of tobacco-mosaic virus by 10M-acetic acid but not by mineral acids (Fraenkel-Conrat, 1957); the acetic acid decreases the surface tension value to the region of over 30 dyn/cm (*International Critical Tables*, 1928). However, even smaller surface-tension value changes can have significant effects on the equilibrium. Thus a decrease of 2.5 dyn/cm in the value of 47 dyn/cm in blood serum results in a greater than 20% decrease in the equilibrium-constant value; such changes can be induced in human sera by freezing and thawing and by increased lipoprotein concentrations (Lewin, 1972). It is relevant that 6M-urea solution reduces the value of the surface tension from approx. 72 dyn/cm to 63 dyn/cm at 25°C while guanidinium chloride solutions reduce it even more (Lewin, 1972). Hence the contribution of high urea and of high guanidinium chloride concentrations to a lowering of interface tension, and therefore to de-adherence of hydrophobic groups, is sufficiently significant to be taken into account when considering helix $\rightleftharpoons$ coil transformations.

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Inhibition of δ -Aminolaevulate Dehydratase by Pyridoxal Derivatives and by Cyanide

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δ -Aminolaevulate dehydratase (EC 4.2.1.24) was purified from extracts of *Rhodospseudomonas spheroides* grown photosynthetically, and assayed as usual by incubating it with substrate (δ -aminolaevulate) in tris buffer, pH 8.5, and determining the product (porphobilinogen) with the Ehrlich reagent (Nandi, Baker-Cohen & Shemin, 1968; S. van Heyningen & D. Shemin, unpublished work). When 1 mM-pyridoxal phosphate was added to the incubation medium the enzyme was inhibited by 30%. At 0.05 mM-pyridoxal phosphate inhibition was almost negligible. Surprisingly the kinetics of inhibition were found to be almost perfectly non-competitive, the K_m for δ -aminolaevulate being

0.8 mM both in the presence and in the absence of inhibitor. The inhibition was not increased by prolonged incubation and activity was fully recovered when pyridoxal phosphate was removed by dialysis.

Nandi & Shemin (1968) showed inhibition of the enzyme by several keto acid analogues of the substrate (e.g. laevulate), but in these cases the inhibition was competitive and presumably due to the binding of the inhibitor at a lysine residue, which they suggested was at the active centre. The effect of pyridoxal phosphate seems to be a specific one of the pyridoxal molecule and not of a carbonyl, since pyridoxal and pyridoxamine phosphate both inhibited to about the same extent as pyridoxal phosphate; but pyridine, benzaldehyde and pyridine-4-carboxaldehyde did not inhibit significantly at 1 mM concentration.

Shemin (1970) has suggested that pyridoxal phosphate, which is a cofactor of δ -aminolaevulate synthetase, which precedes δ -aminolaevulate dehydratase in the pathway of porphyrin biosynthesis, may be involved in a concerted reaction *in vivo* between the two enzymes. Our current work does show some evidence for interaction between pyridoxal phosphate and δ -aminolaevulate dehydratase.

We have also found inhibition of the enzyme by 1 mM-sodium cyanide, and this inhibition shows competitive kinetics. Such competitive inhibition by cyanide has been said to be evidence for a Schiff-base mechanism of the type suggested for this enzyme by Nandi & Shemin (1968); but we consider that it is equally well explained by direct Schiff-base formation between cyanide and δ -aminolaevulate, thereby preventing reaction between the δ -aminolaevulate and the enzyme.

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The Primary Structure of Yeast Aldolase

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Fructose 1,6-diphosphate aldolases fall into two distinct classes that possess different molecular and catalytic properties (Rutter, 1964). Class 1 aldolases occur in animals and higher plants and are tetrameric molecules with molecular weights of about 160 000; they are not inhibited by chelating

agents and each subunit contains a reactive lysine residue capable of forming a Schiff base with the substrate (Kawahara & Tanford, 1966; Grazi, Rowley, Cheng, Tchola & Horecker, 1962). Class 2 aldolases occur in yeasts and bacteria. They are dimeric enzymes with molecular weights of about 80000 and, as they contain essential bivalent metal ions (Kobes, Simpson, Vallee & Rutter, 1969), are inhibited by chelating agents.

