pyrR Identical to pyrH in Salmonella typhimurium: Control of Expression of the pyr Genes

JUST JUSTESEN AND JAN NEUHARD*
Enzyme Division, University Institute of Biological Chemistry B, Copenhagen, Denmark

Received for publication 2 June 1975

Mutants of Salmonella typhimurium showing constitutive synthesis of the pyrimidine biosynthetic enzymes coded for by the pyrA-F genes (G. A. O'Donovan and J. C. Gerhart, 1972) have been reinvestigated. The high rate of expression of the pyrB-F genes in these mutants as well as their pyrimidine excretion is shown to be due to mutations in the gene pyrH encoding uridine 5'-monophosphate kinase. Thus, the term pyrR used for these mutants should be replaced by the designation pyrH.

In Salmonella typhimurium and in Escherichia coli the expression of the nonlinked pyr genes, pyrA-F, is regulated by the endogenous pool of pyrimidine nucleotides (1, 4, 11, 12, 14). Recently an attempt was made by O'Donovan and Gerhart (10) to isolate regulatory mutants in S. typhimurium showing constitutive synthesis of the six pyrimidine biosynthetic enzymes coded for by pyrA-F. Two selections were used: (i) they isolated mutants that would feed the pyrimidine requirement of a pyrimidine requiring tester strain, and (ii) they selected mutants that were resistant to the two pyrimidine analogues 5-fluoracil (FU) and 5-fluorouridine (FUR). Among the mutants they obtained were some that contained high levels of the enzymes responsible for the biosynthesis of uridine 5'-monophosphate (UMP) even when the cells were grown in the presence of exogenous uracil. Since the mutation responsible for this phenotype was not co-transducible with any of the genes pyrA-F, it was tentatively classified as being in a regulatory gene, which they termed pyrR. Preliminary mapping of three pyrR mutants located pyrR around 10 min on the Salmonella chromosome (10).

S. typhimurium strains containing mutations in the structural gene, pyrH, for UMP kinase (EC 2.7.4.-) have been characterized (7). Since UMP kinase is essential for growth, only conditional pyrH mutants can be isolated. Owing to decreased UMP kinase activity pyrH mutants grow with greatly reduced endogenous levels of uridine-5'-triphosphate (UTP). This leads to derepression of the rate of synthesis of the pyrimidine biosynthetic enzymes (7, 12), which in turn results in overproduction of UMP and excretion of pyrimidines into the growth medium. As a result of these alterations in pyrimidine metabolism pyrH strains are resistant to FU and FUR. The gene pyrH is located at 12 min on the Salmonella chromosome (7).

The similarities in phenotypes between the pyrR and pyrH mutants as well as the chromosomal localization of the two genes led to the present investigation, which shows that the pyrR mutants contain partially defective UMP kinases. Thus the term pyrR for these mutations is misleading and should be replaced with the term pyrH.

MATERIALS AND METHODS

Chemicals. Nucleic acid bases, nucleosides, and nucleotides were the products of Sigma Chemical Co. (St. Louis, Mo.), [U-14C]UMP was from The Radiochemical Center (Amersham, England), and carrier-free [32P]orthophosphate in 0.1 N HCl was obtained from Atomenergikommissionens Forsøgsanlaeg (Ris, Denmark).

Bacterial strains and growth conditions. All strains used are derivatives of S. typhimurium LT-2. They are listed in Table 1. Cells were grown with vigorous aeration at 37°C in tris(hydroxymethyl)aminomethane (Tris) minimal medium (5) containing uracil (50 μg/ml), 0.2% Norite-treated vitamin-free casein hydrolysate (Difco), and 0.2% glucose. Cytidine was used in concentrations of 20 μg/ml. Growth was monitored at 436 nm in an Eppendorf photometer model 1101M.

Nucleoside triphosphate pools. Nucleoside triphosphate pools were determined in cultures grown for two generations in liquid medium in the presence of [32P]orthophosphate (specific activity in the medium: 10 μCi per μmol). Extraction and chromatographic separation of the nucleoside triphosphates has been described by Neuhard and Thomassen (9).

Toluene treatment and extraction of cells. Toluene treatment. Glucose-limited overnight cultures (0.05% glucose) were harvested by centrifugation, washed once with 0.9% NaCl, and resuspended in one-twentieth the volume of 0.1 M Tris-hydroxyl-
ride, pH 7.8, 10 mM MgCl₂ (about 6 x 10⁴ to 8 x 10⁴ cells per ml). Toluene was added to the suspensions (10 µl/ml suspension) and they were incubated at 37 C for 15 min in stoppered tubes followed by 15 min of incubation at 0 C with shaking. The toluene-treated suspensions were used for assays within a few hours.

