Biosynthesis of the Monoguanidinated Inositol Moiety of Bluensomycin, a Possible Evolutionary Precursor of Streptomycin*

JAMES B. WALKER

From the Department of Biochemistry, Rice University, Houston, Texas 77001

SUMMARY

The general pattern of biosynthesis of the bluensidine (1D-1-O-carbamoyl-3-guanidino-3-deoxy-scyllo-inositol) moiety of bluensomycin, a monoguanidinated analogue of dihydrostreptomycin, has been studied in extracts of Strep
tomyces hygroscopicus forma glebosus ATCC 14607 (S. glebosus). Our results are consistent with the following biosynthetic pathway: myo-inositol → keto-scyllo-inositol → aminodeoxy-scyllo-inositol → 1-amino-1-deoxy-scyllo-inositol-4-P → 1D-1-O-carbamoyl-3-amino-3-deoxy-scyllo-inositol-6-P → bluensomycin. A major uncertainty concerns the step at which the carbamoyl group is introduced. Carbamoylation might occur, for example, prior to phosphorylation or following transamidination.

myo-Inositol:NAD oxidoreductase activity (C) is reported for the first time in extracts of Streptomyces; activity was assayed by two radiochemical methods, both involving coupling with aminotransferase (D). L-Glutamine:keto-scyllo-inositol aminotransferase (D) was also found to catalyze aminodeoxy-scyllo-inositol:keto-scyllo-inositol, aminodeoxy-scyllo-inositol:pyruvate, and 1,3-diamino-1,3-dideoxy-scyllo-inositol:keto-scyllo-inositol transaminations. L-Arginine:inosamine-P amidotransferase (F) catalyzed transaminations with the following compounds as amidino acceptors: NH₃, 1-amino-1-deoxy-scyllo-inositol-4-P, 1D-1-guanidino-3-amino-1,3-dideoxy-scyllo-inositol-6-P, 1D-1,3-diamino-1,2,3-trideoxy-scyllo-inositol-6-P, and a compound present in S. glebosus extracts believed to be 1D-1-guanidino-1-deoxy-scyllo-inositol-4-P. The latter transamination is markedly enhanced in crude extracts by carbamoyl-P. S. glebosus extracts also have 1-guanidino-1-deoxy-scyllo-inositol-4-P phosphohydrolase activity; neither this enzyme nor the corresponding enzyme from streptomycin producers can dephosphorylate the transamination product presumed to be bluensidine-6-P. Acid hydrolysis of the latter compound gave a compound which, unlike the unhydrolyzed compound, was converted to 1D-1-guanidino-3-amino-1,3-dideoxy-scyllo-inositol by enzymes from a streptomycin producing strain. S. glebosus cannot carry out the above conversion since it apparently lacks at least two enzymes which occur in streptomycin producers: guanidino-1-deoxy-scyllo-inositol dehydrogenase and L-alanine:1D-1-guanidino-3-keto-1-deoxy-scyllo-inositol aminotransferase.

It is suggested that streptomycin producing strains might be descendents of an ancestral strain which, like S. glebosus, produced the monoguanidinated inositol derivative, bluensomycin. It is further suggested that gene duplication and subsequent evolutionary divergence resulted in biosynthesis of the diguanidinated inositol derivative, streptomycin, which is 10 times more effective than bluensomycin as an antibiotic and inhibitor of protein biosynthesis.

Evolutionary mechanisms for the acquisition of novel biosynthetic capabilities remain among the important unsolved problems in biology. It is particularly difficult to understand how biosynthetic pathways involving 20 or more specific enzymatic reactions could have arisen when neither intermediates nor the final end products appear to be required for growth. Such compounds might be termed idiolites, since they are synthesized during the idiophase of the growth cycle, and have a restricted biological distribution.

