor and deoxynucleoside were added with the phage; while in the other they were added 4 min after the phage. When [^{3}H]thymidine is added at the time of infection, incorporation continues until 20 min infection. When [^{3}H]thymidine is added 4 min after infection incorporation continues until 30 min infection. In both cases, the presence of 200 μg deoxyadenosine/ml appears to be slightly inhibitory early in infection and stimulates incorporation at the time when incorporation usually levels off. Other concentrations tested, 50 μg and 100 μg deoxyadenosine/ml (data not shown), also stimulate [^{3}H]-thymidine incorporation at this time.

Figure 2A shows that incorporation of [^{3}H]-thymidine (0.24 μg/ml) at 15 min after infection with T4 is inhibited by 0.125 mM deoxycytidine, deoxyuridine and deoxyadenosine but not 0.125 mM deoxyguanosine. Similarly, in fig.2B it can be seen that incorporation of 0.27 μg [^{3}H]deoxyadenosine/ml is inhibited by all deoxyribonucleosides tested (0.125 mM), but deoxyguanosine is somewhat less effective.

Although little is known about mechanisms of nucleoside transport in T4-infected cells, studies with uninfected *E. coli* indicate that pyrimidine nucleosides and adenosine (or deoxyadenosine) share a common

Fig.1. Effect of deoxyadenosine on [^{3}H]thymidine incorporation in T4-infected cells. (1A) [^{3}H]thymidine, 33 μCi/0.19 μg/ml, was added 15 min after infection. Deoxyadenosine was added at the same time at the concentrations in μg/ml indicated in the graph. (1B) Same as 1A except [^{3}H]-thymidine, 33 μCi/15 μg/ml, was used. (1C) [^{3}H]Thymidine, 33 μg/ml, was added either at the time of infection (○), or 4 min after infection (●). Deoxyadenosine, 200 μg/ml, was added at the time of infection (□), or 4 min after infection (■).

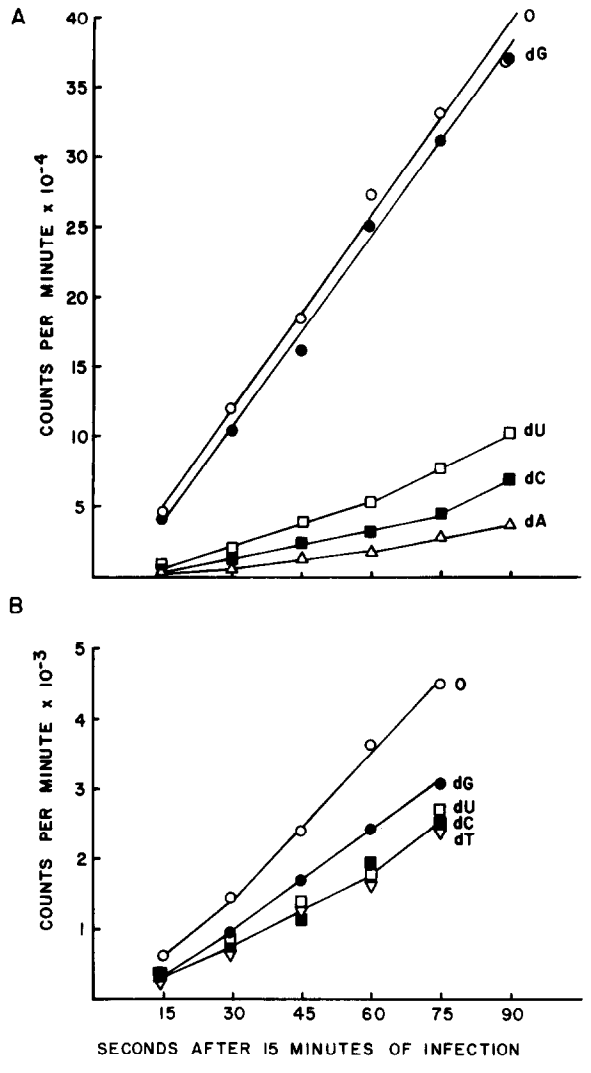


Fig.2. Effect of deoxyribonucleosides on [^{3}H]thymidine and [^{3}H]deoxyadenosine incorporation in T4-infected cells. Label and 0.125 mM deoxyribonucleosides were added 15 min after infection. Symbols: (○) no exogenous deoxyribonucleoside; (●) deoxyguanosine; (□) deoxyuridine; (■) deoxycytidine (△) deoxyadenosine; (▽) deoxythymidine. (2A) [^{3}H]Thymidine incorporation, 50.8 μCi/0.24 μg/ml. (2B) [^{3}H]Deoxyadenosine incorporation 16.5 μCi/0.27 μg/ml.

