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