Our laboratory has been studying one such biosynthetic pathway, the biosynthesis of dihydrostreptomycin, an aminocyclitol antibiotic (I, Fig. 1) secreted by certain strains of filamentous soil bacteria of the genus Streptomyces. Our current concept of the biosynthesis of the streptidine moiety of dihydrostreptomycin is summarized in Fig. 2. Two analogous sequences of five enzymatic reactions each, operating in series, appear to be involved in biosynthesis of the streptidine moiety from myo-inositol (V). Each sequence consists of a hydroxy group to a guanidino group and involves, in order, a dehydrogenation (Reactions C

* This research was supported by Grant C 153 from the Robert A. Welch Foundation, Houston, Texas.
used in this paper, *S. glebosis* cultures were inoculated from slants and grown for 3 days at approximately 26° on a rotary shaker in 2-liter Erlenmeyer flasks containing 500 ml of medium composed of 0.05% glucose-1% soytone-1% tryptone-0.2% yeast extract-0.03% K2HPO4. When accumulation of amidino acceptor compounds was desired (Figs. 7 and 8A), 1% *myo*-inositol was added to the growth medium. Mycelia were harvested by suction filtration through a paper, blotted dry, and stored frozen. Extracts were prepared with a Branson Sonifier by sonication of 6 g of mycelia in 12 ml of water for 4.5 min in 1.5-min segments separated by recooling periods. Sonicates were centrifuged at 30,000 x g for 20 min, and the supernatant solutions were stored frozen. Dialyzed *S. glebosis* extracts employed for studies of Reactions C and D were prepared by thawing a supernatant solution from a sonicate and adding to 2 ml of such an extract 0.15 ml of a solution containing 0.7 m potassium phosphate and 0.3 m EDTA, pH 7.6, plus 0.04 m pyridoxal-P; the solution was dialyzed 16 hours at 4° against deionized water.

*myo*-Inositol preparations from a number of sources were employed with and without recrystallization for the experiments of Fig. 3. Purified alkaline phosphatase from *Escherichia coli* (type III), NAD, NADP, keto-scyllo-inositol, dihydrostreptobiosamine, and *pyridoxal-P* came from Sigma; L-4guanidino-3-carboxyethylamine, 46 Ci per mole, from Schwarz/Mann; 1-amino-1-deoxy-scyllo-[1-14C]-inositol; 3.5 Ci per mole, from Mallinkrodt; Dowex-50(II+), 200 to 400 mesh, resin from Bio-Rad; and *D-chiro*-inositol and L-4-chiro-inositol from Calbiochem. Aminodeoxy-scyllo-inositol was prepared by reduction of the oxime of keto-scyllo-inositol with sodium amalgam (10). Streptidine and streptamine were prepared from dihydrostreptomycin (11). 2-Deoxystreptamine was prepared from kanamycin (11). Aminocyclitols were chemically phosphorylated with P32O5 plus HPO42-, followed by treatment with Ba(OH)2 (11). For these compounds to serve as amidinotransferase substrates, residual Ba2+ should be removed by addition of Na2SO4, and EDTA should be present in the incubation mixture. EDTA also serves to inhibit phosphatase action on amidino acceptors during transamidination.

Separations of labeled compounds were performed on Whatman No. 1 filter paper by (a) ascending paper chromatography, developed with 80% phenol-20% H2O, ammonia atmosphere (provided by adding 1 ml of concentrated NH3OH to the inside of the glass chromatography cylinder); (b) high voltage paper electrophoresis with a refrigerated Savant horizontal plate apparatus. The effective path was 46 cm, 20 volts per cm. Ammonium formate buffer, pH 3.6, at 0.2 ionic strength was used. For both types of separations, strips were cut at 1-cm intervals, put in bottles containing Liquifluor in toluene, and counted with a Nuclear-Chicago liquid scintillation system. In the figures, gross counts per min are given, uncorrected for background.

