A Defect in Histidine Biosynthesis Causing an Adenine Deficiency*

ALEXANDRA E. SHEDLOVSKY† AND BORIS MAGASANIK‡

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston 15, Massachusetts

(Received for publication, May 25, 1962)

In recent years, the role of purine nucleotides in the biosynthesis of histidine has been elucidated (2-5). The early steps of histidine biosynthesis comprise the purine nucleotide cycle shown schematically in Fig. 1. Adenosine triphosphate and 5-phosphoribosylpyrophosphate condense to form N-1-phosphoribosyl adenosine triphosphate; this compound is converted in several steps to Compound III in which the bond between N 1 and C 2 of the purine ring has been cleaved; Compound III subsequently reacts with NH₃ to give imidazoleglycerol phosphate and 3-amino-1-ribosyl-4-imidazolecarboxamide-5'-P; the former is converted to histidine and the latter is converted via inosine monophosphate and adenosine monophosphate back to adenosine triphosphate. Histidine regulates the operation of this cycle by inhibiting the enzyme that catalyzes the condensation of ATP and PP-ribose-P; hence, purine nucleotides are shunted into the cycle only to the extent that they are needed for histidine biosynthesis (2, 3). This feedback inhibition also explains why histidine exerts a sparing effect on the purine requirement of mutants blocked between the carboxamide and IMP. Since these mutants cannot reutilize the carbamoyl which is formed from added purine base in the course of histidine biosynthesis, they lose a portion of their purine supplement unless histidine is present to inhibit this flow (3). However, although histidine can decrease the purine requirement of such a mutant, it should not be able to satisfy completely the purine requirement of this or of any other mutant blocked in the pathway leading to ATP; similarly, purines should not be able to satisfy the histidine requirement of any mutant blocked between ATP and histidine. In other words, no mutant with an alternative requirement for purines and histidine should exist. Nevertheless, there do exist such mutants of Escherichia coli and Salmonella typhimurium. One such mutant of E. coli B/r was isolated and described as a histidine auxotroph by Witkin and Kennedy (6). Studies by Luzzati and Guthrie (7) and Balis et al. (8) have shown that this mutant, strain W-74, could grow fairly rapidly in media containing a purine base in place of histidine, and that it could grow very slowly in an unsupplemented medium. It was suggested by these authors that the mutant might require a compound derived from histidine in a building block for the synthesis of purine nucleotides. In that case, the relationship of histidine to purines would be that of a precursor rather than of a product; in fact, such a relationship has long been known to exist in animal tissues in which carbon 2 of the imidazole ring of histidine can be incorporated into carbon atoms 2 and 3 of the purine ring (9). This utilization of the histidine carbon is dependent on the presence of enzymes that catalyze the degradation of histidine to glutamate and a "single carbon" fragment having the oxidation level of formate (10, 11). It is therefore clear that only those microorganisms which are able to degrade histidine in this manner would be able to utilize histidine as a precursor of a portion of the purine ring. Actually, it has been found that pseudomonads, which liberate carbon 2 of histidine as formic acid, are able to incorporate this carbon into purines, whereas Aerobacter aerogenes, which liberates carbon 2 of histidine as formamide, cannot carry out this incorporation (12). In the case of E. coli, the parent organism of the mutant with the alternative requirement for histidine and purines, neither histidine degradation nor utilization of any portion of histidine for purine synthesis has been clearly demonstrated.

This study was undertaken to determine the relationship of histidine and adenine in the mutant with the alternative requirement for these compounds and to identify the lesion responsible for this nutritional requirement.

**EXPERIMENTAL PROCEDURE**

Bacteria—The organisms used in this study were the prototroph F. coli B/r, supplied by Dr. E. Witkin; a histidine or purine auxotroph derived from this organism by Witkin and Kennedy (6), strain W-74, supplied by Dr. M. E. Balis; and a purine auxotroph, strain B-96, supplied by Dr. J. S. Gots. The strains were maintained on yeast-tryptone agar slants.

