Enzymatic catalysis in non-aqueous media has progressed from using enzymes in aqueous solutions containing relatively low fractions of water-miscible organic solvents to that in biphasic aqueous-organic mixtures, to that in microemulsions and reversed micelles, to that in monophasic organic media containing small amounts of water, to that in anhydrous organic solvents. It is easy to understand why enzymes retain catalytic activity in the first three types of reaction media, for in all of them the enzyme molecules are located in essentially aqueous environments. Conversely, the phenomenon of enzymes vigorously functioning in anhydrous organic solvents goes against the universally accepted dogmas. Nevertheless, it has now been firmly established (see ref. 1 for a review) that this phenomenon exists, that it is quite general, and that enzymes in organic solvents exhibit novel properties, e.g., greatly enhanced thermal stability and dramatically altered substrate specificity and stereoselectivity. In this presentation, the following fundamental questions will be addressed: How does enzymatic activity depend on the nature of the solvent and why? What physicochemical rules govern substrate and stereospecificities of enzymes in organic solvents? Why do enzymes remain catalytically active in organic solvents?, and What additional new properties do enzymes acquire when placed in non-aqueous solvents?


**P-1**

**CLONING, SEQUENCING AND FUNCTIONAL ANALYSIS OF GENES INVOLVED IN PENICILLIN AND CEPHAMYCIN BIOSYNTHESIS.** J.E. Martin, J.L. Barredo, E. Alvarez, B. Díez, E. Montenegro, C. Esmahan, S. Gutierrez, M.G. Dominguez, L. Laz, J.G. Cañadas, J.R. Coque and P. Liras, Área de Microbiología, Facultad de Biología, Universidad de León, 24071 León, Spain.

The p-lactam-thiazolidine ring nucleus of penicillin and the p-lactam-dihydrothiazine nucleus of cephalosporins are formed by cyclization of a precursor \( \gamma-(L-\text{aminoacyl})-\text{L-cysteinyl}-D\text{-valine} \) (ACV). ACV is cyclized by removal of four hydrogen atoms to form isopenicillin N (IPN), an intermediate having an L-\( \gamma\)-aminoacyl side chain attached to the nucleus, by isopenicillin N synthase (IPNS). In the last step of penicillin biosynthesis the \( \gamma\)-aminoacyl side chain is exchanged for aromatic (e.g. phenylacetic acid) side chains. In cephalosporin and cephamycin-producing organisms IPN is converted into cephalosporin C or cephamycins by a series of reactions that involve isomerization of IPN to penicillin N and expansion of the five-membered thiazolidine ring to the six-membered dihydrothiazine ring of cephalosporins.

Two enzymes of the penicillin pathway (IPN and acyl-CoA:AcA-APA acyltransferase) (Alvarez et al., 1987; Animicrob. Ag. Chemother. 31:1675-1682) and three enzymes of the cephamycin biosynthetic pathway (IPNS synthase, IPN isomerase and DAOC synthase) have been purified to homogeneity to determine the amino-terminal ends (Castro et al., 1988; JMG 13:133-144; Cortés et al., 1987; JMG 13:3165-3174).

A gene, pccC, encoding the IPN synthase of \( P. \) chrysogenum was cloned in a 1.3 kb NcoI-BgII fragment that contained an ORF of 996 nucleotides encoding a polypeptide of 331 amino acids with an Mr of about 38,000 (Barredo et al., 1989; MGG, 1991-92). Clones of \( P. \) chrysogenum Ws 54-1255 transformed with the pccC gene showed a higher IPN synthase activity than the untransformed controls.

The acyl-CoA:APA acyltransferase (penDE) gene has been cloned in a 2.4 kb HindIII-BalI fragment. The penDE gene encodes a protein of 327 amino acids with an Mr of 38,943. The cloned pccC and penDE genes complemented several mutants blocked in penicillin biosynthesis.

Both genes pccC and penDE involved in penicillin biosynthesis are clustered in a 5.1 kb Sall fragment in the genome of \( P. \) chrysogenum and are separated by a nontranscribed intergenic region of 1.5 kb. These genes are transcribed in the same orientation from different promoters in two separate transcripts of about 1.15 kb each. The clustering of penicillin biosynthetic genes is of great interest in light of previous claims of horizontal transfer of the pccC gene from \( P. \) lactacidum to filamentous fungi.

A 19.5 kb DNA fragment carrying the pccC and penDE genes of \( P. \) chrysogenum AS-P-78 DNA was cloned in EMBl3 phage vector. The pccC and penDE genes were located by hybridization with probes corresponding to internal fragments of each gene. A low penicillin producing strain (\( P. \) chrysogenum Ws 54-1255) and two high producing strains (AS-P-78 and P2) showed hybridizing fragments of identical size in their chromosomes. By dot-blot hybridization of serial dilutions of total DNA of the three strains it was shown that the intensity of all hybridizing bands was much higher in strain AS-P-78 and P2 than in Ws 54-1255.

A 25 kb DNA fragment that carries both, the pccC and penDE genes is amplified in high penicillin producing strains of \( P. \) chrysogenum. Hybridization of overlapping DNA inserts in different plasmids with probes corresponding to fragments which mapped upstream or downstream of the pccC-penDE region revealed that a fragment of at least 35 kb DNA has been amplified in the high penicillin producing strains. The amplified region did not include the previously cloned pccC gene (Cantoral et al., 1992, Nucleic Acid Research 18: 8177).

Similarly, the IPN synthase genes (from Streptomyces griseus and from Nocardia lactamdurans) and the DAOC synthase genes (from \( N. \) lactamdurans) have been cloned. They seem to form part of a large cluster of cephamycin biosynthetic genes. The IPN synthases of fungal and bacterial origin are very conserved suggesting a common evolutionary origin.