**RESULTS**

The experiments which have been performed on biosynthesis of the bluensidine moiety of bluensomycin (II, Fig. 1) can be most conveniently described by referring to the reaction schemes of Fig. 2. Fig. 2 depicts our previously proposed scheme for biosynthesis of the streptidine moiety of dihydrostreptomycin (I) from glucose-6-P (III). The corresponding bluensidine moiety of bluensomycin contains one guanidino group and a carbamoyl group instead of two guanidino groups (2,4); the location of these two groups was recently established by Barlow and Anderson (5). Fig. 2 also indicates one possible pathway for biosynthesis of the bluensidine moiety; in this scheme the single guanidino group is synthesized by the same enzymes which participate in biosynthesis of the first guanidino group introduced on the inositol ring of dihydrostreptomycin. However, this guanidino group ends up at position 3 of bluensomycin, rather than position 1 as in the case of dihydrostreptomycin biosynthesis. An alternative scheme (not shown) for bluensidine biosynthesis was also considered. In the latter scheme, the single guanidino group is synthesized by the same enzymes which participate in biosynthesis of the second guanidino group introduced on the inositol ring of dihydrostreptomycin, but again ends up at position 3. In this case.
FIG. 2. Our current concept of enzymatic steps involved in biosynthesis of the bluensidine moiety of bluensomycin (II) by S. glebusus, and the streptidine moiety of dihydrostreptomycin (I) by S. humatus, starting from glucose-6-P (III). Participation of epimers of certain intermediates has not been excluded. Extracts of S. glebusus catalyze Reactions C, D, F, and G. Reaction E can be inferred from the presence of its product in mycelial extracts, and slight activity was detected in vitro. The step at which the carbamoyl group is added has yet not been established. Enzyme G apparently dephosphorylates only substrate molecules which have escaped carbamoylation. It is suggested that S. glebusus cannot synthesize dihydrostreptomycin (I) because it lacks enzymes H and I, possibly lacks enzyme J, and enzyme N' is different from enzyme N. S. glebusus extracts catalyze both Reactions F and K. Note that in these schemes, corresponding carbon atoms of the inositol moieties of dihydrostreptomycin (I) and bluensomycin (II) are derived from different carbon atoms of glucose-6-P (III). Abbreviations: DSBA, dihydrostreptobiosamine; KGAM, -ketoglutaramate; ORN, ornithine; PYR, pyruvate; NDP-SUGAR, nucleosidediphosphate-sugar.

Evidence has now been obtained for the occurrence of Reaction C in cell-free extracts of S. glebusus, when coupled with Reaction D. Diazyed extracts of S. glebusus catalyze the conversion of myo-[U-14C]inositol (V) to aminodeoxy-scyllo-[U-14C]inositol (VII) when both NAD+ and an amino donor were provided (Table I). NADP+ was not nearly so active as NAD+ as hydrogen acceptor in this system. The most active amino donors tested were L-glutamine, aminodeoxy-scyllo-inositol, and streptamine, all of which are known to be able to serve as amino donors in Reaction D of streptomycin producers (7, 9). L-Alanine was relatively inactive as an amino donor, an indication that enzyme I was not involved.

The occurrence of Reaction C in S. glebusus was confirmed by employing another version of the coupled dehydrogenase-transaminase assay system described above. In this assay, instead of the use of a low concentration of labeled myo-inositol and a high concentration of nonlabeled glutamine or aminodeoxy-scyllo-inositol as amino donors in Reaction D of streptomycin producers (7, 9), L-Alanine was relatively inactive as an amino donor, an indication that enzyme I was not involved.
myo-inositol was employed to help overcome the unfavorable equilibrium (15) of the dehydrogenation step, and the labeled reactant was the amino donor 1-amino-1-deoxy-scyllo-[1-14C]-inositol. The results are shown in Fig. 3. Both myo-inositol and NAD+ were required for conversion of labeled aminodeoxy-scyllo-inositol to labeled keto-scyllo-inositol by the sum of Reactions 1 and 2.

\[
\text{myo-Inositol} + \text{NAD}^+ \rightarrow \text{keto-scyllo-inositol} + \text{NADH} + \text{H}^+ \tag{1}
\]

Keto-scyllo-inositol + 1-amino-1-deoxy-scyllo-

\[
14\text{C} \text{inositol} \rightleftharpoons \text{aminodeoxy-scyllo-inositol} \tag{2}
\]

\[
+ 1\text{-keto-scyllo-[1-}^{14}\text{C}\text{]inositol} \tag{3}
\]

\[
\text{pyridoxal-P-enzyme} + \text{keto-scyllo-inositol} \rightleftharpoons \text{pyridoxamine-P-enzyme} + \text{aminodeoxy-scyllo-inositol} \tag{4}
\]

\[
\text{L-arginine-glycine amidinotransferase} \quad \text{from vertebrates} \quad \text{and}\]

\[
\text{L-arginine:glycine amidinotransferase} \quad \text{from Streptomyces} \quad \text{and}\]

\[
\text{Streptomyces}\]

\[
\text{bluemsonina} \quad \text{moiety of bluemsonina} \quad \text{have been found to catalyze transfer of an amimidino group}
\]