The bacteria were grown in a modified Werkman's minimal medium, pH 6.5 (13). Glucose was used as the carbon and energy source; it was autoclaved separately from the basal medium and added to a final concentration of 0.5%. Supplements were added to the medium when needed. In all cases when adenine (20 µg per ml) was used as a supplement, 25 µg per ml of thiamine hydrochloride were included in the medium to prevent the possible growth inhibitory effect of adenine (3).

L-Histidine was used as a supplement at a concentration of 10 µg per ml. Cultures were prepared by inoculating 100 ml of
medium in a 250-ml Erlenmeyer flask (or 1 liter of medium in a 2-liter flask) with a portion of a fully grown culture obtained by inoculating a similar flask with a loopful of organisms taken from a slant. The flasks were incubated with vigorous shaking to insure aeration at 37°C.

The growth of the cultures was followed in a Klett-Summerson photoelectric colorimeter (clinical model) with a No. 42 filter.

Chemicals—Adenine and thiamine hydrochloride (Nutritional Biochemicals Corporation), L-histidine (Mann Research Laboratories), adenosine-2-C14 (Nuclear-Chicago), adenosine-8-C14 (Volk Radio-Chemical Corporation), ATP (Pabst Laboratories), the barium salt of ribose-5-P (Schwarz Laboratories) and acetyl phosphate (Cambridge Biochemical) were commercial products.

L-Histidine-2-Cl4 was synthesized by Dr. H. R. B. Revel. Barium salt of ribose-5-P (Schwarz Laboratories) and acetyl phosphate (Cambridge Biochemical) were commercial products.

The structure of Compound III differs from that proposed earlier (2), and is conjectural; it is suggested by the finding that, in contrast to N1-phosphoribosyl-ATP, which possesses an intact purine ring (5), Compound III is readily hydrolyzed by acid to 5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide (ACP) without yielding inorganic phosphate (see below). The sequence of hydrolysis and loss of PP in the conversion of N1-phosphoribosyl-ATP to Compound III is not known. -- --, inhibition; PR, phosphoribosyl; IGP, imidazolglycerol phosphate; HIST, histidine; S-AMP, adenylosuccinic acid.

Compound III was synthesized enzymatically from ATP and ribose-5-P. A crude extract was prepared as described previously from an auxotroph of Salmonella typhimurium which is blocked in the last step of histidine biosynthesis, and the Compound III-synthesizing activity was partially purified by fractionation with 2% protamine sulfate. The fractions containing

