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Lipases: Structure, Function and Applications in Biotransformations.

An International Conference held at the University of Warwick, Coventry CV4 7AL, U.K. from 16-18 July 1991.

INTRODUCTION.

This meeting was organised by the Biotechnology Group of the Industrial Division, and the Bioorganic Group of the Perkin Division, of the Royal Society of Chemistry.

The meeting had a very clear philosophy. It is now well recognised that lipases play an important role in biotransformations, i.e. the transformations of chemical substances into other substances in reactions catalysed by these enzymes. Lipases are the most frequently used enzymes in this area and their use in organic synthesis, particularly where control of stereochemistry is important, is nowadays widespread. The stereochemistry of biologically active compounds is recognised as of considerable, and sometimes critical, importance. Stereoisomers of given compounds always exhibit differences in biological systems, and sometimes these differences are crucial. The case of thalidomide is perhaps the most telling example of this phenomenon. That one stereoisomer of thalidomide was a harmless sedative and the other a dangerous teratogen was recognised too late to spare many hundreds of families the tragedy of children born with extreme deformities. Because of this, and many other examples of differences between stereoisomers of biologically active compounds, drug regulating agencies are nowadays increasingly insisting on registration of pure stereoisomer of active compounds. The phenomenon of differing biological activities of stereoisomers is also of great importance in the agrochemical industry. The identification of the biologically active isomer of a pesticide, and the manufacturing and application of the single isomer, has the desirable outcome that distribution in the ecosystem of an inactive and possibly dangerous compound is avoided. Lipases have been used in numerous procedures for the procuring of single stereoisomers and the rate of application of lipases to problems of this kind continues unabated.

In applying lipases in these highly important areas, organic chemists have been hampered by the fact that too often the enzymes they use are impure preparations, poorly characterised and of unknown structure. Accordingly, much of the work carried out in this area is highly empirical, inasmuch as it is based on a very poor understanding of the biological catalyst being used. It was in an effort to correct this situation that the organisers of the meeting had the idea of bringing together scientists concerned with the fundamental properties of lipases with scientists engaged in studies of their structures and functions, and with scientists working on their applications in organic synthesis, and in particular in the synthesis of chiral compounds in optically pure form.

ORGANISATION OF THE MEETING.

The meeting was based around thirteen lectures and poster presentations. The lecture titles are given in the enclosed programme. Over fifty posters were offered and accepted; their titles are given in the enclosed list of posters presented. Two sessions were reserved for the posters during which poster presenters were asked to be in attendance. However, all of the posters were displayed throughout the meeting and attracted considerable interest.

PARTICIPATION.

Participants were drawn from eighteen countries, as follows:

Country	Number	Country	Number	Country	Number
Austria	5	Germany	7	S. Africa	1
Belgium	7	Holland	9	Spain	2
Canada	1	Israel	1	Sweden	6
Denmark	3	Italy	9	Switzerland	6
Eire	1	Japan	5	U.K.	33
France	10	Portugal	1	U.S.A.	9

SUMMARY OF LECTURES.

The conference began with lectures from two of the leading workers on fundamental properties of lipases, Howard Brockman (University of Minnesota, Minnesota) and Robert Verger (CNRS,Marseilles). Professor Brockman gave an account of our current understanding of the organisation of liquid-liquid interfaces (lipases are active at such interfaces *in vivo*). He described the application of Langmuir-Blodgett techniques to the investigation of the variation of activity with interface concentration of substrate and enzyme. He showed the application of ^{18}O studies to the elucidation of a random-sequential mechanism of lipid hydrolysis at the interface and related this to the distribution of substrate at the interface. Professor Verger concentrated on the stereoselectivity of lipases, and in particular the previously neglected gastric lipases. He described an apparatus for measuring the interfacial tension of lipase/substrate mixtures in contact with an aqueous medium and showed how this could be used to determine the optimum interfacial tension for lipase activity.

There followed lectures by representatives of three groups that had recently published X-ray crystal structures of lipases: Birgitte Huge-Jensen (Novo, Denmark)), Fritz K. Winkler (Hoffman-La Roche, Basel) and Joseph D. Schrag (National Research Council of Canada, Montreal). These lecturers described the structures of the lipases from *Mucor miehei, Geotrichum candidum,* and human pancreas, respectively. Dr Huge-Jensen described the two forms of the *Mucor* enzyme that differed in their glycosylation pattern. She described their purification and properties (pH profiles, temperature optima and regioselectivity with respect to triglyceride hydrolysis). The crystal structure of the *Mucor* lipase showed that it consisted of an eight-stranded β-sheet with an extended α-helix at the N-terminus. The catalytic triad resided in a cleft flanked by amino acid residues with non-polar side chains. Dr Schrag described how the *Geotrichum* enzyme also consisted of isoenzymes with different glycosylation patterns. The crystal structure showed that the enzyme had a eleven-stranded β-sheet with one three-stranded β-sheet perpendicular to the others. Unusually, the catalytic triad included a glutamate residue instead of the normal aspartate. He pointed out a structural motif

common to all of the recently solved lipase structures and named this the "hydrolase fold". The *Geotrichum* lipase was structurally rather similar to the human pancreatic lipase. Dr Winkler described the properties of human pancreatic lipase. He discussed the action of the inhibitor tetrahydrolipstatin and also the calcium binding site and a possible heparin binding site.

