The effect of nucleosides on the phosphorolysis of thymidine by normal and phage-infected cells of E. coli

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The importance of labeling of DNA with radioactive thymine and thymidine has inspired many investigations on this subject. (Munch-Petersen, 1967; Kammen and Strand, 1967). Thymidine usually is a much better precursor than thymine, but is often degraded to thymine by thymidine phosphorylase (Rachmeler et al., 1961). This is an inducible enzyme, but a considerable constitutive level is usually present in bacterial cells sufficient to cause a rapid cleavage of thymidine before the induced synthesis of the enzyme becomes apparent. For the same reason even the cells infected with phage T 4 degrade thymidine (Kammen and Strand, 1967), in spite of the absence of synthesis of bacterial inducible enzymes.

Boyce and Setlow (1962) observed that thymidine phosphorolysis in cultures of E. coli can be inhibited by deoxyadenosine. We have found that many other deoxynucleosides as well as ribonucleosides have the same effect. The phenomenon described here is distinct from that observed by Kammen and Strand (1967); in our experiments the added nucleosides or deoxynucleosides inhibit the phosphorolysis of thymidine and the high efficiency of incorporation is therefore due to a protection of thymidine rather than to the stimulation of thymine uptake.

Methods. The bacteria, E. coli B, were cultivated with shaking in a medium according to Spizizen (1958), containing 0.5% casamino acids. At a density 3 to 5 x 10^8 cells/ml the cultures were infected with a purified suspension of phage T4 (7.6 x 10^{11}/ml) in the presence of tryptophane (25 µg/ml), using 10 phage particles per bacterial cell. The incorporation of [^{3}H] thymidine (Radiochemical Centre, Amersham; specific activity 6 C/mmole) was followed by precipitating the samples with 5% trichloroacetic acid, filtering on nitrocellulose membrane filters and counting on a Frieseke-Hoepfner gas flow counter. In order to determine the extent of thymidine phosphorolysis the samples of the culture fluid were analysed by paper chromatography (Hotchkiss, 1948). The chromatograms were scanned using the gas flow counter fitted with an automatic recorder of radioactivity.

Results. In uninfected as well as in phage-infected cultures of E. coli the incorporation of thymidine (initial concentration 1-2 µg/ml) soon ceases as a result of its phosphorolysis to thymine. Addition of some ribo- or deoxyribonucleosides (usually 50 µg/ml) prevents the cessation of thymidine incorporation. Analyses of the medium, similar to that given in Tab. I and Fig. 1, indicate that the phosphorolysis of thymidine is inhibited by the added nucleosides. Pyrimidine nucleosides and deoxynucleosides as well as deoxyadenosine and adenosine are equally effective; deoxyguanosine is nearly inactive, increasing the level of the plateau slightly (Fig. 1). 6-Azacytidine and 6-azauridine are without effect, but 5-azacytidine is a strong inhibitor of phosphorolysis (Tab. II). In its presence uninfected cells incorporate thymidine normally (Doskočil, Pačes and Šorm, 1967), while in phage-infected cells

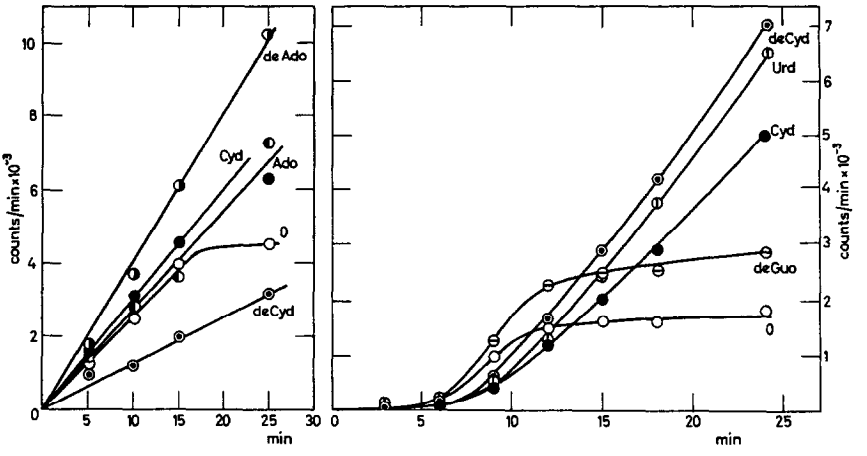

Fig. 1. Effect of Nucleosides on the Incorporation of ^{3}H Thymidine

Initial concentration of thymidine was 2 µg/ml. Nucleosides and deoxynucleosides were added at the concentration of 50 µg/ml. Left: uninfected cells; 25 min after addition of thymidine the media were analysed by chromatography and following values of conversion of thymidine were found: no addition, 100%; adenosine and cytidine, 0%; deoxyadenosine, 21%; and deoxycytidine, 15%. Right: cells infected with phage T4. Curves of incorporation of thymidine in the presence of deoxyuridine and deoxyadenosine (not shown) were similar to those of cytidine and uridine, while the addition of 6-azauridine and 6-azacytidine gave curves similar to that obtained without any added nucleoside. Phage titers obtained were 1.4 to 4.0 x 10^{10}/ml.

