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Effect of Adenosine and Deoxyadenosine on the Incorporation and Breakdown of Thymidine in *Escherichia coli* K-12

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Deoxyadenosine (AdR) and adenosine (AR) enhance the incorporation of thymidine (TdR) into bacterial deoxyribonucleic acid (DNA) by the inhibition of TdR phosphorolysis in vivo. Neither of the purine nucleosides has an effect on the reaction catalyzed by TdR phosphorylase in vitro. AdR induces TdR phosphorylase and both purine nucleosides induce purine nucleoside phosphorylase. AR can stimulate uptake of more TdR into bacterial DNA than AdR.

The incorporation of thymidine (TdR) into deoxyribonucleic acid (DNA) of either *Escherichia coli* or *Bacillus subtilis* is prevented by the induction of a series of enzymes involved in the catabolism of ribonucleosides (1, 11). The first enzyme in this series, TdR phosphorylase (EC 2.4.2.4), catalyzes the following reaction: thymidine + inorganic orthophosphate $\rightleftharpoons$ thymine + deoxyribose-1-P (14, 16). TdR is readily incorporated in a mutant strain lacking TdR phosphorylase activity (8, 9). It has been known for a while that the addition of deoxyadenosine (AdR) prevents the phosphorolysis of TdR enabling its rapid incorporation into DNA of wild-type cells. It was suggested that AdR can transfer its deoxyribose moiety to rebuild TdR (4, 10, 13). However, it was later reported (3, 6, 7) that uridine and adenosine (AR) also promote TdR incorporation; these two ribonucleosides cannot donate deoxyribose and their effect is by the direct inhibition of TdR phosphorylase.

In this study we compare the effect of AdR and AR on the incorporation of TdR, on TdR phosphorolysis, and on the induction of the enzymes TdR phosphorylase and purine nucleoside phosphorylase (EC 2.4.2.1). The latter enzyme catalyzes the phosphorolysis of AdR and AR (12).

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The two bacterial strains used were K-10, an HfrC wild-type derivative of *E. coli* K-12, and K12SH-28, a thymidine phosphorylase-negative mutant of K-12 (8, 9). The cells were grown in glucose M9 minimal medium (17) in a shaker bath at 37 C.

Chemicals. TdR, AR, and AdR were obtained from Sigma Chemical Co., St. Louis, Mo. ^{3}H-TdR was obtained from The Radiochemical Centre, Amersham, England. ^{3}H-thymine was obtained from Schwarz BioResearch Inc., Orangeburg, N.Y.

Radioactive assays. To determine the incorporation of isotopes into acid-precipitable material, 0.2-ml samples of the labeled cultures were transferred to 1 ml of 5% cold trichloroacetic acid. After at least 30 min, the precipitate was collected on membrane filters (0.45 μm pore size) and counted in a toluene-scintillation solution in a Packard Tri-Carb scintillation counter.

For the determination of TdR and thymine in the supernatant, 0.2-ml samples were transferred to 0.2 ml of 10% cold trichloroacetic acid containing 1 mg of thymine and 10 mg of thymidine per ml as carriers. After at least 30 min, the samples were centrifuged for 5 min in a microcentrifuge (model 152; Beckman Instruments, Inc., Fullerton, Calif.). Ascending chromatography was carried out by using Whatman no. 4 filter paper with 20 μliters of the supernatant solution, as described by Bodmer and Grether (3). Results are expressed as per cent counts per minute remaining as TdR.

Enzyme assays. The cells were centrifuged and washed once either with 0.1 M arsenate buffer (*p*H 6.0) or with 0.1 M tris(hydroxymethyl)aminomethane buffer (*p*H 7.2) and resuspended in 0.1 volume so that the cellular concentration was approximately 10^9 cells/ml. The cellular suspension was treated for 2 min at position 5 in a Branson model W 185D sonic oscillator. After centrifugation, the supernatant fluid was used for enzyme assays. Thymidine phosphorylase was assayed either by arsenolysis as described by Razzell (15) or by phosphorolysis as described by Budman and Pardee (5). With arsenolysis, the assay mixture, in a total volume of 0.4 ml of 0.1 M sodium arsenate buffer (*p*H 6.0), contained 0.1 ml of 32 mM TdR and 0.1 ml of extract; after incubation at 45 C, 0.1 ml of the assay mixture was transferred to 1.9 ml of 0.3 N

NaOH and the thymine produced was determined by the increase in optical density at 300 nm. The assay with phosphorolysis was identical except that it was carried out in phosphate buffer (*p*H 5.9). A unit of enzyme activity is equivalent to the formation of 1 μmole of thymine per min. Purine nucleoside phosphorylase was assayed in arsenate buffer by the method of Tsuboi and Hudson (18) with 5 mM deoxyguanosine as substrate. A unit of enzyme activity is equivalent to the formation of 1 μmole of guanine per min.