The next section of the meeting consisted of lectures on aspects of lipase properties with particular emphasis on practical applications. Emmanuelle Charton (Unilever Research, Colworth House, U.K.) described the purification and properties of the lipase from *Geotrichum candidum*. in a lecture that nicely complemented that given by Dr Schrag. She described the purification of the two forms (lipase A, 63 kD and lipase B, 58.3 kD). Lipase B was very specific for the hydrolysis of esters of unsaturated fatty acids. The specificity with respect to fatty acid ester hydrolysis was studied with a variety of synthetic substrates. Lipase B showed a marked selectivity for long-chain unsaturated fatty acids (18:1 (oleic acid), in particular). Lipase B also showed a preference for longer chain fatty acid esters, but was equally active towards 8:0 and 18:0 substrates. In studies of triacetin hydrolysis by lipase A, the typical increase of activity with substrate concentration was observed, hydrolysis not taking place to any significant extent below the critical micelle concentration. Peter Halling (University of Strathclyde, U.K.) described studies on two-phase systems for lipase-catalysed reactions. He stressed the importance of determining and controlling thermodynamic water activity. For example, if water activity is properly controlled, the equilibrium in transesterifications is improved and less free fatty acid is produced. Dr Halling described experiments designed to measure pH in two-phase systems, through the use of hydrophobic pH indicators. He showed that it was possible to determine the pH with reasonable accuracy in the organic phase of two-phase systems. Professor Inada (Toin University of Technology, Japan) gave a lecture on his pioneering work on polyethyleneglycol-modified lipases (PEG-lipases). Through the application of this technique enzymes can be solubilised in organic solvents. He described the application of PEG-lipases in esterifications, transesterifications etc. A novel and intriguing extension of the use of PEG-enzymes was the incorporation of magenetite particles.

This rendered the enzymes magnetic, a property that could be used to isolate the enzyme following an enzyme-catalysed process. A magnetic form of urokinase was being developed for the dissolving of fibrin clots. There are other potential applications of PEG-enzymes in medicine. J.G.T. Kierkels (Andeno, The Netherlands), described the application of lipases to the synthesis of pharmaceuticals, including building blocks for ACE inhibitors and the resolution of α-hydroxy acids. He referred in particular to the properties of emulsions in the lipase-catalysed reactions and demonstrated the importance of controlling emulsion droplet size and lipase concentration. He also discussed the effect of substances such as decanol on lipase activity.

The last section of the conference dealt specifically with the use of lipases in the production of chiral building blocks for organic synthesis in optically active form. Kurt Faber (University of Graz, Austria) described a variety of procedures for optimising lipase-catalysed reactions of bornane derivatives. He described the use of irreversible acyl transfer reagents (oxime esters, enol esters, acid anhydrides) and showed how the best results were obtained with acetic anhydride used in conjunction with celite-supported lipase AY-30. Even better results were obtained with vinyl acetate as acetyl group donor. Dr Faber discussed the relative merits of celite-supported and covalently-immobilised enzymes, demonstrating that the latter gave superior results over several catalytic cycles. Dr Philip Sonnet (USDA, Philadelphia, U.S.A) described a number of studies relating to the use of lipases in esterifications and ester hydrolyses. He discussed the selectivity of the lipase from *Candida cylindracea* in the esterification of (±)-2-octanol with a variety of fatty acids, and the use of lipases to prepare building blocks for the preparation of optically active forms of glycerol acetonide and glycidol. Manfred P. Schneider (University of Wuppertal, Germany) described the use of SAM II (a purified lipase from *Pseudomonas fluorescens*) in the synthesis of many chiral building blocks for organic synthesis. This enzyme is highly effective in catalysing esterolytic reactions of secondary alcohols in which one substituent is small (e.g. methyl) and the other large and hydrophobic. Numerous substrates could be hydrolysed or esterified using this enzyme with exceptionally high stereoselectivity.

CONCLUSION.

The conference was clearly a resounding success. Many of the participants stressed the benefits of bringing together workers from many different but related disciplines working in the general area of lipase properties, structure and function. It is anticipated that the practitioners of biotransformations will have learned much about the fundamental properties of the enzymes they use and that this will lead to a more rational, and therefore more effective use of these marvellous catalysts. On the other hand, it is hoped that the workers on fundamental aspects of lipase activity will have had suggested to them lines of investigation that will further enlighten the work of those applying lipases in organic chemistry.