INHIBITION OF PROTEIN SYNTHESIS BY PYRIMIDONE-(2)-RIBOFURANOSIDE IN *Escherichia coli*

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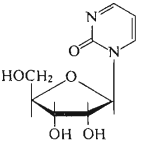
Qualitatively changed inducible enzymes are synthesized in *Escherichia coli* in the presence of pyrimidone-(2)-ribofuranoside.

Pyrimidone-(2)-ribofuranoside (PyR), an analogue of uridine, has been shown recently to inhibit DNA synthesis in *E. coli*[1,2]. Protein synthesis, as measured by the incorporation of radioactive amino acid[2], is inhibited by PyR only slightly. However, as shown in this communication, the quality of the proteins made in the presence of PyR is changed. This change is demonstrated by following the effect of PyR on the activity of inducible enzymes.

EXPERIMENTAL

Escherichia coli strains K 12 and 15 T^- have been used throughout the work. The cells were grown in Erlenmeyer flasks at 37°C on a Dubnoff shaker. Generally a low-P medium[3] was used, supplemented with 0·25% glycerol as a carbon source. In some experiments, medium according to the method of Spizizen[4] or as used by Nakada and Magasanik[5] were employed. For the cultivation of strain 15 T^-, thymine at concentration 1 μg/ml was included in the media.

For induction of β-galactosidase, $5 \cdot 10^{-4}$M isopropyl-β-D-thiogalactopyranoside (IPTG) (Calbiochem) was used. Where catabolite repression was to be excluded, lactose as a sole carbon source was added to the cell suspension. The enzyme activity was determined spectrophotometrically with *o*-nitrophenyl-β-D-galactopyranoside (Calbiochem) as substrate[6]. Tryptophanase was



Pyrimidone-(2)-ribofuranoside

induced by L-tryptophane (Loba-Chemie) and assayed by the modification[7] of the method of Pardee and Prestidge[8].

To follow the incorporation of the radioactive precursors (^{14}C-valine or ^{3}H-thymine) aliquots of cell suspension wese added to equal volumes of ice-cold 10% trichloroacetic acid. The precipitates were collected on membrane filters and radioactivity was measured by scintillation. Alkali stable counts of incorporated ^{32}P were determined by incubation of samples in 0·3M-KOH at 37°C overnight, followed by precipitation with trichloroacetic acid.

All radioactive compounds were obtained from the Institute for Research Production and Application of Radioisotopes in Prague. Pyrimidone-(2)-ribofuranoside, synthesized as described elsewherere[9], was used in concentrations of 60 μg/ml, 2′-deoxythymidine (dThd) and uridine (Urd) (Calbiochem) were used in concentrations of 300 μg/ml.

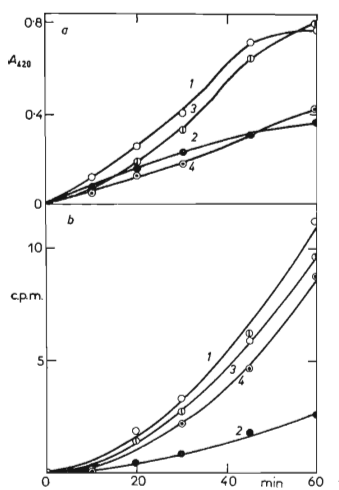


FIG. 1

Effect of PyR on β-Galactosidase Synthesis (*a*) and DNA Synthesis (*b*) in *E. coli* K 12 IPTG, $KH_2{}^{32}PO_4$ (0·25 μCi/ml), PyR, 2′-deoxythymidine or uridine were added at 0 time. Curves: *1* control; *2* PyR; *3* PyR + Urd; *4* PyR + dThd. c.p.m. in *b* represent alkali-stable $^{32}P \times 10^{-3}$.

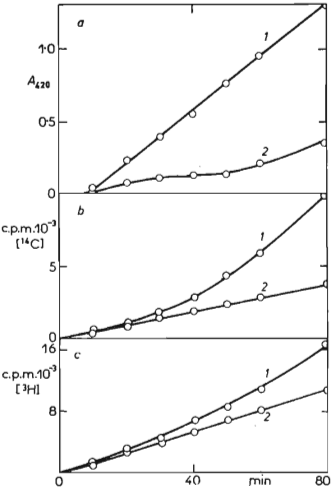


FIG. 2

Effect of PyR on β-Galactosidase Synthesis (*a*), Total Protein Synthesis (*b*) and DNA Synthesis (*c*) in *E.coli* 15T$^-$

IPTG, PyR, valine-[^{14}C] (0·1 μCi/ml), thymine-[^{3}H] (0·5 μCi/ml, 1 μg/ml) were added at 0 time to a culture of *E. coli* 15T$^-$ grown on medium supplemented with 0·1% casamino acids. Curves: *1* control; *2* PyR.

