TRANSAMINATIONS INVOLVING KETO- AND AMINO-INOSITOLS AND GLUTAMINE IN ACTINOMYCETES WHICH PRODUCE GENTAMICIN AND NEOMYCIN

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SUMMARY: Extracts of Micromonospora purpurea and Streptomyces fradiae, which respectively synthesize the 2-deoxystreptamine antibiotics gentamicin and neomycin, catalyze the following transaminations: L-glutamine:keto-scyllo-inositol; aminodeoxy-scyllo-inositol:keto-scyllo-inositol; 2-deoxystreptamine: keto-scyllo-inositol; and streptamine:keto-scyllo-inositol. These results suggest that at least one of the two amino groups of (a) the streptamine moiety of 2-hydroxygentamicin, and (b) the 2-deoxystreptamine moieties of gentamicin and neomycin, is derived by transamination from the 2-amino group of L-glutamine to keto-inositol derivatives.

The aminated inositol derivative, 2-deoxystreptamine(I, Fig. 1), is a component of the clinically important antibiotics neomycin, kanamycin, gentamicin, paromomycin, butirosin, and tobramycin (1). Nothing is known of the enzymic reactions responsible for biosynthesis of 2-deoxystreptamine, although a sound basis for experimentation has been afforded by the in vivo studies of Rinehart and coworkers on the biosynthesis of neomycin (1) and of Daum and colleagues on the biosynthesis of gentamicin (2). We have directed our attention initially to the enzymic reactions responsible for formation of the two amino groups of 2-deoxystreptamine. We reasoned that transamination reactions analogous to those occurring in the biosynthesis of the aminocyclitol moieties of streptomycin (3) and bluensomycin (4) might be involved in biosynthesis of 2-deoxystreptamine, and that the responsible aminotransferase(s) might also react with hydroxylated analogs of deoxyinositol intermediates, as suggested by the in vivo studies of Daum et al. (2).

MATERIALS AND METHODS

The gentamicin producer, Micromonospora purpurea ATCC 15385, and the neomycin producer, Streptomyces fradiae ATCC 10745, were obtained from the American Type Culture Collection. Keto-scyllo-inositol(II; scyllo-inosose; myo-inosose-2) came from Sigma. Aminodeoxy-scyllo-[1-^{14}C]inositol(III; scyllo-inosamine), 3.5 Ci/mol, came from California Bionuclear Corp. 2-Deoxystreptamine and streptamine(IV) were prepared as described elsewhere (5). Keto-scyllo-[1-^{14}C]inositol was prepared by enzymic transamination of aminodeoxy-scyllo-

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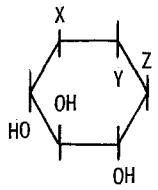

	X	Y	Z
I	NH_2	H	NH_2
II	OH	OH	=O
III	OH	OH	NH_2
IV	NH_2	OH	NH_2
V	NH_2	H	=O
VI	OH	H	=O
VII	=O	H	OH
VIII	OH	H	NH_2
IX	NH_2	H	OH

Fig. 1. Structures of various potential aminotransferase substrates.

[1-^{14}C]inositol with Na pyruvate and separated by passing the incubation mixture through successive columns of Dowex-50(H^+) and Dowex-1(Cl^-) resins; labeled keto-scyllo-inositol appeared in the water wash of each column. The final eluate was evaporated to dryness and taken up in a minimal amount of water. S. fradiae mycelia were grown for 2 days at 26° on 1% glucose-1% peptone-0.2% yeast extract. M. purpurea was grown for 2 days at 35° on the medium described elsewhere (6). Growth and harvesting of mycelia, sonication, high voltage paper electrophoresis, and scintillation counting procedures have been described (7). Transamination assays were performed as described previously (8).

RESULTS

Dialyzed extracts of both M. purpurea and S. fradiae catalyzed the exchange transamination of Reaction 1 (Fig. 2).

$$\text{aminodeoxy-scyllo-[}^{14}\text{C]inositol} + \text{keto-scyllo-inositol} \rightleftharpoons \text{keto-scyllo-[}^{14}\text{C]inositol} + \text{aminodeoxy-scyllo-inositol} \quad (1)$$

A low level of exchange transamination could also be observed in nondialyzed extracts of both organisms, as had been observed in our laboratory several years ago with S. fradiae (unpublished data). Dialysis stimulated the measured rates of exchange 3- to 5-fold, presumably by removing competing endogenous amino donors. The presence of added pyridoxal-P during dialysis was not required for activity, but its presence increased activity by 30% to 100%.

Among the compounds available and tested as amino acceptors, keto-scyllo-inositol had the highest acceptor activity (Fig. 3A), followed by pyruvate (Fig. 3B) and then 2-ketoglutarate.

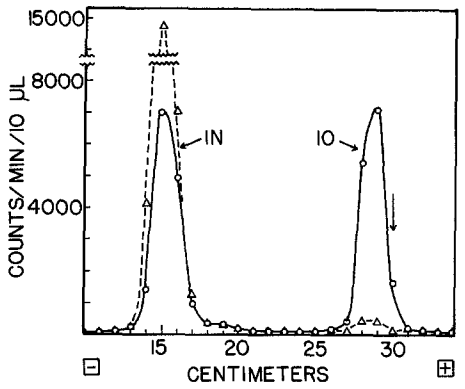

Fig. 2. Aminodeoxy-scyllo-[^{14}C]inositol(IN):keto-scyllo-inositol(IO) exchange transamination catalyzed by a dialyzed extract of M. purpurea. After a 2-hour incubation reactants and products were separated by high voltage paper electrophoresis at pH 3.6. Labeled keto-scyllo-inositol(IO) was formed only when non-labeled keto-scyllo-inositol was present (solid curve) as amino acceptor. Similar results were obtained with extracts of S. fradiae.