1 This method was developed by H. S. Moyed, M. Civen, D. Karibian, and B. Magasanik.

The reaction mixture for the preparation of Compound III contained a total volume of 100 ml: ATP, 0.2 mmole; ribose-5-P, 0.04 mmole; MgCl2, 0.4 mmole; Tris, pH 8.0, 10 mmole; and a sufficient volume of the partly purified extract to convert one-half of the added ATP to Compound III at 37°C during an incubation period of 1 hour. At the end of this time the protein was removed by adding the reaction mixture to an equal volume of 9:1 chloroform-octanol (14); the mixture was vigorously shaken and centrifuged. The lower chloroform-octanol layer and the protein which had collected at the interface were discarded; additional protein was removed by treating the aqueous layer a second time with chloroform-octanol. The deproteinized solution was lyophilized; the residue was taken up in a few milliliters of water, and the insoluble material was separated by centrifugation and discarded. The supernatant solution was brought to pH 6.2 by the addition of acetic acid and was layered on top of a 12-ml column composed of acid-washed charcoal (Norit) and diatomaceous earth (Celite) in the proportion of 1:2. The column was washed with 24 ml of H2O; then the adsorbed material was eluted with a dilute solution of ammonia (concentrated ammonia diluted 20 times with water). The adenine nucleotides and the aminoimidazolcarboxamide ribonucleotide of the reaction mixture are adsorbed but are not eluted from the charcoal by this eluent. Fractions of 10 ml were collected and tested for the presence of material which would yield a diazotizable amine after hydrolysis with dilute acid. The fractions containing such material were combined and concentrated by lyophilization. When a volume of the solution containing approximately 0.2 µmole of the amine was subjected to electrophoresis on paper, a single ultraviolet-absorbing compound could be detected which migrated toward the anode (13 cm per hour at 30 volts per cm in 0.02 M phosphate buffer, pH 7.0); this compound could be diazotized on paper only after heating with dilute acid. The material present in this solution was tentatively identified as 5-(5'-phosphoribosylformamido)-1-(5'-phosphoribosyl)-4-imidazolecarboxamide (Compound III, see Fig. 1) by the fact that hydrolysis with 1 N HCl for 1/2 minutes released a diazotizable amine (which could be identified by electrophoresis on paper as 5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide as described under “Results”) but did not release any inorganic phosphate (see also Reference 5). On incubation with extracts containing the Compound III-cleaving enzyme and NH4 (2), the material was converted to a diazotizable amine, presumably the aminoimidazolcarboxamide ribonucleotide, and to a compound giving the reactions of imidazole-glycerol phosphate (2). The neutralized solution of Compound III was kept at -14°C. After several months of storage increasing amounts of material reacting as diazotizable amines before acid hydrolysis appeared, indicating a progressive breakdown of the labile Compound III.

Analytical Methods—The purines of the nucleic acids and the histidine of the protein were isolated from the bacteria as previously described; the purines were assayed spectrophotometrically and the histidine colorimetrically (3). The radioactivity of these compounds was measured in a windowless proportional flow counter with 95% argon and 5% CO2 as the counting gas; the compounds were counted as infinitely thin films on aluminum planchets. Diazotizable amines were determined before and after treatment of the material under test with hot dilute HCl.
by the method of Bratton and Marshall (2, 15); 5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamidine served as the standard.

**RESULTS**

**Growth of E. coli Strain W-74**—An investigation of the growth of this mutant strain in minimal, in adenine-supplemented, and in histidine-supplemented media at 37° confirms the results reported by Luzzati and Guthrie (7). When the inoculum consisted of cells which had grown to the stationary phase in a histidine-supplemented medium, no growth could be observed for 30 hours in minimal medium; in a histidine-supplemented medium, the cells grew after a short lag (½ to 1 hour) at a rate of 1.34 generations per hour; in an adenine-supplemented medium, the cells grew after a lag of 6 to 10 hours at a rate of 0.66 generation per hour. Cultivation of the inoculum to the stationary phase in an adenine-supplemented medium did not alter the response to histidine, but shortened the period of lag in the adenine-containing medium to 1 to 3 hours, and enabled the cells to grow in minimal medium after a lag of 2 to 5 hours at a rate of approximately 0.19 generation per hour.

Essentially the same pattern of response was obtained when the cells were cultivated at 36°, although the growth rate in any one of the media was reduced by approximately one-half. It seems, therefore, that the lesion responsible for the partial dependence of the mutant on histidine or adenine is not due to the increased temperature sensitivity of an enzyme.

**Utilization of Histidine and of Adenine by E. coli**—To determine whether the amidine carbon of histidine can serve as a source of the ureido carbons of the purine ring of E. coli, cells of the parent strain B/r and of the mutant W-74 were cultivated in media supplemented with histidine-2-C14. The histidine of the cellular protein and the adenine and guanine of the cellular nucleic acids were isolated and examined for their radioactivity. The results presented in Table I show that, at most, a negligible fraction of ureido carbons of the purine ring is derived from histidine; as expected, the exogenous histidine provides the bulk of the histidine of the cell protein. These results are in good agreement with the observations that E. coli cannot use histidine as a sole source of nitrogen and that in extracts of cells cultivated in media containing as much as 0.2% histidine, the enzymes required for histidine degradation cannot be demonstrated. These findings seem to exclude the possibility that the mutant strain W-74 requires histidine as a source of a portion of the purine ring.

