Reversal by Aromatic Amino Acids of 2-Thiazole-DL-Alanine Inhibition of Salmonella typhimurium

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Of 21 L-amino acids tested (at 1.2 × 10⁻⁴ M), only histidine and the aromatic amino acids (phenylalanine, tryptophan, and tyrosine) protect Salmonella typhimurium strains from inhibition of growth and immediately reverse the growth inhibition by 5 × 10⁻⁴ M 2-thiazole-DL-alanine.

In wild-type Salmonella typhimurium LT2 (SQ421), L-histidine is transported by two different permeases, a histidine-specific permease (Kₘ = 8 × 10⁻⁸ M) and a general aromatic permease (Kₘ = 10⁻⁴ M for histidine) (3). The general aromatic permease is able to transport phenylalanine, tryptophan, and tyrosine as well as histidine and a variety of analogues of each of these amino acids (3, 5); the affinity of this permease for the aromatic amino acids is much higher (Kₘ values about 10⁻¹ M) than for histidine. The aromatic amino acids (phenylalanine, tryptophan, and tyrosine) completely inhibit the transport of histidine by the general aromatic permease system but not by the histidine permease system (4). A histidine analogue, 2-thiazole-DL-alanine (TA), mimics histidine in its allosteric "feedback inhibition" of the first (G) enzyme of histidine biosynthetic pathway (2, 8, 9). Since TA thus prevents the synthesis of histidine, wild-type cells fail to grow on minimal medium.

In this report, aspects of the transport of TA (Cyclo Chemical) were investigated since the presence of any of the aromatic amino acids prevents the inhibition of strain LT2 by 5 × 10⁻⁴ M TA. Exogenous 5 × 10⁻⁴ M TA is shown to be transported exclusively by the general aromatic permease and not by the histidine-specific permease. In addition, the novel observation that TA inhibition of growth is immediately reversed by the addition of aromatic amino acids led to an investigation of the possible effects of the aromatic amino acids in the regulation of histidine biosynthesis, namely on "feedback inhibition" and on "derepression." Since these effects of TA could not be demonstrated, it appears that TA may be removed from the cells by exchange with the aromatic amino acids which are transported by the general aromatic permease.

All strains were grown with vigorous aeration at 37°C in minimal medium (7) supplemented as needed with L-histidine (20 μg/ml). Growth was followed by measuring absorbance at 660 nm on a Bausch & Lomb Spectronic 20 colorimeter. The generation time for all strains was approximately 50 min during log-phase growth.

Figure 1A shows the growth response of strain LT2 to 5 × 10⁻⁴ M TA in the presence of six individual L-amino acids at 1.2 × 10⁻⁴ M (histidine, phenylalanine, tryptophan, tyrosine, aspartic acid, or proline). Only the aromatic amino acids and histidine of the 21 amino acids (aspartic acid and proline are shown as representative of the 17 nonaromatic amino acids) tested were able to maintain the growth of TA-treated LT2 cultures at the rate of an untreated culture (amino acids purchased from Sigma Chemical Co.). This observation suggests that, at an exogenous concentration of 5 × 10⁻⁴ M, (i) TA is transported by a permease, perhaps the general aromatic permease which can also transport the aromatic amino acids, and (ii) TA is not transported by the histidine permease since the aromatic amino acids do not block the histidine-specific permease (3).

Study of 12 concentrations of exogenous L-phenylalanine over the range of 5 × 10⁻⁷ to 5 × 10⁻⁴ M shows that 2 × 10⁻⁵ M is the lowest concentration which will completely prevent 5 × 10⁻⁴ M TA from inhibiting the continued
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LOGARITHMIC GROWTH OF STRAIN LT2. APPARENTLY THE GENERAL AROMATIC PERMEASE HAS A MUCH HIGHER AFFINITY FOR THE AROMATIC AMINO ACIDS THAN FOR TA.

