The Biosynthesis and Function of Microbial Wall Components

Carbohydrate Group/Cell Surfaces and Membranes Group of the Society for General Microbiology Joint Symposium held in honour of Sir James Baddiley, F.R.S., at the University of Birmingham on 10 January 1985. Organized by Martin V. Jones (Unilever, Sharnbrook), Harold R. Perkins (Liverpool) and R. Derek Marshall (Glasgow), and edited by R. Derek Marshall

Introduction

A Symposium in honour of Professor Sir James Baddiley, F.R.S., was held on 10 January 1985 in the Howarth Building in the University of Birmingham. The Symposium was organized by the Carbohydrate Group of the Biochemical Society jointly with the Cell Surfaces and Membranes Group of the Society for General Microbiology. It was appropriate that the two Groups, and the two Societies, should have combined for this meeting to mark Sir James's contributions to research, and the occasion also demonstrated the continuing vigour of the Carbohydrate Group, whose existence resulted from discussions between Sir James and Professor W.T.J. Morgan, C.B.E., F.R.S.

Sir James has been a Professor in the University of Newcastle upon Tyne since 1955 when he was appointed to the Chair of Organic Chemistry. Later, in 1977, the University granted him the title of Professor of Chemical Microbiology in recognition of his outstanding contributions to research and of the distinction they brought to the University. In 1983 he was accorded the title Professor Emeritus.

Born in Manchester, where his father was research director at I.C.I., James Baddiley was a pupil at the Manchester Grammar School, then, as now, renowned for its academic excellence and the achievements of its former pupils. At University in Manchester he began research with Alexander Todd, now Lord Todd, F.R.S., Nobel Laureate. In 1945 he moved to Cambridge with Lord Todd so becoming one of the 'Toddlers', that distinguished group of scientists who have made such major contributions to the chemical and biochemical sciences in this country. Cambridge, where he held an I.C.I. Fellowship, was followed by the Wenner Grens Institute in Stockholm (1947–1949), where he was a Fellow of the Swedish Medical Research Council, and then an appointment at the Lister Institute (1949–1955) during which he also held a Rockefeller Fellowship at Harvard (1954). During this period Sir James established a reputation in research such that the late Lord C. Cross, Head of Chemistry at Newcastle, encouraged him to accept the Chair in Organic Chemistry. This early distinction in research arose from a series of contributions to the chemistry of nucleotides, including the first chemical synthesis of adenosine triphosphate, studies on the structure of coenzyme A, and the isolation and characterization of novel compounds including, significantly, two new nucleotides initially referred to as CDP-X and DCP-Y. Early work in Newcastle showed these to be cytidine diphosphate glycerol and cytidine diphosphate ribitol. The prediction, more obvious now than at the time it was made, that the nucleotides might be the precursors of polymers containing glycerol and ribitol, or their phosphates, resulted in the discovery of the teichoic acids. Early work established the principal structural features of these new polymers and this led to the work on their biosynthesis and function and to the problems of cell wall biosynthesis and assembly with which Sir James is still occupied, still productively as was evident from the lecture he gave during the Birmingham Symposium.

Vol. 13 979

SIR JAMES BADDILEY, F.R.S.

While securely founded in an appreciation of the fundamental importance of structure, the Newcastle work extended beyond the confines of chemistry and opened up a new area in bacterial physiology. The Microbiological Chemistry Research Laboratory was internationally recognized and attracted large numbers of foreign students and visiting scientists as well as providing a training ground for M.Sc. and Ph.D. students, many of whom have gone on to achieve distinction in their own right. A feature of the celebration held in Newcastle to honour Sir James on the occasion of his retirement was the large number of these former students, including nearly all of the first 'round' of Newcastle Ph.D. students recruited in 1955, who came back to mark the occasion.

In addition to his major contribution to research Sir James has taken an active part in the work of Scientific Societies and Research Councils. He has been a member of the Council of the Chemical Society (1962–1965), served on the Committee of the Biochemical Society (1964–1967) and the Council of the Society for General Microbiology (1973–1975). He was a member of the Science Research Council (now S.E.R.C.) Enzyme Chemistry and
Structure and serology of the Brucella abortus O-antigen

DAVID R. BUNDLE and MALCOLM B. PERRY
Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6