---

**TABLE I**

**Enzymatic conversion of myo-[U-14C]inositol to aminodeoxy-scyllo-[U-14C]inositol by dialyzed extract of *S. glebosus* in the presence of myo-inositol (1) and NAD+**

| Substrates | Aminodeoxy-scyllo-[U-14C]inositol formed (cpm/10 µL) |
|------------|---------------------------------------------------|
| Experiment 1 | |
| myo-[U-14C]inositol | 40 |
| myo-[U-14C]inositol + NAD+ | 200 |
| myo-[U-14C]inositol + aminodeoxy-scyllo-inositol | 270 |
| myo-[U-14C]inositol + NAD+ + aminodeoxy-scyllo-inositol | 1,260 |
| Experiment 2 | |
| myo-[U-14C]inositol + NAD+ | 120 |
| myo-[U-14C]inositol + NAD+ + aminodeoxy-scyllo-inositol | 1,100 |
| myo-[U-14C]inositol + NAD+ + L-glutamine | 770 |
| myo-[U-14C]inositol + NAD+ + L-alanine | 110 |
| myo-[U-14C]inositol + NAD+ + streptamine | 740 |

---

**Fig. 3.** Enzymatic conversion of 1-amino-1-deoxy-scyllo-[1-14C]-inositol (IN) to 1-keto-scyllo-[1-14C]inositol (IO) by a dialyzed extract of *S. glebosus* in the presence of myo-inositol (1) and NAD+. The complete incubation mixture contained: 1-amino-1-deoxy-scyllo-[1-14C]inositol, 5 µL (68,000 cpm); 100 mM potassium phosphate, pH 7.4, containing 13 mM EDTA and 8 mM pyridoxal-P, 5 µL; 110 mM myo-inositol (twice recrystallized), 5 µL; 3 mM NAD+, 5 µL; dialyzed extract of *S. glebosus*, 10 µL; and water to 30 µL final volume. After incubation at 35° for 145 min, 10-µL aliquots were spotted at 30 cm and separated by high voltage paper electrophoresis at pH 3.6. Picric acid, as a standard marker, migrated 14 cm on an adjacent strip. A, complete incubation mixture minus myo-inositol. B, complete incubation mixture minus NAD+. C, complete incubation mixture. Only in this tube was a significant amount of 1-keto-scyllo-[1-14C]inositol (IO) formed, indicating the necessity for Reaction 1 to occur before the exchange of Reaction 2 can take place.

---

**Fig. 4.** Enzymatic conversion of 1-amino-1-deoxy-scyllo-[1-14C]-inositol (IN) to 1-keto-scyllo-[1-14C]inositol (IO) by a dialyzed extract of *S. glebosus* in the presence of nonlabeled keto-scyllo-inositol (Reaction 3). Conditions employed were identical with those in Fig. 3, except that 5 µL of 28 mM nonlabeled keto-scyllo-inositol replaced myo-inositol plus NAD+ in the complete incubation mixture. This experiment confirmed the Reaction 3 component of the coupled reactions employed in Table I and Fig. 3.
from arginine to hydroxyamine to form hydroxyguanidine, as indicated in Reaction 7. This reaction is believed to be the sum of Reactions 5 and 6 (18). As is the case for streptomycin producing strains (11), extracts of S. glebosus catalyzed L-arginine: L-Arginine + enzyme-SH := L-ornithine

\[ \text{Sum: L-Arginine + NH}_2\text{OH} \rightarrow L-\text{ornithine} \] (7)

NH\(_2\)OH transamidination. As expected, amidinotransferase activity of harvested mycelia varied markedly with the age of culture (cf. 19), composition of growth medium, pH, temperature, and state of inoculum.