RESULTS

In the presence of PyR, the synthesis of total protein is inhibited much less than the synthesis of DNA in cells of *E.coli*[2] K 12. At least part of the protein made in the presence of the inhibitor, however, is either inactive or less active than protein made without inhibitor. This is demonstrated by the inhibition of the synthesis of induced active β-galactosidase (Fig. 1). The effect of PyR on protein synthesis may be separated from that on DNA synthesis. While DNA synthesis inhibition is counteracted by excess of both 2'-deoxythymidine and uridine, only uridine counteracts the effect of PyR on protein synthesis.

The situation is different in the thymine requiring strain (Fig. 2). DNA synthesis is inhibited only slightly, whereas the inhibition of protein synthesis becomes predominant. There is only a small amount of active β-galactosidase formed in this strain in the presence of PyR. Moreover, inhibition of the synthesis of an active enzyme is a difficult process with which to compete (Fig. 3).

Our results indicate that PyR affects the quality of the protein synthesized. An analogous situation arises during the action of another pyrimidine antimetabolite, 5-fluorouracil[10]. Horowitz and Kohlmeier[11] suggest that catabolite-type repression is mainly responsible for the inhibition of β-galactosidase synthesis by 5-fluorouracil.

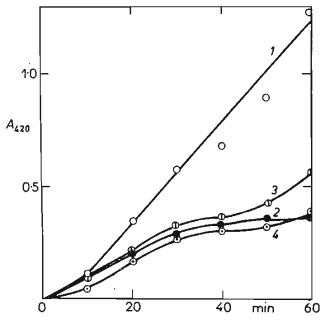


FIG. 3

Lack of Competition by 2'-Deoxythymidine and Uridine of β-Galactosidase Synthesis Inhibition by PyR in *E. coli* 15T⁻

IPTG and nucleosides added at 0 time. Curves: *1*, control; *2*, PyR; *3*, PyR + Urd; *4*, PyR + dThd.

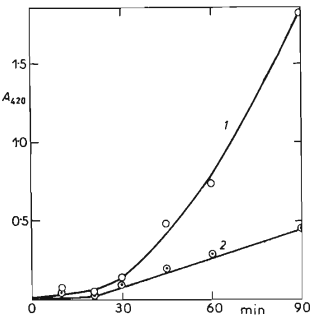


FIG. 4

Lactose Induced β-Galactosidase Synthesis in *E. coli* 15T⁻

Curves: *1*, control; *2*, PyR added simultaneously with lactose at 0 time.

To determine whether any similar effect plays a significant role in PyR action, pre-starved cells were grown in the absence of any carbon source other than lactose. Lactose served as an inducer at the same time. As Fig. 4 shows, catabolite-type repression is excluded by this experiment, since inhibition is persistent under these conditions.

To support the idea that a qualitative change of enzymes synthesized in the presence of PyR is a general phenomenon, the activity of tryptophanase, another inducible enzyme, was measured (Fig. 5). The pattern of tryptophanase inhibition is similar to that of β-galactosidase inhibition.

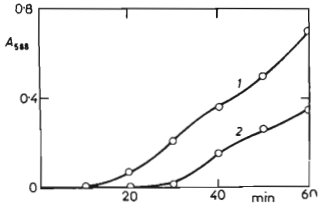


FIG. 5
Inhibition ot Tryptophanase Synthesis by PyR
Inducing L-tryptophane was added at 0 time. Curves: *1*, control; *2*, PyR added simultaneously with L-tryptophane.

DISCUSSION

The foregoing experiments show that the effect of PyR on protein synthesis can be separated from its effect on DNA synthesis by performing the experiments either with *E.coli* 15 T⁻ or *E. coli* K 12 and excess of 2'-deoxythymidine. In general, the action of PyR resembles that of 5-fluorouracil, since both compounds inhibit DNA synthesis and affect protein synthesis qualitatively rather than quantitatively. Thus, far, the only observed difference is the ineffectiveness of catabolite-type repression mechanism in PyR treated cells. It should be noted that 5-fluorouracil is effective as a base. The base of PyR, pyrimidone-(2), has no inhibitory activity, most probably because it does not penetrate the cell.

In analogy with 5-fluorouracil, the mechanism of PyR action may be visualized by means of incorporation. If PyR is incorporated into RNA, it may well change its coding properties[2].

In thymine requiring strain 15 T⁻, DNA synthesis is inhibited only slightly if at all. The inhibition observed in Fig. 2*c* may be a consequence of growth inhibition. This strain lacks thymidylate synthetase[12]. It is possible therefore, that inhibition of this enzyme by PyR in strain K 12 is the cause of DNA synthesis inhibition in this strain.

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