Table I

Conversion of Thymidine to Thymine by Infected and Uninfected Bacteria

One portion of the culture was infected with phage T4. 7 min after infection, both infected and uninfected portions (15 ml each) were filtered on a Millipore filter and resuspended in 3 ml of Spizizen medium without glucose. Thymidine (4 µC, 5 µg/ml) was added and the mixtures incubated, in separate 1-ml portions with added nucleosides, at 37°C without aeration for 5 min. The media were then analyzed by paper chromatography.

Additions	Percent conversion of thymidine	
	Infected bacteria	Uninfected bacteria
none	100	100
Cytidine, 200 µg/ml	14.2	20.8
Deoxycytidine, 200 µg/ml	11.8	17.7

incorporation of thymidine is strongly inhibited (Doskočil and Šorm, 1967).

In infected cells the added nucleosides and deoxynucleosides have small effects on the rate of incorporation of thymidine. This is evident from Fig. 1, when the slopes of the curves in the interval from 7 to 12 min, (i.e. before thymidine has

Table II

Inhibition of Phosphorolysis of Thymidine by 5-Azacytidine

[^{3}H]Thymidine (1 μC, 2 μg/ml) was added to 8 ml of a culture and 4 ml of it were infected with phage T4. Portions (2 ml) were taken from both infected and uninfected cultures and 5-azacytidine (50 μg/ml) was added. 15 min later the media were analyzed by paper chromatography.

Additions	Percent conversion of thymidine	
	Infected bacteria	Uninfected bacteria
none	100	90.1
5-Azacytidine, 50 μg/ml	18.5	22.9

decomposed) are compared. In uninfected cells, deoxyadenosine increases the rate of incorporation of thymidine by 50%, while deoxycytidine reduces this rate about two times (Fig. 1). The ribonucleosides prevent the phosphorolysis of thymidine, but are without effect on the rate of its incorporation.

The inhibition of phosphorolysis by nucleosides and deoxynucleosides is observed only with intact cells. In a cell-free system nucleosides have no appreciable effect (Tab. III). Similarly 5-azacytidine, a strong inhibitor *in vivo*, is inactive in a cell-free system (Doskočil, Pačes and Šorm,1967).

Table III

Effect of Cytidine and Adenosine on the Phosphorolysis of Thymidine in a Cell-free Extract

To a bacterial culture (5 x 10^8 cells/ml) thymidine (50 µg/ml) was added as inducer of thymidine phosphorylase. 20 min later the bacteria were harvested by centrifugation, resuspended in 1 ml 0.1M phosphate, pH 6.0 and sonicated. The reaction mixture contained [^{3}H] thymidine, (5 µC, 1 µg), the nucleosides (25 µg) and the cell-free extract (0.02 ml) in 0.5 ml 0.1M phosphate, pH 6.0. Samples (0.1 ml) were taken in 10 min intervals and chromatographed.

Additions	Per cent conversion of thymidine	
	after 10 min	after 20 min
none	52.1	73.8
Cytidine, 50 µg/ml	48.4	62.2
Adenosine, 50 µg/ml	48.5	68.7

Discussion. The specificity pattern of the inhibitory action of nucleosides on thymidine phosphorolysis in vivo is entirely different from that of substrates and inducers of thymidine phosphorylase (Razzell and Casshyap, 1964). Deoxycytidine, deoxyuridine as well as the corresponding ribonucleosides are in vivo inhibitors, but only deoxyuridine is the substrate of the enzyme. Deoxyguanosine and deoxyadenosine are inducers, but only the latter has an inhibitory activity. The inhibition is limited to whole cells, infected or uninfected, while no comparable inhibition can be detected in cell-free extracts. Therefore direct interaction of the nucleosides with thymidine phosphorylase is probably not the cause of inhibition. The findings suggest the presence of a binding site on the surface of the cells, where thymidine has to be bound before the phosphorolysis can take place; this binding site could be susceptible to blocking by nucleosides. On the other hand the nucleosides or deoxynucleosides have surprisingly little effect on the rate of thymidine incorporation, especially

in phage-infected cells. Deoxycytidine, reducing the rate of incorporation of thymidine in uninfected bacteria, is without such effect in phage-infected cells, in spite of being a potential precursor of phage DNA.

These findings open a new way for improving the efficiency of labeling of DNA, namely by protecting radioactive thymidine against phosphorolytic breakdown by addition of a nucleoside.

A more detailed account concerning the action of pyrimidine nucleoside analogs will be published in Collection of Czechoslovak Chemical Communications.

References

Boyce, R.P., and Setlow, R.B., Biochim. Biophys. Acta, 61, 618 (1962).
Doskočil, J., Pačes, V., and Šorm, F., Biochim. Biophys. Acta, 145, 779 (1967).
Doskočil, J., and Šorm, F., Biochim. Biophys. Acta, 145, 771 (1967).
Hotchkiss, R.D., J. Biol. Chem., 175, 315 (1948).
Kammen, H.O., and Strand, M., J. Biol. Chem., 242, 1854 (1967).
Munch-Petersen, A., Biochim. Biophys. Acta, 142, 228 (1967).
Rachmeler, M., Gerhart, J., Rosner, J., Biochim. Biophys. Acta, 49, 222 (1961).
Razzell, W.E., and Casshyap, P., J. Biol. Chem., 239, 1789 (1964).
Spizizen, J., Proc. Natl. Acad. Sci. U.S., 44, 1072 (1958).