RESULTS

Effect of AdR and AR on thymine incorporation. Figure 1 shows the effect of AdR and AR on the incorporation of radioactive-labeled thymine into the acid-precipitable material. Only AdR can stimulate thymine incorporation, as it can donate to thymine the deoxyribose moiety which is lacking in the cellular pool, to form TdR (4, 10, 13). AR, which itself cannot promote thymine uptake, causes partial inhibition of the uptake promoted by AdR. It should be pointed out that neither AdR nor AR has an effect on the bacterial growth rate.

Effect of AdR and AR on TdR incorporation and breakdown in vivo. Figure 2A shows the incorporation of labeled thymidine into the acid-precipitable material; here both purine nucleosides stimulate incorporation. Figure 2B shows TdR breakdown to thymine in the supernatant of the same experiment. In the control, TdR is degraded to thymine within 30 min at which time incorporation ceases (3, 14). In the cultures treated either with 1 mM AdR or 1 mM AR TdR, phosphorolysis is strongly inhibited for at least 90 min by the former and 120 min (approximately two generations) by the latter. The inhibition is eventually overcome, probably because of the induction of the enzyme purine nucleoside phosphorylase (EC 2.4.2.1) which cleaves either purine nucleoside to the free base (12; *see below*).

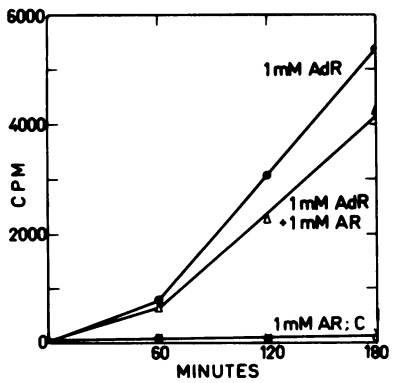

FIG. 1. *Incorporation of thymine by E. coli K-10 treated with AdR and AR. Exponentially growing cells of strain K-10 were treated with* ^{3}*H-thymine (2 mg/ml; 0.87 μc/ml) and divided into four subcultures treated with AdR AR, or both AdR and AR; C represents the untreated control. At various intervals, samples were withdrawn for determination of radioactive acid-precipitable material.*

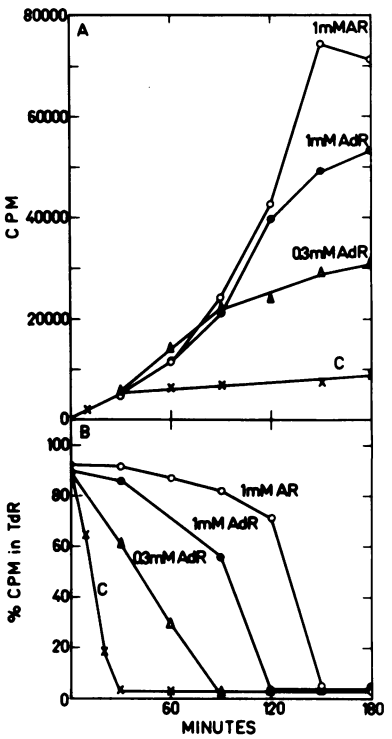

FIG. 2. *Effect of AdR and AR on the incorporation (A) and breakdown (B) of TdR. Exponentially growing cells of strain K-10 were treated with* ^{3}*H-TdR (5 μg/ml; 16 μc/ml) and divided into subcultures treated with either AdR or AR. At various intervals, samples were withdrawn for radioactive determination of the acid-precipitable material (A), and other samples were taken for the determination of radioactive TdR and thymine in the culture solution (B).*

The data of Fig. 2 clearly show that, on completion of the breakdown of exogenous TdR, there is a marked change in the kinetics of TdR incorporation. In the AR-treated culture it stops at once, whereas in the AdR-treated culture uptake continues after TdR breakdown but at a much slower rate. This slow uptake is probably due to the presence of deoxyribose in the cellular pool which is the product of AdR phosphorolysis.

