[S5-3] [10/11/2003(Sat) 11:10-11:50/ Grand Ballroom 102]

# Biosynthesis of 2-deoxystreptamine Containing Aminoglycosides

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## Introduction:

Aminoglycoside antibiotics are among the chlinically important antibiotics and a major structural feature is the existence of characteristic aminocyclitol aglycones [1]. Considering the structural features, aminoglycosides are classified in to two classes. The first are those containing fully substituted aminocyclitol such as streptomycin, hygromycin, fortimycin, etc. These antibiotics are proposed to be biosynthesized from *myo*-inositol, which is ubiquitous in plants, animals and microorganisms [2]. The second class of antibiotics belong kanamycin, gentamicin, tobramycin, ribostamycin etc. which contain a unique aminocyclitol, 2-deoxystreptamine (DOS), which is only found in these antibiotics representing the typical secondary metabolic product [3].

Although several efforts have been carried out for understanding the molecular mechanism and biosynthetic routes of several catarogies of antibiotics; for example, β-lactams (penicillin and cephalosporins), nonribosomal peptides (cyclosporins, gramicidins), polyketides (macrolides, tetracyclins, anthracyclines etc) and fully substituted aminocyclitols (streptomycin, bluensomycin etc.), a little information exists about the biosynthesis of DOS-containing aminoglycosides, which represent of the majority of clinically important aminoglycosides. A few biosynthetic steps of butirosin have been verified recently [4], which represents the sole biochemical and genetic information for the DOS containing aminocyclitols (C). Among the genes in the cluster, L-glutamine: 2-deoxy-*scyllo*-inosose (DOI) aminotransferase (*btr*S), DOI synthase (*btr*C) and glycosyltransferase (*btr*M) have been characterized by gene expression or disruption experiments [4-5]. Those are the sole information available regarding the genetics and biochemistry of DOS-containing aminoglycosides.

Being the most of such aminoglycosides are obtained from actinomycetes, not a single has been isolated and characterized yet. Due to the lack of information about the aminoglycoside biosynthetic routes, cloning and of such genes is rather difficult. In this context, we have afforded the isolation of these gene clusters (gentamicin, kanamycin and tobramycin) from *Streptomyces* and *Micromonospora* species and their characterization. Furthermore, a systematic approach has been proposed for cloning of such genes.

Biosynthesis of DOS begins with carbocycle formation catalyzed by 2-deoxy-*scyllo*-inosose synthase converting glucose-6-phosphate (fig.1, A) to 2-deoxy-*scyllo*-inosose (B) in the presence of $NAD^+$ coenzyme [6]. Further, the biosynthetic intermediate is believed to undergo the first transamination to yield C followed by dehydrogenation to yield D, and second transamination to yield DOS (E) [4]. Other amino-sugars become appended to DOS by the activities of one or more glycosyltransferases resulting into the generation of myriads of bioactive aminoglycosides (Fig.1).

## Results and Discussions

Partial gene sequences of DOI synthase (345 bp) and DOI aminotransferases (255) were isolated form the different aminoglycosides producer actinomycetes (*S. kanamyceticus*, *S. tenebrarius*, *S. ribosidificus* and *M. inyoensis*). Genomic library of *S. kanamyceticus*, *M. purpurea* and *S. tenebrarius* were constructed, and screened out some cosmids positive to DOI synthase and L-glutamine: DOI aminotransrerase probes. PGEN01 from genomic library of *M. purpurea* (gentamicin producer), pSKC2 from *S. kanamyceticus* (kanamycin producer) and pST51 from *S. tenebrarius* (tobramycin producer) were taken for the sequencing. The sequencing of those cosmids revealed the several open reading frames (ORFs); DOI synthase, L-glutamine: DOI aminotransferases, oxidoreductase, dehydrogenase, glycosyltransferase, carbamoyl transferase etc. Their possible biosynthetic functions were assigned on the basis of amino acids identities with the proteins in the databases. The genes were found to be in the cluster, and one or more resistance genes (ribosomal methylases or phosphotransferases or acetyltransferases) were found together with the biosynthetic genes (Fig. 2). Transport and efflux proteins found in each cluster may have been involved in the export of antibiotics out from the cells.

DOI synthases from each cluster were cloned into either pET32a or pRSET B vectors for the expression in *E. coli* BL21 (DE3) under control of T7 promoter. Induction conditions were optimized for the over expression of DOI synthase as soluble protein. When the assay of DOI synthase was carried out using the soluble crude cell extract, conversion of glucose-6-phosphate to DOI was detected in TLC, HPLC, MALDI-Mass, and finally confirmed by $^1$HNMR. DOI synthases retained all the proposed substrate and cofactor binding domains [7]. In a parallel experiment, gentamicin resistant ribosomal methylase (ORF3) from gentamicin biosynthetic pathway was cloned and expressed in *E. coli.* The bacteria harboring the recombinant plasmid showed higher level of resistance against gentamicin when cultured in Luria Bertani (LB) supplemented with antibiotic (*100* μg/ml).

Similarly, L-glutamine: DOI aminotransferase and dehydrogenase from kanamycin biosynthetic gene cluster were also characterized. These results led us to the conclusion that the isolated genes have active role in the biosynthesis of aminoglycosides. Similar organizations of biosynthetic genes were found in all cases studied. Several hypothetical proteins were assigned in each cluster that can have novel activities, and they may have been involved in the biosynthesis of other several unique sugar moieties.