Studies of the primary structure of class 1 aldolase (as exemplified by muscle aldolase) are in progress in several laboratories (Sajgó, 1969; Anderson & Perham, 1970; Gracy, Lacko & Horecker, 1969), and it was therefore decided to study the primary structure of yeast aldolase in an attempt to ascertain whether the two different classes of aldolase are related structurally.

Yeast aldolase was prepared in pure crystalline form by a modification of an existing procedure (Richards & Rutter, 1961). It gave a single band by polyacrylamide-gel electrophoresis in the presence of 0.1% sodium dodecyl sulphate and the molecular weight of the subunit was shown to be 42000 ± 2000 , thus confirming the dimeric structure of the enzyme. Carboxymethylation with iodo[2- 14 C]acetate, as well as titration with 5,5'-dithiobis-(2-nitrobenzoic acid) in 4M-urea, indicated 10 cysteine residues/dimer, and the results of amino acid analyses show that the monomer consists of a single polypeptide chain containing about 360 amino acid residues, corresponding to a molecular weight of 40000. Moreover five radioactive peptides of unique sequence, each containing one residue of S-[2- 14 C]-carboxymethylcysteine, have been isolated and characterized from enzymic digests of the carboxymethylated enzyme.

A comparison of the sequences around the cysteine residues in these peptides (which together account for approx. 25% of the molecule) with the sequences of tryptic peptides containing cysteine derived from the rabbit (Sajgó, 1969) and sturgeon (Gibbons, Anderson & Perham, 1970) enzymes has so far shown no evidence of sequence homology between the class 1 and the class 2 aldolases.

In contrast with other glycolytic enzymes such as glyceraldehyde 3-phosphate dehydrogenase (G. M. T. Jones & J. I. Harris, 1971, in preparation), it therefore appears that aldolases from yeast and muscle are not structurally related and that they have evolved independently.

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Light-Induced Development of Enzymes of Glycollate Metabolism in Etiolated Bean Leaves

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Phosphoglycollate and glycollate are products of photosynthetic CO₂ fixation by the chloroplasts, but the subsequent metabolism of glycollate occurs outside the chloroplasts. In parallel to a study of the development of photosynthetic activity in the plastids of greening leaves, the activities of some of the enzymes of the glycollate pathway were determined. Greening was brought about by transferring 14-day-old dark-grown plants of *Phaseolus vulgaris* cultivar. Alabaster to continuous illumination of 700 μ W/cm². Of the activities investigated, phosphoglycollate phosphatase and NADPH-glyoxylate reductase have been reported to be wholly located in the chloroplasts (Thompson & Whittingham, 1967), catalase has been located exclusively in the peroxisomes, and glycollate oxidase and hydroxypyruvate reductase are also peroxisomal, although this may not be their only location (Tolbert, Yamazaki & Oeser, 1970).

With the exception of the chloroplast component of the measured NADPH-glyoxylate reductase activity, which declined, all of these enzymes increased about tenfold in activity/leaf during 96h of greening, but their activities remained constant or declined during similar periods in darkness. A lag phase in development of about 5h was found. Their behaviour thus resembled that of those photosynthetic carbon-cycle enzymes that are located exclusively in the chloroplasts (ribulose diphosphate carboxylase, phosphoribulokinase and NADP-linked triose phosphate dehydrogenase) and of ribose phosphate isomerase, which occurs both inside and outside the chloroplasts (Bradbeer, 1969, 1970). Those other carbon-cycle enzymes that occur both in the chloroplast and in the cytoplasm did not show a lag period and had smaller overall increases

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