The next question concerned the amidino acceptor specificity of S. glebosus amidinotransferase. The L-arginine:inosamine-P amidinotransferase occurring in streptomycin producing strains of Streptomyces has a substrate specificity which can be depicted in part as shown in Fig. 5 (18). Certain of these substrates were tested with S. glebosus extracts, with the results shown in Fig. 6. The following inosamine derivatives, prepared by nonspecific chemical phosphorylation (11), were found to serve as amidino acceptors with L-[guanidino-\(^{14}\)C]arginine as donor: Compound XVa (Fig. 6A); Compound XVb (Fig. 6B); Compound XVc (Fig. 6C); and the 2-deoxy derivative of Compound XVc (Fig. 6D). Fig. 6A corresponds to Reaction F of Fig. 2, and Fig. 6B corresponds to Reaction K of Fig. 2. The substrate specificity of S. glebosus amidinotransferase could not be distinguished by these experiments from the substrate specificity of amidinotransferase of streptomycin producers. The results of Fig. 6B suggest that streptidine phosphate, and presumably dihydrostreptomyein, would be produced by S. glebosus mycelia if Compound XIII could be synthesized. Evidently the absence of enzymes II and I preclude that possibility.

Detection of Physiological Amidino Acceptors in S. glebosus—A search for physiological amidino acceptors (X-NH\(_2\)) in extracts of S. glebosus was next undertaken, employing Reaction 8 as an assay.

L-[guanidino-\(^{14}\)C]arginine + X-NH\(_2\) \[ \rightarrow \] L-ornithine + X-NH-\(^{14}\)C=NH\(_2\)+NH\(_2\) (8)

In these experiments the supernatant solutions from sonicated mycelia of S. glebosus were used as a source of both amidino acceptors and amidinotransferase activity. When such extracts were incubated with labeled arginine as amidino donor, a single peak containing radioactive products was obtained after paper chromatographic separation, as shown in Fig. 7. In contrast, it will be recalled that two distinct peaks were obtained with extracts of streptomycin producing strains, corresponding to Compounds IX and XIV of Fig. 2 (11). The presence of myo-inositol in the growth medium increased the concentrations of amidino acceptors in S. glebosus mycelia. Furthermore, when the radiochemical enzymatic assay for amidino acceptors (Reaction 8) was con-

FIG. 6. Some reactions catalyzed by S. glebosus amidinotransferase with chemically phosphorylated aminocyclitols as amidino acceptors. In each case the enzyme selected the proper positional isomer from the mixture of phosphorylated derivatives, presumably according to the specificities indicated in Fig. 5 (cf. 11). Each incubation mixture contained: l-[guanidino-\(^{14}\)C]arginine, 33 μCi per ml; 5 μl; 0.5 μl Tris, pH 7.4, containing 13 mM EDTA, 5 μl; 0.5 ml mercaptoethanol, 1 μl; dialyzed S. glebosus extract, 10 μl; and 10 μl of a solution containing the indicated chemically phosphorylated aminocyclitol. After incubation at 35° for 150 min, 10 μl were spotted and separated on ammoniacal phenol paper chromatograms. A, chemically phosphorylated aminodeoxy-scyllo-inositol as acceptor, giving l-guanidino-1-deoxy-scyllo-inositol-4-P (IGP). B, chemically phosphorylated monomonomidinated streptamine isomers as acceptor, giving l-streptidine-6-P (IGG). C, chemically phosphorylated streptamine as acceptor, giving 1D-1-amino-3-guanidino-1,3-dideoxy-scyllo-inositol-6-P (INGP). D, chemically phosphorylated 2-dideoxystreptamine as acceptor, giving 1D-1-amino-3-guanidino-1,2,3-trideoxy-scyllo-inositol-6-P (diINGP).

FIG. 7. Physiological amidino acceptors in extracts of S. glebosus grown in presence of myo-inositol. A nondialyzed supernatant solution from sonicated mycelia was the source of both amidinotransferase and amidino acceptors. More labeled products were formed when carbamoyl-P was present during incubation (solid curve) than when it was omitted (dashed curve). The single peak formed subsequently was shown to contain two different monoguanidinated, monophosphorylated compounds, 1-guanidino-1-deoxy-scyllo-inositol-4-P (XGP) and an unknown compound (XGP). The complete incubation mixture contained: l-[guanidino-\(^{14}\)C]arginine, 33 μCi per ml, 5 μl; 0.5 μl Tris, pH 7.4, containing 13 mM EDTA, 5 μl; 33 mM dithiolum carbamoyl-P, 5 μl; nondialyzed S. glebosus extract, 10 μl. After incubation at 36° for 115 min, 10 μl aliquots were spotted and separated by paper chromatography with ammoniacal phenol.
Transamidination products were not separated from each other. The peak component refractory to dephosphorylation, XGP, was eluted first with 0.5 M HCl (tubes 1 to 20), followed by elution of guanidino-deoxy-scyllo-inositol (IG) with 1.0 M HCl (tubes 21 to 29).