Since most of the aminoglycoside antibiotics have several side effects, search of new antibiotics or modified aminoglycosides or development of hybrid aminoglycosides are attractive areas for the biochemists. Development of such bioactive materials by synthetic approach is trivial due to the complications in the control of stereochemistry. So, indeed, the pathway modifications, amplification or regulations for the production of secondary metabolites by genetic manipulations can be a valuable tool. In this context, the gene clusters of above-mentioned aminoglycosides provide a ground to pave the way for the discovery of novel antibiotics that can fulfill the current clinical demands.

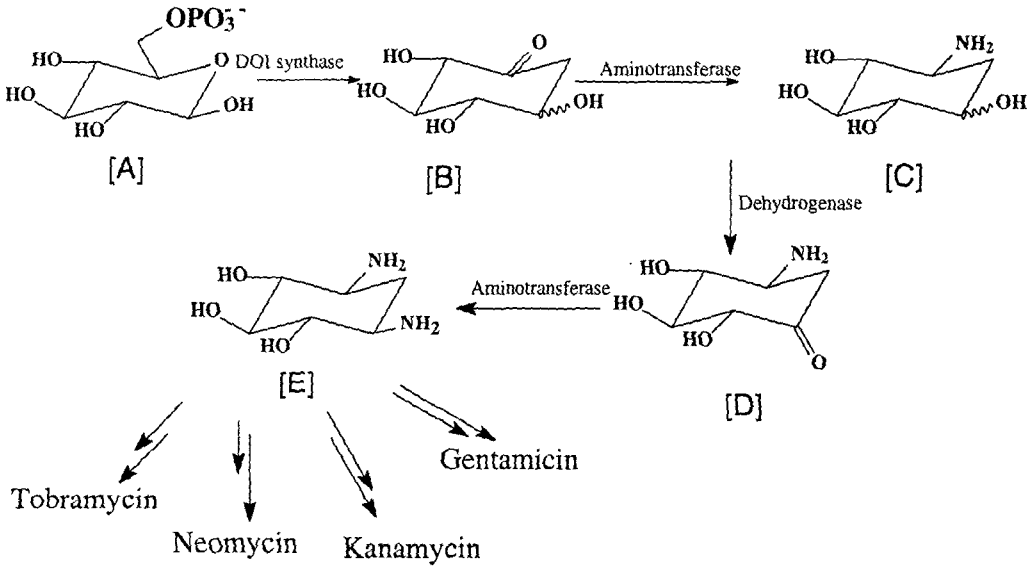


**Figure 1.** Schematic representation of DOS biosynthetic pathway

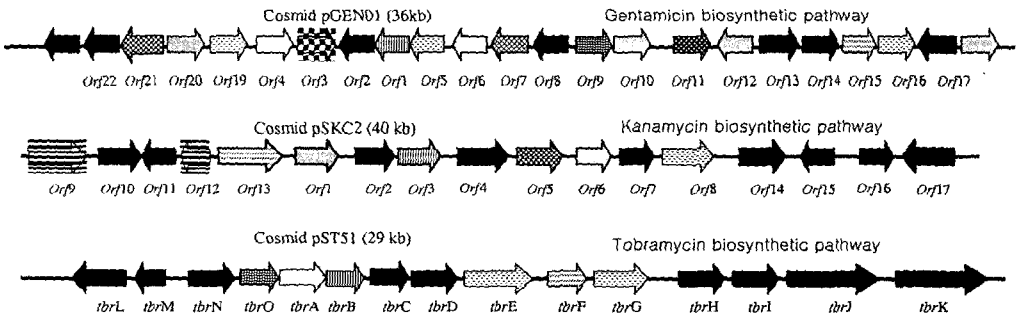


**Figure 2.** Biosynthetic gene clusters of gentamicin, kanamycin and tobramycin. The similar types of arrows indicated the proteins having similar functions.

## References


1. Rinehart, K. L. Jr. and R. M. Stroishane. *J. Antibiot.*, *29*, 319-353 (1976).
2. Munro, M. H. G.; Taniguchi, M.; Rinehart, K. L. Jr.; Gottlieb, D.; Stoudt, T. H. and T. O. Rogers. *J. Am. Soc.*, **97**, 4782-4783 (1975).
3. Yamauchi, N. and Katsumi Kakinuma: *J. Org. Chem.*,**60**, 5614-5619 (1995).
4. Ota, Y.; Tamegai, H.; Kudo, F.; Kuriki, H.; Takeshita-Koike, A.; Eguchi, T. and K. Kakinuma. *J. Antibiot.* **53**, 1158 -1167 (2000).
5. Tamegai, H.: Nango, E; Kuwahara, M.; Yamamoto, H.; Ota, Y.; Kuriki, H.; Eguchi, T. and K. Kakinuma. *J. Antibiot.* **55,** 707-714 (2002).
6. Yamauchi, N. and K. Kakinuma. *J. Antibiot.* **45,** 774-780 (1992).
7. Eguchi, T.; Sasaki, S.; Huang, Z and K. Kakinuma. *J. Org.Chem.*, **67**, 3979-3984 (2002).