C₁ Compounds as Substrates for the Production of Single-Cell Protein

By D. E. F. HARRISON and G. HAMER. (*Shell Research Ltd., Borden Microbiological Laboratory, Sittingbourne, Kent, U.K.*)

The cultivation of micro-organisms that utilize methane and/or methanol as their carbon energy sources for growth will be discussed. Emphasis will be placed on the methods employed for increasing the yield of protein-rich biomass and for improving the productivity of the systems employed. Some experimental results obtained in laboratory-scale fermenters will be reviewed.

Allosteric Binding of Coenzymes to Alcohol Dehydrogenases

By A. WISEMAN. (*Department of Biochemistry, University of Surrey, Guildford GU3, Surrey, U.K.*)

NAD⁺ or NADP⁺ preparations have been shown to accelerate the thermal inactivation of both yeast and liver alcohol dehydrogenases during incubation, before assay, for 20 or 50 min respectively at 43°C at pH 8.8 (Wiseman, Hanley & Williams, 1971).

This effect was concentration-dependent in the range 1½–4 mM approximately; 50% recovery, of the activity obtained after heating in the absence of NAD⁺ or NADP⁺, was found at about 2½ mM-NAD⁺ or -NADP⁺. This suggests a K_d of this order for some other binding site present, because the catalytic site of alcohol dehydrogenases shows a much lower K_m for these coenzymes. For example the K_m of liver alcohol dehydrogenase for NAD⁺ was reported to be 51 μM at pH 8 (Theorell & McKinley-McKee, 1961). Clear evidence for other such additional binding sites has been reported (Weiner, 1969). Each molecule of liver alcohol dehydrogenase (two subunits/molecule) bound a spin-labelled coenzyme analogue (an inhibitor competitive with NAD⁺) at two strong (K_d = 17 ± 8 μM) and five or six somewhat weaker sites (K_d = 75 ± 9 μM) in metal-free phosphate buffer at pH 6.0. Also, NAD⁺ has been found to quench the fluorescence of NADH bound to liver alcohol dehydrogenase (Theorell & McKinley-McKee, 1961).

The thermal inactivation of alcohol dehydrogenases promoted by NAD⁺ or NADP⁺ preparations could be due to a conformational change caused by allosteric binding of these coenzymes, with relatively weak affinity for that site. Recent work here (A. Wiseman, unpublished work) with yeast alcohol dehydrogenase bound to DEAE-cellulose (Miles-Seravac Ltd., Maidenhead, Berks., U.K.) has demon-

strated a much higher thermal stability for this bound enzyme as compared with free enzyme. This followed the same preincubation treatment, with or without NAD⁺ as appropriate, followed by the addition of ethanol, then rapid shaking for 2 min and immediate filtration and spectrophotometric determination at 340 nm (the K_m for NAD⁺ was found to be about 3 times that of the free enzyme). The allosteric site for thermal inactivation by coenzyme may be blocked during the binding procedure in this case.

Theorell, H. & McKinley-McKee, J. S. (1961). *Acta chem. scand.* **15**, 1811.

Weiner, H. (1969). *Biochemistry*, Easton, **8**, 526.

Wiseman, A., Hanley, H. O. & Williams, N. J. (1971). *Biochem. J.* **123**, 8P.

Quaternary Structure of Oligomers: a New Theory and its Application

By E. J. MILNER-WHITE. (*Department of Chemistry and Biochemistry, Guy's Hospital Medical School, London, S.E.1, U.K.*)

This approach to the study of self-assembly of proteins with identical globular subunits co-ordinates and extends current ideas about symmetry in protein structures (Klug, 1967) with the concept of isologous and heterologous association (Monod, Wyman & Changeux, 1965). In the simplest possible type of self-assembly a subunit or protomer is assumed to contain two corresponding, but different, sites on its surface. It is proposed that any two such sites on a protomer are called a 'site-pair'. It is shown that the quaternary structure of almost all proteins may be described in terms of only a few simple binding patterns characterized by the number of site-pairs on each protomer and by their isologous or heterologous nature. The geometry of multisubunit proteins is further clarified by defining four types of angle, called θ , ϕ , ϵ and ζ , which are parameters of the arrangement of the binding sites on each subunit. This present communication is limited to a consideration of oligomers.

Three main types of oligomer assembly can be distinguished: the protomer has, in each case, (a) one site-pair, (b) two isologous site-pairs and (c) one isologous and one heterologous site-pair. The range of possible structures is shown to correspond to proteins with respectively (a) cyclic symmetry (mostly dimers), (b) dihedral symmetry and (c) cubic symmetry. [Exceptions to this scheme should be noted but seem less likely. Dihedral symmetry is possible with the type of binding in class (c), and cubic symmetry may be generated from protomers

with two heterologous site-pairs; alternatively three appropriate site-pairs may be present.] Oligomeric proteins discussed include haemoglobin, aldolase, glutamate dehydrogenase, pyruvate dehydrogenases, immunoglobulins and spherical virus coats.

This analysis reveals some unusual possible protein structures when the subunits are markedly non-spherical. A dihedral structure for annelid chlorocruorin is proposed. Valentine & Chignell (1968) suggested that, because of the flat rhombic shape of the muscle phosphorylase tetramer, the molecule must be composed of two different types of subunit. However, chemical evidence suggests

that they are identical. To reconcile these findings it is proposed that the tetramer is dihedral with identical subunits.

I thank Dr W. L. G. Gent, Mrs B. Moreland and Dr D. C. Watts for help, and the Muscular Dystrophy Group of Great Britain for a grant.

Klug, A. (1967). In *Formation and Fate of Cell Organelles*, p. 1. Ed. by Warren, K. B. New York and London: Academic Press.

Monod, J., Wyman, J. & Changeux, J.-P. (1965). *J. molec. Biol.* **12**, 88.

Valentine, R. C. & Chignell, D. A. (1968). *Nature, Lond.*, **218**, 950.