Sonic disruption. Two hundred milliliters of stationary night cultures were harvested by centrifugations, washed once with 0.9% NaCl, and resuspended in 4 ml 0.1 M Tris hydrochloride, pH 7.8, 10 mM MgCl₂, and 2 mM mercaptoethanol. The suspensions were sonically oscillated three times for 30 s (Branson Sonifier) and centrifuged for 30 min at 20,000 x g in a Sorvall refrigerated centrifuge at 0 C. The supernatant fluids were treated with one-fifth volume of 10% streptomycin sulfate. After 30 min at 0 C the suspensions were centrifuged and the supernatant fluids were dialyzed for 2 h against 200 volumes of 0.1 M Tris-hydrochloride, pH 7.8, 10 mM MgCl₂, and 2 mM mercaptoethanol. The dialyzed extracts were used directly for enzyme assays.

Enzyme assays. For assays either toluene-treated cells or streptomycin-treated sonic extracts were used. They gave similar results.

ATCase. Aspartate transcarbamylase (ATCase) was assayed according to Gerhart and Pardee (6).

UMP kinase. UMP kinase activity was determined as described by Ingraham and Neuhard (7). One unit of enzyme is the amount that catalyzes the conversion of 1 nmol of substrate per min at 37 C. Specific activities are expressed as units per milligram of protein.

Protein. Proteins were determined by the method of Lowry et al. (8).

Heat treatment. One milliliter of streptomycin-treated sonic extract (3 to 6 mg of protein/ml) was placed in a glass tube in a waterbath at 62 C. At times, 0, 2, 5, 10, and 20 min, 100-µl samples were transferred to centrifuge tubes kept in an ice bath. After centrifugation the supernatant fluids were assayed for UMP kinase activity.

RESULTS AND DISCUSSION

FUR is toxic to S. typhimurium because it is converted by the cells to 5-fluorouridine-5'-triphosphate which may be incorporated into ribonucleic acid rendering it nonfunctional. Thus, mutants unable to convert FUR to 5-fluorouridine-5'-monophosphate are resistant to this analogue. It was previously shown (3) that FUR may be converted to F-Ump by two pathways in S. typhimurium: (i) by direct phosphorylation catalyzed by uridine kinase (EC 2.7.1.48), and (ii) by phosphorylase to F-Ump followed by conversion of F-Ump to 5-fluorouridine-5'-monophosphate by the action of uracil phosphoribosyltransferase (EC 2.4.2.9). Thus, two mutations, udk and upp, are required to make the cells resistant to FUR. Recently, however, it was observed, independently by O'Donovan and Gerhart (10) and by Ingraham and Neuhard (7) that single mutations, resulting in overproduction of pyrimidines may also cause resistance to FUR. In one class (7) the mutation causing pyrimidine overproduction was shown to be in the structural gene, pyrH, coding for the essential enzyme UMP kinase (EC 2.7.4.-). Cells containing a partially defective UMP kinase contain a low pool of UTP and, as a result, they contain high levels of the pyrimidine biosynthetic enzymes (7, 12). The derepressed synthesis of the biosynthetic enzymes explains the excess production and excretion of pyrimidines by these mutants. Co-transduction studies, using the general transducing phage P1, showed 30% linkage between the pyrH and the pan loci on the Salmonella chromosome (7).
The second class of mutants resistant to FU and FUR due to overproduction of pyrimidines has also high endogenous levels of the pyrimidine biosynthetic enzymes, even when grown in the presence of uracil in the growth medium. The affected gene was shown by P22 transduction to be unlinked to any of the structural genes, pyrA-F. Preliminary mapping studies by conjugation placed the mutations between leu and pro on the Salmonella chromosome ([10]; J. Roth, personal communication). It was suggested that this class of mutants were mutated in a gene, pyrR, coding for a protein involved in the control of expression of the pyr genes (10).

Since recent evidence indicate that the six pyr genes do not belong to one single regulon (12, 13) this possibility seems at present unlikely. An alternative explanation is that they all, like the first class of mutants, are mutated in the structural gene for UMP kinase, and thus are pyrH mutants.

Table 2 (columns 3 and 4) gives the levels of ATCase and UMP kinase in the six HD strains investigated. For comparison we have included the data obtained with a known pyrH mutant JL1269. In all the pyrR strains the specific activities of UMP kinase are less than 15% of that in the wild type. Three alternative explanations should be considered to explain the simultaneous appearance of high levels of ATCase (as well as the other pyrimidine biosynthetic enzymes [12]) and the low levels of UMP kinase: (i) the mutation affects a gene (pyrR) involved in the regulation of expression of one or more of the pyrA-F genes, and that the gene product(s) of one or more of these genes inhibits the expression of the pyrH gene; (ii) the high levels of the pyrimidine biosynthetic enzymes interfere with the in vitro assay of UMP kinase; (iii) the pyrR gene is identical to pyrH, which means that the HD strains, like JL1269, are mutated in the structural gene for UMP kinase.

The first alternative cannot, at present, be discarded. However, the physiological significance of such a regulatory mechanism is not apparent. The second alternative mentioned above is ruled out by an experiment in which the specific activities of ATCase and UMP kinase were determined in a pyrimidine auxotrophic strain, JL1018, after 2 h of pyrimidine starvation. The results obtained (Table 3) show that derepression of the synthesis of the pyrimidine biosynthetic enzymes (represented by the ATCase values in Table 3) does not affect the specific activity of UMP kinase.