To determine whether the mutant synthesizes histidine from ATP by the usual pathway, its ability to incorporate carbon 2 of adenine into cellular histidine was compared with that of the parent strain. As might be expected from the role of ATP in histidine biosynthesis (3), carbon 2 of the exogenously supplied adenine was readily incorporated into histidine by both parent and mutant (Table I).

The results presented in this section indicate that the mutant during growth in a histidine-containing medium produces adenine nucleotides without the contribution of a “single carbon” fragment from histidine; during growth in an adenine-containing medium, it produces histidine by the usual pathway from adenine nucleotides. The question arises why it should be unable to grow at a fast rate in a medium supplemented with neither histidine nor adenine. The fact that only histidine fully restored its rate of growth suggested that the malfunction of one of the reactions between ATP and histidine was responsible for this inability. Examination of the compounds excreted by the organism in minimal and in adenine-containing media supported this view.

**Excretion of Diazotizable Amines by Strain W-74**—Mutants that lack one of the enzymes essential for the biosynthesis of a cell constituent frequently excrete the substrate of the missing enzyme into their culture fluid. Such excretions occur in “leaky mutants,” that is, mutants which have not lost the essential enzymatic activity entirely but have retained enough to grow at a suboptimal rate. The accumulation of intermediates of histidine biosynthesis in the medium can be readily recognized. The condensation product of ATP with PP-ribose-P reveals itself by its characteristic spectrum (5), “Compound III” by the fact that mild acid hydrolysis converts it to a diazotizable amine (2), and the subsequent intermediates by the fact that they are imidazole derivatives which react with diazotized sulfanilic acid (16).

The examination of the culture fluids of the mutant showed the presence of material which could be converted to a diazotizable amine by hydrolysis with 0.2 N HCl at 100° for 5 minutes. This material was excreted by the mutant during growth and accumulated to the extent of 0.02 μmole per ml and 0.01 μmole per ml in cultures which had reached full growth in the adenine-supplemented and the minimal medium, respectively. It could be shown by feeding adenine-8-C14 and isolating the diazotizable amine as described below that the exogenous adenine could serve as the major source of the aminoimidazolecarboxamide moiety of the excreted material. The substance failed to appear in cultures containing histidine, which supported the view that the excreted material is indeed a histidine precursor or derived from a histidine precursor. The inhibition by histidine of the condensation of ATP with PP-ribose-P accounts for the suppression of the excretion of histidine precursors by histidine (2). The further observation that the cultures did not contain imidazole derivatives reacting with diazotized sulfanilic acid suggested that

---

2 P. Lund and B. Magasanik, unpublished observations.

---

**TABLE I**

Incorporation of radioactivity from histidine-2-C14 and from adenine-3-C14 into cellular purines and histidine

The cells were grown in media supplemented with either 20 μg per ml of L-histidine-2-C14 (7700 c.p.m. per μmole) or 16 μg per ml of adenine-2-C14 (7690 c.p.m. per μmole). The bacteria were harvested from the exponential phase of growth. The results are expressed as relative molar activity (R. A.).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Supplement</th>
<th>Guanine</th>
<th>Adenine</th>
<th>Histidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/r</td>
<td>Histidine-2-C14</td>
<td>0.4</td>
<td>0.0</td>
<td>61</td>
</tr>
<tr>
<td>W-74</td>
<td>Histidine-2-C14</td>
<td>1.8</td>
<td>2.2</td>
<td>76</td>
</tr>
<tr>
<td>B/r</td>
<td>Adenine-2-C14</td>
<td>67</td>
<td>92</td>
<td>69</td>
</tr>
<tr>
<td>W-74</td>
<td>Adenine-2-C14</td>
<td>64</td>
<td>83</td>
<td>63</td>
</tr>
</tbody>
</table>
The partial failure of histidine biosynthesis is due to a deficiency in the ability of the mutant to convert all of the Compound III which forms to imidazoleglycerol phosphate.