The detection of bovine brucellosis is based upon the detection of antibodies directed against the cell-surface polysaccharide of the causative organism Brucella abortus (FAO/WHO, 1971). A related pathogen, Brucella melitensis, represents one of several antigenically cross-reactive bacteria and is itself a more virulent pathogen, especially in humans. Despite the early recognition of the so-called A and M antigens of B. abortus and B. melitensis (Wilson & Miles, 1933), the precise composition and structure of these antigens has remained unresolved for 50 years. In fact one of the most incisive observations was an early paper which reported that the A and M determinants were present on a single complex molecule, an aminopolysaccharide component or 'AP substance', which also contained formyl residues (Miles & Pirie, 1939). Work during the subsequent quarter of a century contributed little more to this basic picture, although several important cross-serological reactions involving B. abortus and other Gram-negative bacteria were reported. The most marked is that involving Yersinia enterocolitica 0-9 (Ahvonen et al., 1969) but also of note are those with enterobacteria of the Kauffmann-White serogroup N (Corbel, 1975) and Vibrio cholerae (Barua & Watanabe, 1972). The molecular species responsible for the cross-serological reactions between B. abortus and Y. enterocolitica 0-9 were established as the O-antigen of the respective lipopolysaccharide (LPS) molecules (Lindberg et al., 1982).

When initial attempts to extract and purify the LPS of B. abortus failed to yield amounts of antigen suitable for structural studies, an alternate strategy for the purification and analysis of the A antigen was conceived. Monoclonal antibodies toward the cross-reactive Y. enterocolitica 0-9 LPS antigen were generated with the intention of using them to recover the B. abortus A antigen. To comprehend the nature of this serological cross-reaction and hence gain insight into the B. abortus O-antigen structure, the readily accessible antigen of the less pathogenic Y. enterocolitica was also subjected to structural studies. In the event only one arm of this dual strategy was essential to the structural elucidation of the A antigen.

The aqueous phenol extraction (Westphal et al., 1952) of Y. enterocolitica 0-9 cells yielded two LPS and in agreement with previous results (Sandulache & Marx, 1978) these were recovered in the aqueous and phenol phases of the extract. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Tsai & Frasch, 1982) showed that the material in the aqueous phase was a rough, R-type LPS and that the phenol-soluble component was a smooth LPS, a conclusion confirmed by mild acid hydrolysis [1% (v/v) acetic acid] to split-off lipid A. The smooth LPS gave a high-M_o component, indicative of O-polysaccharide linked to core oligosaccharide, whilst the aqueous material gave only low-M_o core oligosaccharides. Structural studies employing 1H and 13C n.m.r. data established that the O-chain repeating unit consisted of a single sugar, a 6-deoxy-hexopyranosyl unit, bearing a amino dideoxy functionality acylated by formic acid. Recalling the cross-serological reactions between B. abortus, Y. enterocolitica 0-9 and V. cholerae and the unique 4-amino-4,6-dideoxy hexose of the latter organism's O-antigen (Kenne et al., 1979; Redmond, 1979), special hydrolysis conditions were employed to isolate the constituent hexose of Yersinia O-polysaccharide. Hydrolysis with anhydrous hydrogen fluoride yielded the sugar 4-amino-4,6-dideoxy-β-D-mannose (perosamine) identified by g.c.-m.s. and 13C n.m.r. spectra. Methylation analysis of the polysaccharide indicated a 1,2-linkage between perosamine residues. In combination with 1H and 13C n.m.r. measurements, the structure of Y. enterocolitica 0-9 O-chain might then be unambiguously to be a linear homopolymer of 1,2-linked 4,6-dideoxy-4-formamido-O-D-mannopyranosyl units (Caroff et al., 1984a).

When killed B. abortus cells were extracted on a scale large enough to yield at least 20 mg of LPS by the phenol/ water method (Westphal et al., 1952), the material present in aqueous and organic phases could be easily analysed. 13C n.m.r. data for the polysaccharide of the smooth LPS recovered from the phenol phase were identical to those recorded for the Y. enterocolitica 0-9 LPS, and detailed chemical analysis further substantiated this conclusion. Although the B. abortus LPS antigen required careful purification from contaminating lipid, the pure O-antigen was indeed structurally identical with the Y. enterocolitica 0-9 polysaccharide O-chain (Caroff et al., 1984b). Both 13C and 1H n.m.r. spectra (Fig. 1) show the presence of two conformations of the N-formyl group, E and Z. The Z predominates but within the polymer, adjacent residues experience neighbours of either conformation. Therefore, the spectra are quite complex due to this random microheterogeneity, which may however be abolished by de-N-formylation to yield the free amino form of the polymer. Also in the N-acetylated derivative no E conformer is present.