According to the third explanation, the low UMP kinase activities of the HD strains are the result of mutations in pyrH rendering the UMP kinases of the strains partially defective. If so, it would be highly probable that the structurally altered enzymes show increased temperature sensitivity in vitro, as was found for the UMP kinase of JL1269 (7). The stability of UMP kinase activity to heating at 62 C was tested using crude extracts from HD-11, HD-12, HD-47, HD-87, and the wild-type KP-1001. The UMP kinases of the mutants are significantly more labile to heating than is the wild-type enzyme, indicating that the HD strains contain structurally altered UMP kinases (Fig. 1).

If the increased rate of synthesis of the pyrimidine biosynthetic enzymes of the HD strains is a result of their low UMP kinase

---

**Table 2. ATCase, UMP kinase, and pyrimidine nucleoside triphosphate contents of the pyrR mutants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>ATCase activity*</th>
<th>UMP kinase activity*</th>
<th>UTP</th>
<th>Cytidine 5'-triphosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>KP1001</td>
<td>Wild type</td>
<td>1,26</td>
<td>1.24</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>HD-11</td>
<td>pyrR</td>
<td>19,3.5</td>
<td>0.41</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>HD-12</td>
<td>pyrR</td>
<td>30,5.2</td>
<td>0.70</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>HD-46</td>
<td>pyrR</td>
<td>21,2.3</td>
<td>0.46</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>HD-47</td>
<td>pyrR</td>
<td>32,6.6</td>
<td>0.63</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>HD-58</td>
<td>pyrR</td>
<td>35,ND*</td>
<td>0.52</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>HD-87</td>
<td>pyrR</td>
<td>22,4.7</td>
<td>0.66</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>JL1269</td>
<td>pyrH</td>
<td>175,3.0</td>
<td>0.20</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

* ATCase values are given relative to the fully repressed value observed in KP1001 grown in the presence of uracil in the growth medium.
* Nanomoles of UMP phosphorylated per minute per milligram of protein at 37 C, as measured in sonicated, streptomycin-treated extracts (see Materials and Methods).
* ND, Not determined.

**Table 3. Effect of pyrimidine starvation on ATCase and UMP kinase activities in the pyrimidine requiring strain JL1018**

<table>
<thead>
<tr>
<th>Exp no.</th>
<th>Growth conditions*</th>
<th>Relative ATCase activity</th>
<th>UMP kinase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose limitation</td>
<td>1</td>
<td>1780</td>
</tr>
<tr>
<td>2</td>
<td>Uric acid limitation</td>
<td>11</td>
<td>1950</td>
</tr>
</tbody>
</table>

* Enzyme activities were determined in tolenuenzed cell suspensions containing in both experiments 6 x 10^6 cells per ml.
* Cells were grown overnight in Tris minimal medium containing in experiment 1: 0.05% glucose and 50 µg of uracil per ml and in experiment 2: 0.2% glucose and 4 µg of uracil per ml.
* UMP kinase activities are expressed as counts per minute of [14C]UDP + [14C]UTP formed per minute from [14C]UMP in a 10-µl aliquot of a standard assay.
activity, we would expect these strains to contain subnormal levels of UTP even when grown with uracil in the medium. Table 2 column 5 shows that this expectation is fulfilled; the UTP pools of the mutants are between 25 and 50% of that found in the wild type. Such a decrease is sufficient to cause the observed derepression of the biosynthetic enzymes (12).

The rate of synthesis of two of the enzymes of the de novo pathway, i.e., dihydroorotase and dihydroorotate dehydrogenase are specifically repressed when cells are grown with high endogenous cytidine-5' triphosphate pools, independently of the size of the UTP pool (12). Such a condition may be created by growing mutants lacking cytidine deaminase (EC 3.5.4.5) (cdd) in a cytidine-containing medium. A cdd derivative of HD-11 has been constructed (KP1407) and assayed for dihydroorotase and dihydroorotate dehydrogenase after growth in the presence or in the absence of cytidine. Cytidine is fully capable of repressing the rate of synthesis of these two enzymes in KP-1407 (R. Keln, personal communication).

From the results presented we conclude that pyrimidine excretion, resistance to FU and FUR, and "constitutivity" of the pyrimidine biosynthetic enzymes (10) of the HD strains investigated in this study, are the results of mutations in pyrH, the structural gene coding for UMP kinase. We suggest that the gene designation pyrR should be reserved for a gene coding for a regulatory protein directly involved in the control of expression of one or more of the genes pyrA, B, C, D, E, and F.

ACKNOWLEDGMENTS

Part of this work was done in the laboratory of G. A. O'Donovan, Texas A & M University, College Station, Tex. We would like to express our gratitude to Dr. O'Donovan for his hospitality during the stay, for making the HD strains available to us, and for numerous stimulating discussions. This work was supported by a NATO grant (no. 629) to J. Neuhard and G. A. O'Donovan.

LITERATURE CITED