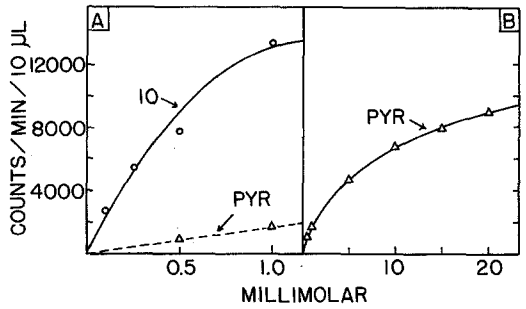

Fig. 3. Comparison of (A) keto-scyllo-inositol(IO) and (B) pyruvate(PYR) as amino acceptors from aminodeoxy-scyllo-[^{14}C]inositol in transaminations catalyzed by a dialyzed extract of M. purpurea. After a 2-hour incubation the labeled keto-scyllo-inositol formed was measured following separation by high voltage paper electrophoresis at pH 3.6.

A number of amino acids were tested as potential amino donors to keto-[^{14}C]-inositol. The most active of these was L-glutamine (Fig. 4A). The next most active amino acid donor tested was L-alanine, followed by L-glutamate, but the latter compounds were significantly active only at concentrations above 10 mM. Compounds relatively inactive as amino donors included: L-asparagine, D-glutamine, L-aspartate, glycine, D-glucosamine, L-glutamic-γ-hydroxamate,

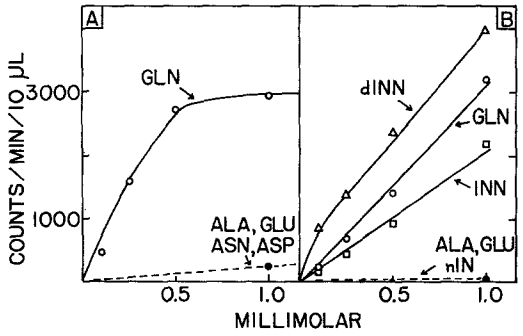

Fig. 4. Comparison of (A) various amino acids and (B) various aminocyclitols as amino donors to keto-scyllo-[^{14}C]inositol in transaminations catalyzed by dialyzed extracts of M. purpurea for (A) and S. fradiae for (B). After a 2-hour incubation the labeled aminodeoxy-scyllo-inositol formed was measured following separation by high voltage paper electrophoresis at pH 3.6. 2-Deoxystreptamine(dINN); streptamine(INN); 2-amino-2-deoxy-neo-inositol(nIN).

L-methionine-DL-sulfoximine, L-2-amino-3-ureidopropionate, O-carbamoyl-L-serine, S-carbamoyl-L-cysteine, L-methionine, and DL-phenylalanine. D-Alanine was slightly less active than L-alanine in these crude extracts, which presumably contain alanine racemase.

Both 2-deoxystreptamine and streptamine were very active as amino donors with keto-scyllo-[^{14}C]inositol as acceptor (Fig. 4B). 2-Amino-2-deoxy-neo-inositol was not an active amino donor. We have not yet established which amino group of 2-deoxystreptamine participates in this transamination, but preliminary data suggest that it is probably position 1, giving Compound V as the transamination product.

DISCUSSION

Cultures of a 2-deoxystreptamine-less idiotroph of M. purpurea have recently been reported by Daum et al. (2) to convert exogenous keto-scyllo-inositol(II), aminodeoxy-scyllo-inositol(III), and streptamine(IV) to 2-hydroxygentamicin, and to convert exogenous Compound VI (or VII), as well as 2-deoxystreptamine(I), to gentamicin. Our cell-free enzymic studies (Figs. 2 and 4A) suggest that the first step in the conversion of keto-scyllo-inosi-

tol to the streptamine moiety of 2-hydroxygentamicin by M. purpurea involves Reaction 2.

L-glutamine + keto-scyllo-inositol $\rightleftharpoons$ 2-ketoglutaramate + aminodeoxy-scyllo-inositol (2)

L-glutamine + Compound VI(or VII) $\rightleftharpoons$ 2-ketoglutaramate + Compound VIII(or IX) (3)

A similar transamination occurs during biosynthesis of the guanidinocyclitol moieties of streptomycin (3,8) and bluensomycin (4) by other species of actinomycetes, which might indicate a common genetic origin for key steps in biosynthesis of these diverse aminocyclitol antibiotics. We suggest that keto-scyllo-inositol is an analog of the actual amino acceptor occurring in gentamicin and neomycin producers, and that the aminotransferase responsible for Reaction 2 normally catalyzes Reaction 3 as an early step in biosynthesis of the 2-deoxystreptamine moieties of gentamicin and neomycin (cf. 2).

It is of particular interest that these mycelial extracts can catalyze transaminations involving 2-deoxystreptamine (Fig. 4B). Studies are in progress to determine (a) which amino group of 2-deoxystreptamine is involved in transamination with keto-scyllo-inositol, and (b) whether a single aminotransferase or two different aminotransferases participate in biosynthesis of the 2-deoxystreptamine moieties of gentamicin, neomycin, and related antibiotics.

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