fication studies, as follows. (a) Enzyme activity was destroyed by treatment with diethyl pyrocarbonate at pH 6.0, implicating histidine in activity. Although the inhibition by α -oxoglutarate was unaffected by this reagent, the pH-dependence of α -oxoglutarate inhibition further suggests the involvement of histidine in this regulation. (b) Treatment with 2-hydroxy-5-nitrobenzyl bromide at pH 4.6 caused a rapid loss of enzyme activity with no alteration of NADH or α -oxoglutarate inhibition. When one tryptophan residue/enzyme subunit was attacked by the reagent 10% of the initial activity remained. Modification of methionine by H_2O_2 at pH 6.0 caused no loss of enzyme activity or regulation. Together with the results of 2-hydroxy-5-nitrobenzyl bromide treatment this suggests that the third photo-oxidizable amino acid implicated in catalytic activity is tryptophan rather than methionine. (c) Modification of the enzyme with the thiol-specific reagent 5,5'-dithiobis-(2-nitrobenzoate) resulted in complete loss of NADH inhibition and, more slowly, of enzyme activity, but no loss of α -oxoglutarate inhibition. This confirms that cysteine is essential for NADH regulation and activity. The resistance of the active-site cysteine residue(s) to photo-oxidation may result from a quenching effect exerted by the photo-oxidation of other neighbouring residues.

Weitzman, P. D. J. (1966a) *Biochim. Biophys. Acta* **128**, 213

Weitzman, P. D. J. (1966b) *Biochem. J.* **101**, 44c

Wright, J. A., Maeba, P. & Sanwal, B. D. (1967) *Biochem. Biophys. Res. Commun.* **29**, 34

The Regulation of *Acinetobacter* sp. α -Oxoglutarate Dehydrogenase Complex

By MALCOLM G. PARKER and P. DAVID J. WEITZMAN
(Department of Biochemistry, University of Leicester,
Leicester LE1 7RH, U.K.)

Preliminary investigations on unpurified preparations of the α -oxoglutarate dehydrogenase complex from *Acinetobacter* sp. revealed the following regulatory properties (Weitzman, 1972). (a) AMP and ADP stimulate enzyme activity by lowering the K_m for α -oxoglutarate, whereas ATP is slightly inhibitory; (b) NADH inhibits the complex; (c) AMP and ADP overcome the NADH inhibition.

The complex has now been purified to homogeneity as indicated by gel electrophoresis and sedimentation-velocity analysis. The purified α -oxoglutarate dehydrogenase complex responds to NADH and adenylates as above. We have attempted to identify the effector sites of action by means of assays specific for the component enzymes.

The first enzyme component, E1 (α -oxoglutarate

dehydrogenase), has been assayed by electron transfer to 2,6-dichlorophenol-indophenol by following the reaction at 578 nm. The effects of AMP and ADP on component E1 were similar to those observed for the whole complex. The effect of NADH on component E1 could not be tested in this way owing to the non-enzymic reduction of 2,6-dichlorophenol-indophenol.

The third enzyme component, E3 (lipoamide dehydrogenase), has been assayed spectrophotometrically and polarographically. NADH was a competitive inhibitor with respect to NAD^+ , whereas the adenine nucleotides were without effect on enzyme activity and on the NADH inhibition of component E3. This result contrasts with the relief of NADH inhibition by AMP and ADP observed with the whole complex, suggesting an additional site of action for NADH.

Evidence to support this suggestion has been obtained from kinetic studies: (a) the inhibition of the complex by NADH is competitive with respect to α -oxoglutarate; (b) although NADH inhibition is competitive with respect to NAD^+ it is not overcome completely at high NAD^+ concentrations; (c) detailed kinetic analysis indicates two binding sites for NADH. These results are consistent with an NADH-inhibitor site on component E1.

Studies on the adenylate regulation of the complex have shown that the effectors act allosterically. Treatment with 1 M-urea desensitizes the complex to AMP and ADP without loss of activity. This desensitization is accompanied by a lowering of the K_m for α -oxoglutarate.

The effects of adenylates and NADH on the α -oxoglutarate dehydrogenase complex are consistent with a regulatory role within the tricarboxylic acid cycle. As might be expected, these controls operate on the first enzyme component and the NADH inhibition represents a feedback mechanism within the complex itself.

Weitzman, P. D. J. (1972) *FEBS Lett.* **22**, 323-326

A Simple Method for the Determination of the Endo or Exo Nature of Carbohydrases Acting on Polymeric Substrates

By P. M. DEY, I. R. RACHED and K. WALLENFELS
[Department of Biochemistry, Royal Holloway
College (University of London), Englefield Green,
Surrey, U.K., and Chemisches Laboratorium der
Universität, 78 Freiburg i. Br., German Federal
Republic]

Several methods are known for distinguishing between the endo and exo nature of some carbohydrases acting on their polymeric substrates (Nelson

et al., 1963; Drummond *et al.*, 1969, 1971; Wallenfels *et al.*, 1969; Tung & Nordin, 1969; Marshall & Whelan, 1971). Some of these methods involve chemical modification of the substrate and are, in general, time-consuming. We have now developed a rapid and universal method for such investigations by using an ultrafiltration technique.