Induction of TdR phosphorylase and purine nucleoside phosphorylase. Figure 3A shows the kinetics of TdR phosphorylase induction by TdR, AdR, and AR. The small amount of TdR present in the previous experiment (0.02 mM) is

insufficient for the induction of the enzyme. Nevertheless, the basal amount of enzyme suffices for the rapid phosphorolysis of TdR (Fig. 2B; 3, 6). AdR, which inhibits TdR breakdown, is a potent inducer of the enzyme and AR, which is the strongest inhibitor of TdR phosphorolysis, does not induce the enzyme (5, 14, 16). Figure 3B shows the effect of these nucleosides on the induction of purine nucleoside phosphorylase; this enzyme catalyzes the phosphorolysis of AdR and AR (12). Both purine nucleosides and TdR induce this enzyme, although only twofold.

Effect of AdR and AR on TdR breakdown in vitro. Doskočil and Pačes (6, 7) reported that AdR and AR can inhibit TdR breakdown in whole cells only. This observation led them to propose that the inhibitor occupies a membranous site in the intact cell where the reaction takes place. On the other hand, Budman and Pardee (5) demonstrated a competitive inhibition of TdR phosphorylase by AdR and by uridine in a sonic extract of induced *E. coli* B. Figure 4 shows a Lineweaver-Burk plot of TdR phosphorylase activity, in the presence of AdR, AR, or uridine in a cell-free extract of induced *E. coli* K-10 cells. Only uridine shows competitive inhibition of enzyme activity, whereas AdR and AR have no effect. The data of Fig. 4 concerning AdR and AR are therefore in agreement with those reported by Doskočil and Pačes. The K_m for TdR was 5.7 mM in the presence 0.1 M phosphate buffer (*p*H 5.9); K_m values of 1.3 and 1.1 mM have been previously reported (5, 14).

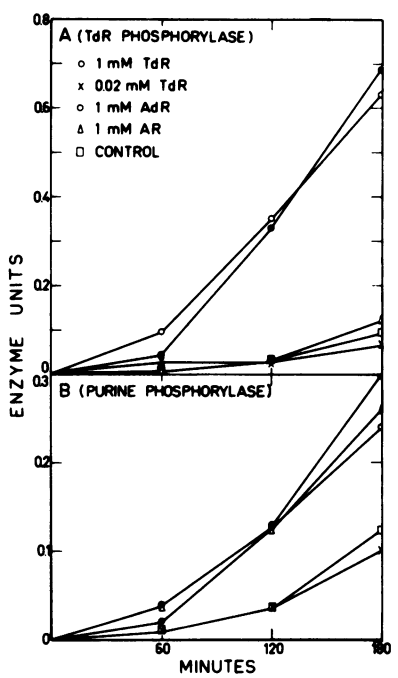

FIG. 3. *Induction of TdR phosphorylase (A) and of purine nucleoside phosphorylase (B) by TdR, AdR, and AR. K-10 cells were grown exponentially; at t = 0 min, different nucleosides were added. At various intervals, samples were withdrawn for the determination of TdR phosphorylase (A; determined by arsenolysis) and purine nucleoside phosphorylase (B).*

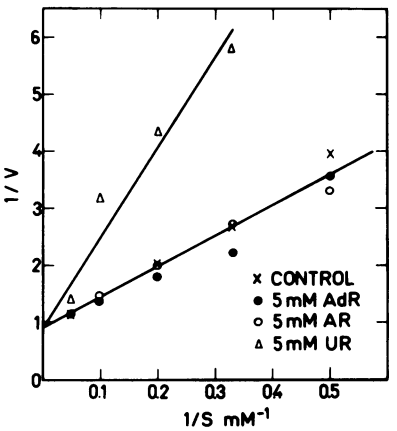

FIG. 4. *Lineweaver-Burk plot of TdR phosphorylase activity in a sonic extract of induced E. coli K-10. Exponential cells were induced for 90 min with 2 mM TdR and a sonic extract was prepared. Assay was carried out in phosphate buffer with various concentrations of TdR. Concentration of AdR, AR, and uridine was 5 mM. Units of velocity are expressed as micromoles thymine produced per minute.*

Efficiency of AdR as promoter of TdR incorporation. Fangman and Novick (8, 9) have shown that TdR is readily incorporated into DNA of a mutant strain lacking TdR phosphorylase. To test the extent of AdR efficiency in the promotion of TdR uptake, we compared the differential rate of TdR incorporation in the wild-type strain (K-10), in the presence of AdR, to that of a TdR phosphorylase-deficient strain (K12SH-28) in the absence of AdR (Fig. 5). Since the differential rate of incorporation in the treated wild-type strain is identical to that of the untreated mutant strain, we conclude that AdR can completely overcome the inhibitory effect of TdR phosphorolysis on TdR incorporation. Furthermore, the fact that AdR has no effect on TdR incorporation in the mutant strain (Fig. 5B) indicates that the effect of AdR in the wild-type cells (Fig. 5A) is specific to TdR phosphorylase.