To isolate the compound for purposes of identification, the mutant was grown in a 500-ml adenine-supplemented culture. At the end of the growth period, the culture fluid from which the bacteria had been removed by centrifugation was found to contain a total of 5.4 µmoles of material that reacted as diazotizable amine after acid hydrolysis. The culture fluid was evaporated to dryness from the frozen state, and the residue was extracted with 7 ml of water. The extract, which contained approximately 5 µmoles of the bound diazotizable amine, was passed over a 6-ml column consisting of charcoal (Norit) and diatomaceous earth (Celite) in the proportion of 1:2. All the material yielding diazotizable amine was adsorbed and was not removed by washing with 50 ml of H₂O. Most of this material could be eluted from the column with approximately 20 ml of an aqueous solution containing 1.1% concentrated ammonium hydroxide (Fraction a). Additional material was obtained by elution with 12 ml of aqueous solution containing 2.8% concentrated ammonium hydroxide and 50% ethanol (Fraction b). The material in the two fractions was concentrated by lyophilization and subjected to electrophoresis on filter paper in 0.02 m phosphate buffer, pH 7.0, at 30 volts per cm. The results are given in centimeters traveled per hour toward the anode (+), or the cathode (−). 0, ultraviolet-absorbing, diazotizable amines. O, ultraviolet-absorbing compounds converted to diazotizable amines by acid hydrolysis.

The presence of Compound III in Fractions a and b was confirmed by an enzymatic procedure. A crude enzyme preparation of the wild strain of E. coli, which was capable of liberating in the presence of NH₄ a diazotizable amine from Compound III, acted on the material in Fractions a and b in a corresponding manner (2).

The compound in Fraction b which moved slowly toward the anode was presumably a partially dephosphorylated derivative of Compound III. It was eluted, subjected to hydrolysis in 0.1 N HCl at 100° for 5 minutes and again subjected to electrophoresis on paper. The material in the hydrolysate migrated toward the cathode at the same rate as 5-amino-4-imidazolecarboxamide and could be diazotized directly. This result suggests that the more slowly moving acidic compound in Fraction b was 5-(5'-phosphoribosylformamidino)-4-imidazolecarboxamide or the corresponding riboside. The presence of the 5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide is in all likelihood explained by the nonenzymatic breakdown of the very labile Compound III.

The results of these experiments show that the mutant does indeed excrete the histidine precursor, Compound III, and is presumably deficient in the ability to produce imidazoleglycerol phosphate. This assumption would readily explain the need of the mutant for histidine; it would not explain the ability of adenine to support growth, unless one assumes that the excretion of Compound III causes a purine deficiency. The experiments described in the next section were devised to test this hypothesis.

Effect of 2-Thiazolealanine—The observations reported so far are compatible with the view that the excretion of Compound III is responsible for the inability of strain W-74 to grow well in an unsupplemented medium. The excretion of Compound III may make the cell deficient in purine nucleotides, either because of the actual loss of purine nucleotides as Compound III or because Compound III inhibits one of the reactions of purine biosynthesis. Replacement of the purine nucleotides by exogenous adenine, or prevention of Compound III excretion by exogenous histidine, would restore the ability of the mutant to grow at a rapid rate. To test the hypothesis that Compound III excretion rather than the need for histidine is primarily responsible for the behavior of the mutant, an attempt was made to halt specifically the excretion of Compound III without providing the mutant with histidine.