The amidinated reaction products produced in a scaled up incubation mixture could be separated from other labeled compounds in a single peak on a Dowex 50(H\(^+\)) column, as shown in Fig. 8A. The isolated radioactive compounds migrated similarly to Compound IX during paper chromatography at pH 3.6 and pH 10.4. These compounds could be dephosphorylated by incubation with *Escherichia coli* alkaline phosphatase to give compounds with mobilities similar to Compound X. Evidently the labeled peak contained compounds possessing one guanidino group and one phosphate ester group. The occurrence of enzyme E in *S. glebosus* could be inferred from the fact that the amidino acceptors are phosphorylated. Weak enzyme E activity was occasionally detected in *S. glebosus* extracts, but enzyme J was not detected in the same extracts.

A hint that the labeled peak of Fig. 8A contained a compound, XGP, different from Compound IX came from its slightly higher RF value on ammoniacal phenol paper chromatograms; its dephosphorylated derivative also had a slightly higher RF than Compound X. The question then arose whether the postulated XGP was a carboxamidylated derivative of Compound IX. Such a compound would be difficult to distinguish from Compound IX by the methods described above, but it might react differently from Compound IX when incubated with extracts containing enzymes G, H, and I of Fig. 2.

**Presence of Reaction G Activity in S. glebosus—Contrary to our expectations,** we have isolated a compound, XGP, in significant amounts from extracts of *S. glebosus* grown on arginine in the presence of Mg\(^2+\) and absence of EDTA. The isolated radioactive compound XGP was only partially dephosphorylated with *E. coli* alkaline phosphatase, as shown in Fig. 9A. However, complete dephosphorylation of 1-[\(^{14}C\)]guanidino-1-deoxy-scyllo-inositol-4-P (IGP) was achieved by incubation with enzyme G, as shown in Fig. 9C. Alternatively, enrichment of the XGP component could be obtained by performing the initial transamidination reaction with *S. glebosus* and labeled guanidino compounds, followed by chromatography on a Dowex-50 column as before, as shown in Fig. 8B. The resulting XGP component was resistant to dephosphorylation by enzyme G, as shown in Fig. 9C. Alternatively, enrichment of the XGP component could be obtained by performing the initial transamidation reaction with *S. glebosus* and labeled guanidino compounds in the presence of Mg\(^2+\) and absence of EDTA, to allow any Compound IX formed to be dephosphorylated by enzyme G present in the same extract.

**Further Characterization of Unknown Physiological Transamidination Product (XGP)—** When the labeled preparation (XGP) of Fig. 8B was dephosphorylated with *E. coli* alkaline phosphatase, the resulting compound (XG) could not serve as a substrate for enzymes H plus I from a streptomycin producer, as shown in Fig. 10A. These results demonstrated that XG is not Compound X. However, hydrolysis of XGP with 6 N HCl at 100°C for 28 hours gave a compound which did serve as a substrate for enzymes H plus I, as shown in Fig. 10B. This treatment with 6 N HCl removed the phosphate group and would hydrolyze any carboxamidyl ester present (4). The reaction product (IGN) of Fig. 10B appears to be Compound XII. These results are consistent with XGP being bluensidine-6-P, but this has not yet been rigorously established, of course.