DISCUSSION

The data presented show that AdR and AR are inhibitors of TdR phosphorolysis in vivo. This inhibition, which in the presence of 1 mM purine nucleoside holds for 90 to 120 min, is

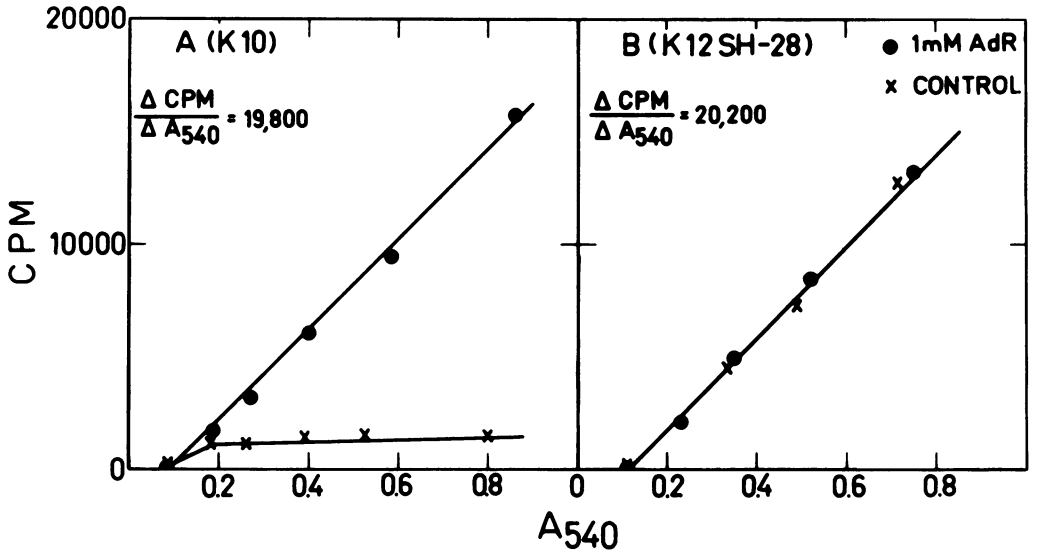

FIG. 5. *Incorporation of TdR by E. coli K-10 (A) and K12SH-28 (B) treated with 1 mM AdR, plotted as a function of culture density ($A_{540\ nm}$; 0.1 unit of absorbancy is approximately equal to 4 × 10^7 cells/ml). Cells growing exponentially were treated with ^{3}H-TdR (2 μg/ml; 1.15 μc/ml) and divided into two subcultures, one of which was treated with 1 mM AdR. At various intervals, samples were withdrawn for radioactive determination of the acid-precipitable material.*

complete as judged by the ability of the cells to incorporate TdR compared to cells lacking TdR phosphorylase (Fig. 5). AR can inhibit TdR phosphorolysis for a longer time than AdR and it can therefore promote uptake of more TdR into bacterial DNA (Fig. 2). This is probably because, in contrast to AdR, it does not induce TdR phosphorylase (Fig. 3); it may also have a weaker affinity for purine phosphorylase than does AdR and thus remains uncleaved for a longer period.

The mechanism by which AdR and AR inhibit phosphorolysis is not yet clear. Our results, which show that AdR and AR inhibit TdR phosphorolysis in vivo but not in a cell-free extract (Fig. 4), support the idea proposed by Doskočil and Pačes (6) that both purine nucleosides exert their inhibitory effect by occupying a binding site on a surface of the intact cell where the reaction occurs.

AdR, as a donor of deoxyribose, can stimulate uptake of thymine (Fig. 1; reference 10). Since AdR is an inhibitor of TdR phosphorylase, it can be expected to transfer its deoxyribose moiety to thymine by means of another enzyme, trans-*N*-deoxyribolase (EC 2.4.2.6), as proposed by Munch-Petersen (13). This, however, is incompatible with the findings of Budman and Pardee (5) who demonstrated that, in strain K12SH-28 which lacks TdR phosphorylase activity, AdR cannot promote uptake of thymine. Furthermore, according to Beck and Levin (2), *E. coli* 15T$^-$ has almost undetectable quantities of *trans-N*-deoxyribolase. It seems, therefore, that the breakdown of TdR and its synthesis from thymine in the presence of AdR are both catalyzed by TdR phosphorylase.

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