The special properties of the histidine analogue, 2-thiazolealanine, made such an experiment possible. It has been shown by Moyed (17) that this compound is not incorporated into protein in place of histidine but exerts its inhibitory effect on a prototrophic strain of E. coli by mimicking histidine in inhibiting the enzyme that yields Compound III. The effect of 2-thiazolealanine on the growth of the mutant strain W-74 and of its parent, strain B/r, was now examined. It was found that the histidine analogue at a level of 10 µg per ml in an otherwise unsupplemented medium reduced the growth rate of strain B/r from 1.34 generation to 0.76 generation per hour but increased the growth rate of strain W-74 from 0.19 to 0.66 generation per hour; higher levels inhibited the growth of both strains. The analogue closely resembled adenine in its stimulatory effect on the growth of the
mutant: cells cultured in an adenine-containing medium grew after a short lag and at the same rate when transferred to media supplemented with either adenine or 2-thiazolealanine, while cells cultured in a histidine-containing medium grew only after a long lag when transferred to either an adenine-supplemented or a 2-thiazolealanine-supplemented medium. On the other hand, unlike adenine but like histidine, 2-thiazolealanine completely prevented the excretion of Compound III.

These findings support the idea that Compound III excretion rather than the lack of histidine is responsible for the inability of the mutant to grow in minimal medium at a rapid rate. The experiments described in the next section attempt to discover whether the quantity of purine nucleotides lost as Compound III could account for the limitation on the growth rate in minimal medium.

**Compound III Excretion and Purine Synthesis**—To estimate the relation between the rate of purine synthesis and growth, the uptake of adenine-8-C\(^{14}\) by a purine-requiring mutant, strain B-96, which is also derived from *E. coli* B, was determined. This mutant, which is unable to convert 5-amino-1-(5'-phosphoribosyl)-4-carboxamide to IMP, was grown in media containing 20 \(\mu g\) per ml of histidine and different levels of adenine-8-C\(^{14}\). By examination of the radioactivity incorporated into the cells and that remaining in the medium, it was found that the formation of \(10^8\) cells required the uptake of 7.4 mmoles of adenine. One may therefore assume that the strains of *E. coli* that produce the purine nucleotides by synthesis de novo form 7.4 mmoles for every \(10^8\) cells produced. Consequently, if growth proceeds at the normal rate of 1.3 generations per hour, the rate of purine synthesis is 1.3 \(\times\) 7.4 \(\times\) \(\ln 2\) = 6.7 mmoles per hour per \(10^8\) cells.

The rate of purine synthesis and of Compound III excretion by strain W-74 was determined by placing adenine-grown cells in minimal medium and measuring the increase in cell density and the increase in bound diazotizable amine (Fig. 3). It can be seen that the formation of \(10^8\) cells occurred concomitantly with the excretion of 5.5 mmoles of Compound III. According to the previous experiment the purine nucleotide content of \(10^8\) cells amounts to 7.4 mmoles. It seems, therefore, that a total of 12.9 mmoles synthesized, 5.5 mmoles or approximately 40% was excreted as Compound III. The growth rate of the mutant in minimal medium was only 0.13 generation per hour in this experiment. The rate of purine synthesis is therefore 12.9 \(\times\) 0.13 \(\times\) \(\ln 2\) = 1.2 mmoles per hour per \(10^8\) cells. The mutant thus produces in the unsupplemented medium purine nucleotides at a rate only approximately one sixth that of the parent strain. This seems to contradict the idea that the loss of Compound III has made the mutant purine-deficient, for the feedback inhibition exerted by purine derivatives (18) would lead one to expect purine deficiency to result in an increased rather than a decreased rate of synthesis of purine precursors.

To clarify this issue, the rate of excretion of a purine precursor by cells of *E. coli* strain B-96, the organism which had been used to demonstrate the feedback inhibition exerted by purines, was measured. This mutant is unable to convert 5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide into IMP, and excretes the corresponding nucleosides when no exogenous purine bases are present (18). It can be seen (Fig. 4) that the diazotizable amines are excreted by the mutant at a rate which is not much greater than the rate at which Compound III is excreted by strain W-74: 0.9 mmoles of the amine is excreted by \(10^8\) cells in 1 hour; an additional small amount of the amine is retained in the cells. It therefore appears that purine deficiency can lead to a decrease rather than an increase in the rate of purine precursor synthesis, perhaps because ATP is required at several steps of this biosynthetic sequence.