**Discussion**

At the outset of this investigation, little was known of the enzymatic steps involved in biosynthesis of bluensomycin, although suggestions had been made on the basis of preliminary findings (11). Our experimental results are consistent with the general scheme shown in Fig. 2 for biosynthesis of the mono-guanidinated inositol (bluensidine) moiety of bluensomycin (11).
The step at which carbamoylation occurs is not yet known. ISL>TA, 1~1; and 0.33 M EDTA, 1~1; and 0.33 M L-alanine, 2~1. After further incubation at 35° for 180 min, 10-~1 aliquots were spotted at 30 cm and separated by high voltage paper electrophoresis at pH 3.6. Picric acid migrated 13 cm. No phosphorylated substrate remained. A, curve showing no further enzymatic transformation of dephosphorylated unknown transamination product (XG) by enzymes H plus I; R, curve showing formation of compound with mobility of 1D1-1-guanidino-3-amino-1,3-dideoxy-scyllo-inositol (IGN) from XG previously hydrolyzed with 6 N HCl.

The biosynthetic scheme of Fig. 2 is consistent with the recent stereospecific assignments for the bluensidine moiety by Barkow and Anderson (5). Their assignments, arrived at by physicochemical means (5), are compatible with the substrate specificities of (a) enzyme F, assuming that R can also be a carbamoyl ester (Fig. 5); and (b) enzymes L and M, assuming that their substrates must be phosphorylated and at the position indicated in Fig. 2.

Although myo-inositol dehydrogenase has been studied in a number of organisms (15, 21, 22), the enzymatic experiments described in Table I and Fig. 3 have provided the first evidence for Reaction C obtained in cell-free extracts of Streptomyces. It was necessary to couple Reaction C with Reaction D to demonstrate this dehydrogenation. Our findings in vitro thus complement the extensive studies in vitro by Horrier and others on the roles of myo-inositol (23-25), keto-scyllo-inositol (13, 26), and scyllo-inositol (12, 13) as precursors of the streptidine moiety of streptomycin. It is still not known why this particular myo-inositol dehydrogenation reaction has been so difficult to demonstrate in vitro in Streptomyces which produce the streptomycin family of antibiotics. myo-Inositol dehydrogenases from other sources can readily be assayed in the reverse direction i.e., reduction of keto-scyllo-inositol by NADH (15, 21), but this reverse assay has not so far proved useful with Streptomycetes extracts. Further studies will be necessary to determine whether this myo-inositol dehydrogenase, as an early enzyme in a biosynthetic pathway, has an important regulatory function; regulatory enzymes often present assay or stability problems. The details of its linkage with electron transport enzymes should also prove of interest.

Although the stage in bluensomycin biosynthesis at which carbamoylation occurs is not yet known, despite our efforts in that direction, the unknown transamination product (XGP) obtained on incubation of S. globosus extracts with labeled arginine (Figs. 7 to 10) has many of the properties expected for bluensidine-6-P. The substrate specificity of enzyme F (Fig. 5) would probably permit a carbamoyl group at the indicated position. Any intermediates which escaped carbamoylation would probably be enzymatically converted to Compound X, in view of the presence of enzyme G activity in S. globosus (Fig. 9). No kinase activity with Compound X as acceptor has been detected in S. globosus or S. bikijiiensis.

The mechanism of the marked enhancement of transamination reactions by carbamoyl-P observed in vitro with nondialyzed mycelial extracts (Fig. 7) remains unknown. Enhancement might result from: (a) carbamoylation of an inosamine derivative to give a substrate with a lower K_m or higher V_max with amidinotransferase; (b) carbamoylation of ornithine, a strongly inhibitory amidinotransferase, to form noninhibitory citrulline; or (c) an allosteric activation of amidinotransferase. This phenomenon will be examined further.

It is too early in our investigation to draw definitive conclusions concerning the evolutionary relationships between the respective enzymatic pathways for biosynthesis of bluensomycin and dihydrostreptomycin. However, two possibilities will be briefly considered as a framework for future experiments.

1. One possibility is that bluensomycin producers resemble an ancestral strain, or contain an ancestral epimorph, which has not undergone gene duplication in the guanidinocyclopentitol biosynthetic pathway. Bluensomycin producers can add only one guanidine group to the inositol ring, because they lack genes coding for enzymes II and I. If it turns out that enzymes E and J are coded by different genes, gene J would be missing. Bluensomycin producers presumably require an additional gene which codes for a carbamoylation enzyme. In this scenario, dihydrostreptomycin producing strains are descendants of the above ancestral strain, or contain epimorphs, which have undergone duplication and subsequent independent mutation of genes coding for Reactions C and D, and possibly E, F, and G. The nature of any selective pressure is not known since the physiological functions of these idiobites have not been established (9). However, dihydrostreptomycin is approximately 10 times more effective than bluensomycin as an antibiotic and inhibitor of protein synthesis (6), and therefore might represent a later evolutionary product.