channel of transport, whereas guanosine (or deoxyguanosine) does not [12–15]. We therefore have interpreted these results as indicating that exogenously supplied deoxycytidine, deoxyuridine, and deoxyadenosine inhibit thymidine transport whereas exogenously supplied deoxyguanosine does not. Correspondingly, exogenously supplied pyrimidine deoxynucleosides could inhibit deoxyadenosine transport. Deoxyguanosine also inhibits deoxyadenosine incorporation, however to a lesser extent. Thus, it might appear that in T4-infected cells, thymidine seems to be taken up by the primary transport system established for uninfected cells [12–15]. Since deoxyguanosine does inhibit [^{3}H]deoxyadenosine incorporation, the latter must be taken up in T4-infected cells in a more complex, or less well-understood manner. It is possible, that exogenous deoxyribonucleosides are exerting their effect by inhibiting the direct phosphorylation of thymidine by thymidine kinase or possibly at a subsequent step. We feel that this is less likely than the above interpretation for the following reasons:

(i) Deoxyadenosine is a competitive inhibitor of thymidine phosphorylase [3] and should cause an accumulation of thymidine precursor and thus stimulate incorporation

(ii) Deoxycytidine has been shown to have no effect on host thymidine kinase

(iii) TMP and GMP are common substrates for T4-induced deoxyribonucleotide kinase [16] and deoxyguanosine does not inhibit thymidine incorporation as measured above.

Although in the above short-term experiments where low concentrations of thymidine were used, incorporation measurements could have reflected the direct phosphorylation of thymidine by thymidine kinase, the conversion of 2.5 μg thymidine/ml by thymidine phosphorylase to thymine and deoxyribose-1-phosphate [5] is complete 5 min after thymidine addition to T4-infected cells at 3 min infection. Incorporation of thymine into T4-infected cells [5] can be stimulated by the simultaneous addition of deoxyribonucleosides. Similar results depicting the stimulatory effect of exogenous deoxyribonucleosides on thymine incorporation are shown in fig.3. Thymidine does not stimulate thymine incorporation, and would not be expected to since thymidine breakdown to thymine and deoxyribose-1-phosphate would dilute the radioactive pool. Figure 4 depicts an experiment where 10 μg/ml thymidine was added at 10 min after T4 infection. Under these conditions, incorporation levels off after 20 min infection. If 200 μg/ml deoxyadenosine is added at 22 min infection,

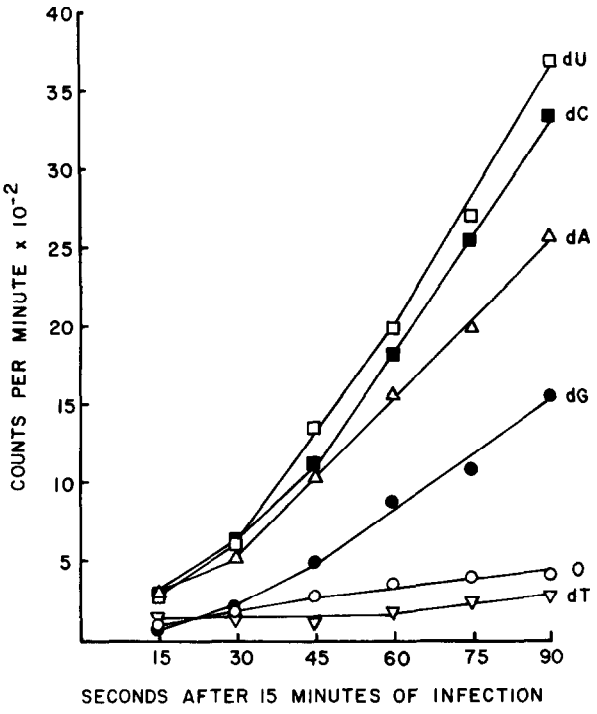


Fig.3. Effect of deoxyribonucleosides on [^{3}H]thymine incorporation in T4-infected cells. [^{3}H]thymine, 33 μCi/2 μg/ml, and various deoxyribonucleosides as indicated in the graph (80 μg/ml), were added 15 min after infection.

