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Conference Report

Biotrans '95, University of Warwick, Coventry, UK, 5–8 September 1995

The University of Warwick provided the venue for Biotrans '95, an international conference on all aspects of Biocatalysis. The meeting was the second of its kind, following on from the highly successful Biotrans '93 organised by Professor Herfried Griengl in Graz, Austria. This time the symposium was hosted by Professor David Crout and was located in the excellent facilities provided by the University of Warwick.

The theme of the meeting, as before, covered all aspects of biocatalysis including the use of enzymes and whole cells in organic synthesis, the production of fine chemicals by pathway engineering and directed evolution, scale-up of biotransformations, structural and mechanistic studies on enzymes, zenobiotic transformations and industrial use of biocatalysts in chemical production. A notable sub-theme addressed by a number of the speakers was the use of enzymes in the synthesis of biologically active carbohydrates and oligosaccharides. In addition to the 27 oral presentations there was an impressive total of 131 posters. Below is a summary of some of the highlights of the meeting.

The meeting was opened by Professor Christian Wandrey (Jülich, Germany) who gave an overview of his group's work in the area of large-scale enzyme-catalysed carbohydrate, especially oligosaccharide, synthesis. The production of *N*-acetyl neuraminic acid (sialic acid) using a membrane reactor had been successfully carried out using sialic acid aldolase, leading to the production of 100 g per day of sialic acid. The sialic acid could then be converted to cytidine monophosphate *N*-acetyl neuraminic acid (CMP–NANA) using the enzyme CMP–NANA synthetase which had been over-expressed in *Escherichia coli*. Other enzymic transformations in the process of being scaled-up included transketolase, an α-1,2-mannosyl transferase, and a β-1,4-galactosyl transferase. Later on in the conference, Dr. Mike Dawson (Glaxo–Wellcome, UK) described his company's successful synthesis of sialic acid on a large scale, using sialic aldolase immobilised onto Eupergit C. A 500 l fermentation of the over-expressed organism provided 2×10^6 units of enzyme which could be immobilised directly without purification. By exploiting differences in the solubility of GlcNAc and ManNAc it proved possible to deliver the substrate at high concentrations resulting in a high yield ($> 95\%$) of the product on a multi-kilo scale. Glaxo–Wellcome require sialic acid as the starting material for an anti-influenza drug that is currently in development.

Dr. Vladimir Kren (Institute of Microbiology, Czech Republic) described the enzymic synthesis of the trisaccharide sialyl(α-2,3)-Gal(β-1,3)-Gal, known as the Sialyl T-Antigen, which has been implicated in haemolytic-uraemia syndrome in babies. The synthesis involved the initial use of β-galactosidase from bovine testes for the construction of the β-1,3 linkage between galactose and glucose followed by silalylation using an α-2,3 sialyl transferase from pig

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liver, resulting in an overall yield of 35% of the trisaccharide.

Two presentations dealt with the powerful and elegant approach of genetically engineering biosynthetic pathways to generate complex chiral molecules. Professor David Hopwood (John Innes Centre, UK) gave a fascinating account of the results of genetically manipulating polyketide biosynthesis in *Streptomyces*. The results of several years work have led to the identification of a 22 kilobase gene cluster that is responsible for the biosynthesis of the polyketide actinorhodin. By studying the function of the genes it has been possible to elucidate the 'design rules' that result in the programmed synthesis of the complex polyketide product. Dr. Gregg Whited (Genecor International, USA) described the progress made in the use of the aromatic amino acid pathway in *E. coli* for the production of fine chemicals from the cheap, renewable carbon source glucose. By careful control of the expression of the genes involved in the pathway, it was possible to enhance the level of the important enzymes. Using this approach, Dr. Whited described how it was possible to prepare in an efficient way substituted indoles, indole acetic acid, and ultimately the dye indigo.

Professor Herbert Waldmann (University of Karlsruhe, Germany) outlined his work on the synthesis of glycopeptides and peptide conjugates that play an important role in signal transduction. A key element of his research was the development of new enzyme-based protection group strategies that allowed the synthesis of these complex glycoconjugates in a more efficient manner. For example, he described the use of two new carbamate protecting groups for amines that could be cleaved under mild conditions namely (4′-acetyloxybenxyloxycarbonyl) whose release is triggered using an acetyl esterase and a β-D-glucopyranosyl carbamate that can be removed using β-glucosidase.

Professor Mike Page (University of Huddersfield, UK) described a number of interesting ring-opening and ring-closure reactions catalysed by hydrolases. He reported that phenyl substituted butyrolactones and 5-membered ring carbonates were good substrates for pig liver esterase (PLE) resulting in high enantiomeric excesses (96–98%). In the reverse reaction, ω-amino acids and nitriles could be converted to the corresponding lactams using either PLE or a nitrile hydrolase, although so far the use of this approach for the synthesis of β-lactams had proved unsuccessful.