The results of these experiments are therefore in keeping with the idea that the excretion of Compound III has depleted the cell of purine nucleotides. However, these results do not disprove the possibility that the accumulated Compound III may directly produce a purine deficiency by inhibiting one of the reactions responsible for the biosynthesis of purine nucleotides. Compound III added at a level of 50 \(\mu\)moles per ml, which is much higher than that found in cultures of strain W-74, failed to inhibit the growth of the parent strain, but it is possible that
this lack of an effect is due to impermeability of the cell to this compound.

DISCUSSION

The excretion of the histidine precursor, Compound III, by strain W-74 during its slow growth in a minimal medium suggests that the lesion responsible for its partial auxotrophy is a defect in the enzyme that converts Compound III to imidazoleglycerol phosphate. Such a defect would be expected to lead to a requirement for histidine for rapid growth; it is, however, not clear why adenine should replace histidine as growth factor unless additional assumptions are made.

One hypothesis that could account for the effect of adenine would be that the Compound III-cleaving enzyme of the mutant has less affinity for its substrate than that of the parent strain. Exogenous adenine might then be converted to Compound III rapidly enough to bring about an increase in the intracellular level of this compound and thus increase the rate of its enzymatic cleavage to imidazoleglycerol phosphate. The observation that adenine stimulates the formation of Compound III is compatible with this assumption. However, the finding that 2-thiazolealanine inhibits Compound III synthesis but stimulates the growth of the mutant clearly eliminates this hypothesis.

Another hypothesis which accounts for the growth stimulatory effects of histidine, 2-thiazolealanine, and adenine assumes that the excretion of Compound III makes the organism deficient in purine nucleotides. In this case, histidine and 2-thiazolealanine would correct the deficiency by inhibiting the excretion of Compound III, while adenine would correct the deficiency by serving as a source of purine nucleotides. The purine deficiency may be due either to the loss of endogenously synthesized purine nucleotides as Compound III or to the inhibition of purine biosynthesis by the accumulated Compound III. Although there is no a priori reason why Compound III should inhibit purine biosynthesis, its structural similarity to purine nucleotides suggests that it might mimic a purine nucleotide which controls this process by feedback inhibition.

Most of the mutants of Salmonella typhimurium that have an alternative requirement for histidine or adenine can grow slowly in minimal medium and excrete diazotizable amines (19). The explanation given here for such an alternative requirement in strain W-74 of E. coli may therefore well apply to other mutants with similar requirements.

SUMMARY

A mutant, strain W-74, of Escherichia coli, grows rapidly in a medium supplemented with histidine, somewhat more slowly in a medium supplemented with adenine, and very slowly in an un-supplemented medium. The mutant was found to excrete the histidine precursor Compound III in a minimal or in an adenine-containing medium; histidine prevents this excretion. The histidine analogue, 2-thiazolealanine, which is not incorporated into protein but which mimics histidine in inhibiting the enzyme system that forms Compound III was also found to stimulate the growth of the mutant and to prevent the excretion of Compound III. These results suggest that the mutant is defective in the enzyme responsible for the conversion of Compound III to the histidine precursor imidazoleglycerol phosphate and that the purine precursor 5-amino-1-(5'-phosphoribosyl)-4-imidazolcarboxamide. This partial block brings about the excretion of a portion of the endogenously produced purine nucleotides as Compound III. The resulting purine deficiency can be corrected either by the exogenous replacement of the lost purines or by the prevention of the loss by the feedback effect of histidine or 2-thiazolealanine.

REFERENCES

A Defect in Histidine Biosynthesis Causing an Adenine Deficiency
Alexandra E. Shedlovsky and Boris Magasanik


Access the most updated version of this article at http://www.jbc.org/content/237/12/3725.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/237/12/3725.citation.full.html#ref-list-1