FIGURE 1B INDICATES THAT TA IS TRANSPORTED BY THE GENERAL AROMATIC PERMEASE. STRAIN aroP504 (SQ454), WHICH HAS A DEFECTIVE GENERAL AROMATIC PERMEASE (3), IS ABLE TO GROW NORMALLY IN THE PRESENCE OF $5 \times 10^{-4}$ M TA. A MUCH HIGHER CONCENTRATION ($10^{-2}$ M) OF TA DOES, HOWEVER, INHIBIT GROWTH OF THIS MUTANT. PHENYLALANINE AT $10^{-2}$ M IS UNABLE TO PREVENT GROWTH INHIBITION BY $10^{-2}$ M TA OF STRAIN aroP504 (FIG. 1B) OR OF STRAIN LT2 (NOT SHOWN), WHICH SUGGESTS THAT AT THIS HIGH CONCENTRATION TA ENTERS THE CELLS INDEPENDENTLY OF THE GENERAL AROMATIC PERMEASE. ALSO, $10^{-2}$ M TA APPEARS TO ENTER CELLS INDEPENDENTLY OF THE HISTidine-SPECIFIC PERMEASE; AT THIS HIGH CONCENTRATION, SUFFICIENT TA TO INHIBIT GROWTH PROBABLY ENTERS BY SIMPLE DIFFUSION. THE GROWTH OF STRAIN hisP1650 (SQ455), WHICH HAS A DEFECTIVE HISTidine-SPECIFIC PERMEASE (10), IS INHIBITED BY $10^{-2}$ M TA IN THE PRESENCE OF $10^{-2}$ M PHENYLALANINE WHICH BLOCKS THE GENERAL AROMATIC PERMEASE (RESULTS NOT SHOWN).

FIGURE 1C SHOWS THAT A CULTURE OF LT2, IN WHICH THE GROWTH HAD BEEN COMPLETELY INHIBITED BY $5 \times 10^{-4}$ M TA FOR 20 MIN, IMMEDIATELY RESUMED EXPONENTIAL GROWTH AFTER THE ADDITION OF $5 \times 10^{-4}$ M TYROSINE. THE ADDITIONS OF EITHER PHENYLALANINE OR TRYPtophan ($5 \times 10^{-4}$ M) WERE EQUALLY EFFECTIVE (NOT SHOWN).

ACIDS ($1.2 \times 10^{-4}$ M) PROTECT WILD-TYPE STRAIN LT2 FROM GROWTH INHIBITION BY TA ($5 \times 10^{-4}$ M). SYMBOLS: O, NO ADDITION; D, TA; @, TA + PHENYLALANINE; △, TA + HISTidine; ■, TA + TYROSINE; †, TA + TRYPtophan; ‡, TA + ASPArcic acid; Δ, TA + PROline.

FIG. 1B. GROWTH RESPONSE (ABSORBANCE AT 660 nm) OF SALMONella TYPHIMURIUM STRAINS. AT ZERO TIME (ARROW), PORTIONS OF A LOG-PHASE CULTURE WERE DISTRIBUTED INTO VARIOUS FLASKS. THE GROWTH OF STRAIN aroP504, WHICH HAS A DEFECTIVE GENERAL AROMATIC PERMEASE, IS NOT INHIBITED BY $5 \times 10^{-4}$ M TA, BUT IS INHIBITED BY $10^{-2}$ M TA EVEN IN THE PRESENCE OF $10^{-1}$ M PHENYLALANINE. SYMBOLS: O, NO ADDITION; @, $5 \times 10^{-4}$ M TA; ‡, $10^{-2}$ M TA; △, $10^{-2}$ M TA + $10^{-2}$ M PHENYLALANINE.

FIG. 1C. GROWTH RESPONSE (ABSORBANCE AT 660 nm) OF SALMONella TYPHIMURIUM STRAINS. AT ZERO TIME (ARROW), PORTIONS OF A LOG-PHASE CULTURE WERE DISTRIBUTED INTO VARIOUS FLASKS. L-TYROSINE ($5 \times 10^{-4}$ M) REVERSES THE GROWTH INHIBITION BY $5 \times 10^{-4}$ M TA OF WILD-TYPE STRAIN LT2. SYMBOLS: AT 20 MIN AFTER THE ADDITION OF TA, L-TYROSINE WAS ADDED TO ONE SUBCULTURE (@); NO TYROSINE ADDED TO ONE SUBCULTURE (O). BOTH SUBCULTURES PRIOR TO ADDITION OF TA AT ZERO TIME (@).
Fig. 2. Phosphoribosyltransferase relative activity in extracts of strain hisEIF135. Each reaction mixture contained (per ml): 50 μmoles of tris(hydroxymethyl)aminomethane—hydrochloride buffer (pH 8.0), 4 μmoles of MgCl₂, 2.5 μmoles of adenosine triphosphate, 0.6 μ mole of phosphoribosylpyrophosphate magnesium salt (PRPP), and 0.2 mg of protein from an extract of strain hisEIF135. Reactions were followed at 37°C in a Zeiss PMQII spectrophotometer at 290 nm against a blank without PRPP. A, TA inhibits phosphoribosyl-adenosine triphosphate synthesis in the presence or absence of L-tyrosine. Strain hisEIF135 grown in 0.03 mM (limiting) L-histidine. Symbols: ○, reaction mixture; □, reaction mixture + 10⁻⁴ M TA; ●, reaction mixture + 10⁻³ M TA + 10⁻⁴ M tyrosine. B, Aromatic amino acids do not reverse repression of the histidine G enzyme by 0.07 mM L-histidine. Strain hisEIF135 grown in 0.03 mM histidine (・); 0.07 mM histidine + 0.5 μmole of each aromatic amino acid (phenylalanine, tryptophan, and tyrosine (▲)).