Professors Claudine Augé (Université de Paris-Sud, France) and Wolf-Dieter Fessner (RWTH Aachen, Germany) both presented work on the use of aldolases for the synthesis of complex sugars. Professor Augé discussed the use of pyruvate dependant aldolases, e.g. sialic acid aldolase, and reported that this enzyme was able to accept not only a range of analogues of *N*-acetyl mannosamine but also fluoropyruvic acid in place of pyruvic acid. Professor Fessner focused on the use of those aldolases that utilise dihydroxyacetone phosphate as the nucleophilic component of the reaction. He described potential solutions to the problems of enzyme availability, *via* over-expression in *E. coli*, and availability of dihydroxyacetone phosphate by in situ generation from glycerol phosphate. Most of these features have now been incorporated in an 'aldolase kit' that is marketed by Boehringer Mannheim.

Professor David Hawkins (Huntingdon Research Centre, UK) and Professor Bert Holland (Brock University, Canada) described recent work in the emerging area of biotransformations using mammalian whole cells. Professor Hawkins discussed how the study of xenobiotic metabolism in animals can shed light on the fate of various organic molecules in vivo. Typical reactions that occur in the liver and intestinal tract are glucuronidation, sulphation, nitro reduction and glutathione conjugation. In addition to species-dependent biotransformations, there are also notable differences between the sexes. Professor Holland described his recent work aimed at harnessing human cell cultures for carrying out preparative biotransformations. He outlined the extra care that must be taken when

culturing mammalian cells compared to their microbial counterparts. For example, human cells grow very slowly, doubling their cell mass only every 5–30 hours and must be grown under carefully controlled conditions of pH, oxygen tension, stir-rate and temperature control. His initial studies were being directed towards reproducing known biotransformations on a variety of commercially important therapeutic drugs, including Glaxo's anti-ulcer compound ranitidine (Zantac).

Professor John Holbrook (University of Bristol, UK) gave an impressive account of his worked aimed at redesigning lactate dehydrogenases thereby providing useful catalysts for the reduction of α-ketoacids to enantiomerically pure α-hydroxy acids on a preparative scale. By employing the technique of side-directed mutagenesis, the Bristol group had successfully remodelled the D-lactate dehydrogenase from *Lactobacillus bulgaricus* so that it was able to reduce 4-cyclopentyl-2-ketobutyric acid to the corresponding (*R*)-hydroxy acid, an intermediate for the synthesis of the peptoid inhibitor captopril. The key to the improvement in catalytic activity was to weaken binding of the cofactor NAD^+ which led to reduction of inhibition by the keto acid substrate.

Continuing with the theme of structural-based studies, Dr. Jenny Littlechild (University of Exeter, UK) gave an account of a multi-disciplinary project aimed at using transketolase for the large scale asymmetric synthesis of carbon–carbon bonds. The enzyme had been successfully over-expressed in *E. coli* (43% of total cell protein) providing large amounts of enzyme for both preparative transformations and crystallisation studies which had provided crystals. The structure of transketolase has been solved, by molecular replacement, to a resolution of 1.9 Å. The thiamine pyrophosphate cofactor and calcium ion are both bound at the interface of the dimeric protein.

Dr. Andy Kiener (Lonza, Switzerland) gave an elegant presentation describing the ways in which whole cell biotransformations can be applied successfully to the biotransformations of N-heterocycles on an industrial scale. For example, β-nicotinic acid can be hydroxylated at either the 2-position (*Alcaligenes* sp.) or 6-position (*Pseudomonas* sp.) depending upon the choice of organism. Similarly, 2-methyl-5-ethyl-pyridine underwent conversion to 5-ethyl-α-nicotinic acid (*P. putida*) or (4′-methylpyridine) acetic acid (*P. oleovorans*). Finally, the use of a *Rhodococcus* sp. containing a nitrile hydratase allowed the efficient conversion of 3-cyanopyridine to nicotinamide.

The final session on the meeting on Friday morning began with a lecture from Professor Manfried Schneider (University of Wuppertal, Germany) describing the use of lipases for the synthesis of optically active intermediates in the synthesis of δ-lactones (flavour constituents) and carba-analogues of phospholipids (second messengers). Following on, Professor Roland Furstoss (University of Marseille, France) gave an impressive account of his work on the isolation and application of microbial epoxide hydrolases for the stereoselective hydrolysis of epoxides. The epoxide hydrolase from *Aspergillus niger* has been shown to hydrolyse a range of epoxides (e.g. epoxyacrolein diethylacetal, > 98% e.e.) with excellent selectivity. Intriguingly, Professor Furstoss revealed that the same epoxide hydrolase was able to hydrolyse a racemic epoxide to a single isomer of the corresponding diol as a consequence of a pair of stereospecific reactions. Significantly, cell-free preparations of the enzyme have been obtained suggesting that a commercial stable biocatalyst may soon be available.

The final presentation was delivered by Professor Tomas Hudlicky (University of Florida, USA) representing an elegant symbiosis of biocatalysis and organic synthesis. By using mutant strains of *Pseudomonas putida* he described how it was possible convert a range of aromatic derivatives to the corresponding homochiral cyclohexadienediols which could then be elaborated to complex natural products. In addition to the synthesis of various cyclitols and pseudo-

sugars, Professor Hudlicky's group had recently completed the first total synthesis of the anti-cancer agent pancratistatin.

The date and venue for Biotrans '97 has yet to be decided although a European location seemed likely. This biennial symposium has quickly established itself as the principal meeting point for international scientists, in the field on biotransformations, from both academe and industry. We all look forward to a meeting in two years time that is as stimulating and enjoyable as Graz and Warwick have been.

Nicholas J. Turner, Editor