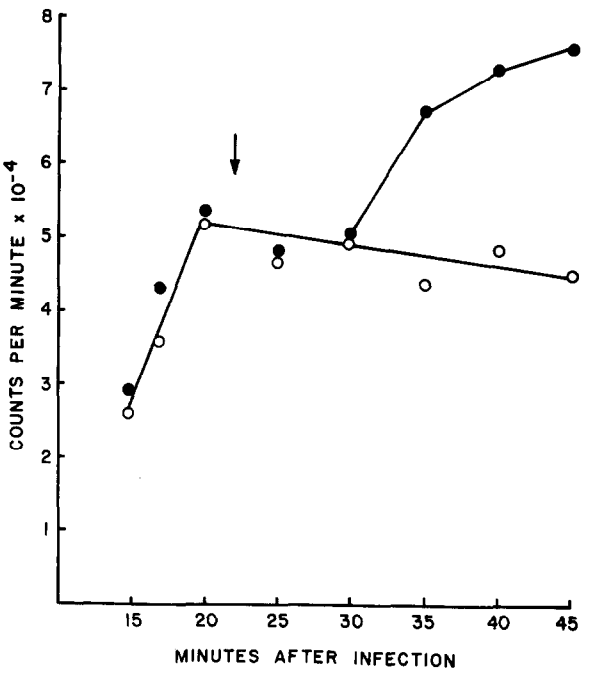


Fig.4. Reincorporation of ^{3}H-label after addition of deoxyadenosine to T4-infected cells. [^{3}H]Thymidine, 33 μCi/10 μg/ml, was added 10 min after infection. Deoxyadenosine, 200 μg/ml, was added at 22 min after infection. Symbols: (○) no deoxyadenosine; (●) 200 μg/ml deoxyadenosine.

a further increase of incorporation is observed. Similar results were obtained when deoxyadenosine was added at 30 min infection (data not shown). We have interpreted these data as indicating that thymidine incorporation ceases when the intracellular pools of deoxyribosyl donors are depleted, and that deoxynucleosides present at this time stimulate incorporation by replenishing these pools. This phenomenon can explain the stimulation of thymidine incorporation when exogenous deoxyribonucleosides are present at times when incorporation usually ceases, as seen in fig.1B,C. Stimulation by exogenous deoxyribonucleosides under these conditions is analogous to the stimulation observed when incorporation of thymine is directly measured as in fig.3, or to the stimulation observed when endogenous deoxyribosyl pools are normally small as in uninfected *E. coli* [1–4]. Thus, in T4-infected cells, incorporation of low concentrations of [^{3}H]thymidine into the acid-insoluble fraction is inhibited by deoxyribonucleosides involved in a common pathway(s) of transport; deoxyadenosine, deoxycytidine, deoxyuridine but not deoxyguanosine. The effect is concentration dependent, that is, the higher the concentration of labeled thymidine supplied, the higher the concentration of added deoxyadenosine necessary to inhibit its uptake. Similar results have recently been reported for uninfected *E. coli* [15]. Incorporation of deoxyadenosine behaves in a somewhat similar manner. In contrast, incorporation of thymine in T4-infected cells is stimulated by exogenous deoxyribonucleosides as has been found [5]. When the T4-infected cells have converted [^{3}H]thymidine to [^{3}H]thymine plus deoxyribose-1-P, and when intracellular supplies of the latter have been exhausted, exogenously supplied deoxyribonucleosides stimulate incorporation by supplying deoxyribosyl donors to the [^{3}H]thymine product of [^{3}H]thymidine as when incorporation of the former is directly measured.

In conclusion, in T4-infected cells, exogenously

supplied deoxyribonucleosides inhibit thymidine incorporation when short-term, high specific activity, low thymidine concentrations are used for labeling. Deoxyribonucleosides stimulate long-term incorporation when low specific activity, high thymidine concentrations are used for labeling. Therefore, depending on the measurement desired, careful examination of the effects of exogenously added deoxyribonucleosides on thymidine incorporation in T4-infected cells is required.

Acknowledgement

This research was supported by grant number CA 21342 awarded by the National Cancer Institute, US DHEW.

References

[1] Boyce, R. P. and Setlow, R. B. (1962) Biochim. Biophys. Acta 61, 618–620.
[2] Kammen, H. P. (1967) Biochim. Biophys. Acta 134, 301–311.
[3] Budman, D. R. and Pardee, A. B. (1967) J. Bacteriol. 94, 1546–1550.
[4] Jensen, F., Leer, J. C. and Nygaard, P. (1973) Eur. J. Biochem. 40, 345–354.
[5] Kammen, H. O. and Strand, M. (1967) J. Biol. Chem. 242, 1854–1863.
[6] Munch-Petersen, A. and Schwartz, M. (1972) Eur. J. Biochem. 27, 443–447.
[7] Kozloff, L. M. (1953) Cold Spring Harbor Symp. Quant. Biol. 18, 209–220.
[8] Melamede, R. J. and Wallace, S. S. (1977) J. Virol. 24, 28–40.
[9] Shimizu, K. and Sekiguchi, M. (1974) J. Virol. 13, 1–8.
[10] Dewey, M. J. and Frankel, F. R. (1975) J. Virol. 68, 387–401.
[11] Cunningham, R. P. and Berger, H. (1977) Virology 80, 67–82.
[12] Doskocil, J. and Paces, V. (1968) Biochem. Biophys. Res. Commun. 30, 153–158.
[13] Komatsu, Y. (1963) Biochim. Biophys. Acta 330, 206–221.
[14] Mygind, B. and Munch-Petersen, A. (1975) Eur. J. Biochem. 59, 365–372.
[15] Vyacheslavov, L. G. and Mosevitsky, M. I. (1977) Eur. J. Biochem. 74, 313–318.
[16] Duckworth, D. H. and Bessman, M. J. (1967) J. Biol. Chem. 242, 2877–2885.