The actions of several enzymes were examined on their respective substrates, such as pullulanase on pullulan, α -amylase on amylose and β -amylase on amylose. All the substrates were washed before enzymic reaction, by ultrafiltration with an Amicon cell with PM 10 membrane (Amicon Corp., Lexington, Mass., U.S.A.). This resulted in exclusion of polysaccharides with molecular weights less than 10000. The enzymic reactions were carried out in the ultrafiltration cells at 20°C with a flow rate of 0.4 ml/min, and the initial products were fractionated as they were formed.

Analysis by t.l.c. of the fractions from the reactions of pullulanase and α -amylase showed the appearance of a dark spot at the base-line. The intensity of this immobile spot increased with increasing fraction number along with the appearance of faster-moving spots. The latter were the homologues of maltotriose and maltose for the two enzymes respectively. The later fractions contained only maltotriose and maltose for these enzymes. The spot on the base-line in the first fraction would presumably consist of carbohydrate units with molecular weights below 10000. This therefore must be the result of the endo action of the two enzymes. If the reaction is not carried out in the ultrafiltration cell and the enzymic digest as such is analysed, one would not be sure whether the spot at the base-line is a product or the unchanged substrate. The exo action of an enzyme, on the other hand, would produce a single fast-moving spot on t.l.c. This was confirmed by the experiment with β -amylase and amylose.

It is expected that the ultrafiltration technique could also be applied for the proteases and the nucleases.

We are grateful to The Royal Society (London) for financial support. This work was carried out at the University of Freiburg, Freiburg i. Br., German Federal Republic.

- Bender, H., Lehman, J. & Wallenfels, K. (1959) *Biochim. Biophys. Acta* **36**, 309
 Drummond, G. S., Smith, E. E. & Whelan, W. J. (1969) *FEBS Lett.* **5**, 85
 Drummond, G. S., Smith, E. E. & Whelan, W. J. (1971) *FEBS Lett.* **15**, 302
 Marshall, J. J. & Whelan, W. J. (1971) *Anal. Biochem.* **43**, 316
 Nelson, T. E., Scaletti, J. V., Smith, F. & Kirkwood, S. (1963) *Can. J. Chem.* **41**, 1671
 Tung, K. K. & Nordin, J. H. (1969) *Biochemistry* **29**, 84
 Wallenfels, K., Bender, H. & Rached, I. R. (1966) *Biochem. Biophys. Res. Commun.* **22**, 254

Wallenfels, K., Rached, I. R. & Hucho, F. (1969) *Eur. J. Biochem.* **7**, 231

The Presence of a Sulphated Polysaccharide in the Cell Envelopes of *Halobacterium halobium*

By MACIEJ KONCEWICZ (introduced by DAVID J. ELLAR) (*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.*)

Media containing at least 4M-NaCl are required for optimal growth of the obligate halophilic bacterium *Halobacterium halobium*. This Gram-negative rod-shaped organism lacks the peptidoglycan component typically found at the surface of most other bacteria. The anionic nature of envelope proteins and lipids in this organism (Stoeckenius & Kunau, 1968; Kates *et al.*, 1967) suggested that acidic groups might be involved in its survival in high-salt media. In this context studies by Doyle & Birdsell (1972) indicated that charged glucose-containing polymers may change conformation with varying salt concentrations. No uronic acids were detected in envelope polysaccharides of this organism. The possibility of bound sulphate or phosphate rendering these polysaccharides acidic was the basis for the present studies.

Polysaccharides and glycoproteins were extracted from cell envelopes of *H. halobium* with aqueous phenol and fractionated on a column of Sephadex G-100–Sephadex G-200 (2:1, w/w). One carbohydrate fraction containing mainly mannose but no protein could not be precipitated from aqueous solution by ethanol in the absence of bivalent cations. The presence of sulphate residues in this fraction was initially demonstrated by studying the incorporation of [³⁵S]sulphate. The organism was grown in sulphate-free medium for ten generations, and 0.5mCi of [³⁵S]sulphate was added to give a final concentration of 1 μ M. The envelope polysaccharides were isolated and chromatographed under normal conditions on Sephadex G-100–Sephadex G-200. The bulk of the radioactivity was eluted with the polysaccharide peak. Evidence for the presence of sulphate was obtained after acid hydrolysis for 24h, when 92% of the recovered radioactivity could be precipitated from acidic solutions by BaCl₂ plus carrier sulphate.

Confirmation of the presence of sulphate hemi-ester groups in this material was obtained by i.r. spectroscopy. A strong absorption at 1240cm⁻¹ characteristic of S=O in sulphate esters was observed together with other peaks characteristic of hexopyranoses. Phosphate was not detected.