2. Another possibility is that bluensomycin producers are derived from dihydrostreptomycin producers. In this scenario,
addition of a gene coding for a carbamoylation enzyme resulted in synthesis of bluensidine-6-P, which cannot be dephosphorylated by enzyme G. Bluensidine-6-P reacts in the presence of enzyme L and subsequent enzymes to form bluensomycin rather than dihydrostreptomycin. There would be no further need for enzymes H through K, since their substrates would no longer be formed. Genes coding for these latter enzymes could then be lost, further mutated to serve new functions, or be repressed. Again, the selection pressures are not known.

REFERENCES
1. Dyer, J. R. & Todd, A. W. (1963) J. Amer. Chem. Soc. 85, 3896–3897
2. Bannister, B. & Argoudelis, A. D. (1963) J. Amer. Chem. Soc. 85, 119–120
3. Bannister, B. & Argoudelis, A. D. (1963) J. Amer. Chem. Soc. 85, 234–235
4. Naito, T. (1962) Penishirin Sono Ta Koseibusshitsu 16, 373–379 (CA, 60, 4230e (1964))
5. Barlow, C. B. & Anderson, L. (1972) J. Antibiotics 25, 281–286
6. Okanishi, M., Koshiyama, H., Ohmori, T., Matsuzaki, M., Ohashi, S. & Kawaguchi, H. (1962) J. Antibiotics, Ser. A 15, 7–13
7. Walker, J. B. & Walker, M. S. (1969) Biochemistry 8, 763–770
8. Walker, M. S. & Walker, J. B. (1971) J. Biol. Chem. 246, 7034–7040
9. Walker, J. B. (1971) Lloydia 34, 363–371
10. Anderson, L. & Iarrot, H. A. (1960) J. Amer. Chem. Soc. 72, 3141–3147
11. Walker, M. S. & Walker, J. B. (1966) J. Biol. Chem. 241, 1262–1270
12. Breton, J., Horner, W. H. & Russ, G. A. (1967) J. Biol. Chem. 242, 813–818
13. Horner, W. H. & Thaker, I. H. (1968) Biochim. Biophys. Acta 165, 306–308
14. Barnett, J. E. G., Rasheed, A. & Corina, B. J. (1973) Biochim. Biophys. J. 131, 21–30
15. Vidal-Leiria, M. & Van Uden, N. (1973) Biochim. Biophys. Acta 293, 295–303
16. Walker, J. B. (1960) J. Biol. Chem. 235, 2357–2361
17. Walker, J. B. (1968) J. Biol. Chem. 231, 1–9
18. Walker, J. B. (1974) Enzymes 9, 97–500
19. Walker, J. B. & Hinrichs, V. S. (1964) Biochim. Biophys. Acta 89, 473–482
20. Bruce, R. M., Ragheb, H. S. & Weiner, H. (1968) Biochim. Biophys. Acta 158, 499–500
21. Berman, T. & Magasanik, B. (1966) J. Biol. Chem. 241, 800–806
22. Candy, D. J. (1967) Biochem. J. 103, 666
23. Majumdar, S. K. & Kutner, H. (1962) Appl. Microbiol. 10, 157–168
24. Horner, W. H. (1964) J. Biol. Chem. 239, 2256–2258
25. Hedinger, H. (1964) Science 143, 983–984
26. Horner, W. H. & Russ, G. A. (1969) Biochim. Biophys. Acta 129, 352–354
Biosynthesis of the Monoguanidinated Inositol Moiety of Bluensomycin, a Possible Evolutionary Precursor of Streptomycin

James B. Walker

*J. Biol. Chem.* 1974, 249:2397-2404.

Access the most updated version of this article at [http://www.jbc.org/content/249/8/2397](http://www.jbc.org/content/249/8/2397)

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/249/8/2397.full.html#ref-list-1](http://www.jbc.org/content/249/8/2397.full.html#ref-list-1)