The G enzyme, phosphoribosyltransferase, was assayed (2) under various conditions to check if aromatic amino acids could either reactivate TA-inhibited G enzyme or reverse repression by histidine. The protein and nucleic acid concentrations of the cell extracts (1) were determined by measuring optical densities of the solutions at 260 and 280 nm with a Zeiss PMQII spectrophotometer and by use of an E. Adams nomograph (distributed by California Corp. for Biochemical Research) which is based on the extinction coefficient for enolase and nucleic acid (11). The protein concentration of the final extracts was approximately 8 mg/ml. Ethylenediaminetetraacetate was purchased from Fisher Scientific Co.; Tris buffer, adenosine-5'-triphosphate disodium salt and phosphoribosylpyrophosphate magnesium salt from Sigma Chemical Co.

The possible activation of the G enzyme by the aromatic amino acids in the presence of an inhibitor, TA or L-histidine, in a phosphoribosyl-ATP generating system, was investigated by assaying G enzymatic activity in the presence of an aromatic amino acid and an inhibitor (TA or histidine) over a wide range of ratios of aromatic/inhibitor concentrations, from 0.3 to 120. Figure 2A, a representative example, shows that there was no relief by an aromatic amino acid of the inhibition by TA of the G enzyme.

The possibility that the aromatic amino acids might induce the formation of the histidine biosynthetic enzymes was also ruled out as follows. Strain hisEIF135 (SQ437, a spontaneous multisite mutation in the hisE, I, and F genes), which cannot metabolize phosphoribosyl-ATP (2), was grown in three similar cultures in the presence of (i) 0.03 mM L-histidine (derepressed), (ii) 0.07 mM L-histidine (repressed), and (iii) 0.07 mM L-histidine + 0.5 μmole of each of three aromatic amino acids. Cell extracts were prepared from cultures grown to an absorbance at 660 nm of 0.6 and assayed in parallel for the relative activities of the G enzyme. Figure 2B illustrates that growth in the presence of excess aromatic amino acids and 0.07 mM histidine was not accompanied by derepression of the histidine biosynthetic enzymes.

These results indicate that at an exogenous concentration of 5 × 10⁻⁴ M TA is transported in strain LT2 only by the general aromatic permease. The sudden switch from no growth to full exponential growth of TA-inhibited LT2 culture, upon addition of an aromatic amino acid, suggests efficient elimination of the inhibitor. The aromatic amino acids do not directly interfere with the regulation of histidine biosynthesis since they are able neither to reactivate the TA-inhibited G enzyme in an assay mixture nor stimulate derepression of the histidine biosynthetic enzymes in mutant hisEIF135 on limited (yet not so low as to cause
derepression of the histidine enzymes) 0.07 mM histidine.

The quick return of TA-inhibited cells to full growth rate may be caused by efflux of TA by the general aromatic permease which is simultaneously transporting in aromatic amino acids. The possibility of such an exchange reaction is consistent with recent findings (6) in _Escherichia coli_ K-12 that rapid exchange of a preexisting labeled aromatic amino acid pool by external amino acids and analogues takes place through the general aromatic transport system (K_m for each aromatic amino acid approximately 5 x 10^-7 M) as well as through the specific transport systems for each aromatic amino acid (K_m for each approximately 2 x 10^-6 M). An aroP mutation in _E. coli_ inhibits exit and exchange of the small pool generated by specific transport. The quick reversal of TA inhibition by aromatic amino acids is distinct from the slow, spontaneous recovery, which can be seen in the gradually rising slopes of the lower curves of Fig. 1A to C. This partial recovery has been reported previously in _E. coli_ and is due in part to derepression of the histidine enzymes which results in some synthesis of histidine (8).

The entry of exogenous 10^-2 M TA is independent of the histidine-specific and of the general aromatic permease systems. High concentrations of L-histidine may also enter cells by simple diffusion, since 10^-3 M L-histidine permitted normal growth in the presence of tryptophan of a histidine-requiring strain lacking in the histidine-specific permease hisHB22 hisP1657 (5).

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**LITERATURE CITED**