ADP produced mixed inhibition (Webb, 1963) with respect to NADH, which was non-linear (Cleland, 1963b), K_i tending to 0.6 mM. Inhibition was non-competitive with nitrate (K_i 15 mM) or BV^+ (K_i 50 mM) and apparently competitive with phosphate ($K_i \geq 15$ mM). ATP also produced mixed inhibition (K_i 6 mM) with NADH. 5'-AMP and 2':3'-cyclic AMP inhibited competitively (K_i 4 mM approx.), whereas 3':5'-cyclic AMP produced mixed inhibition (K_i 0.4 mM). Notwithstanding differences in kinetics, ATP, ADP, 5'-AMP and 2':3'-cyclic AMP induced co-operative kinetics for NADH with negative and positive phases (Levitzki & Koshland, 1969) and ADP itself was co-operative ($n = -3$ approx.). Nitrite inhibited non-competitively with nitrate (K_i 6 mM) and synergism (Webb, 1963) occurred with ADP. In fresh compared with aged enzyme ADP produced stronger inhibition and co-operativity by NADH was accentuated, but K_m for uninhibited enzyme (2.5 μ M) was smaller. Nitrate showed slight negative co-operativity induced by ADP.

The suggestion that ADP exerts allosteric control of nitrate reductase (Nelson & Ilan, 1969) implies compartmentation of enzyme and ADP relative to supply of NADH if the observed kinetics apply *in vivo* in relation to probable concentrations of ADP (40–140 μ M) and NAD (+NADH₂) (20–60 μ M) in spinach cells or chloroplasts (Ogren & Krogmann, 1963; Jozwiak & Leyko, 1969). Alternatively, the enzyme in chloroplasts (Coupé, Champigny & Moysé, 1967) may possess different kinetics.

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The Mechanism of Action of Yeast Invertase

By S. SHALL, A. BASEER and A. WAHEED. (*Biochemistry Laboratory, University of Sussex, Brighton BN1 9QG, U.K.*)

The mechanism of action of yeast invertase has been investigated by chemical modification and kinetic studies on external and internal yeast invertase. The external yeast invertase is a glycoprotein containing about 50% carbohydrate (Neumann & Lampen, 1967; Waheed & Shall, 1967). Internal invertase is found inside the cell (Friis & Ottolenghi, 1959; Gascon & Ottolenghi, 1967) and contains no carbohydrate (Gascon & Lampen, 1968). The two enzymes are reported to differ in amino acid composition (Gascon, Neumann & Lampen, 1968); in particular, the internal enzyme does not contain cysteine.

External invertase is inhibited to about 50% by low concentrations of iodine (von Euler & Landergren, 1922). This inhibition is reversible by mercaptoethanol and β -mercaptoethylamine but not by α -carboxyl- β -mercaptoethylamine (Shall & Waheed, 1968).

Internal invertase is inhibited by iodine and reactivated by mercaptoethanol in the same way as external invertase. The kinetics of the two enzymes are very similar. Both require an acid with pK_a about 6.8 in its protonated form. The internal invertase shows a pH-dependence of the K_m , which has not previously been reported for external invertase, although P. Ottolenghi (personal communication) has shown that the K_m of external invertase also is dependent on pH.

Iodoacetamide inhibits external yeast invertase but iodoacetic acid does not. The group that is alkylated has a pK_a well in the alkaline region.

Both the external and the internal enzyme are inhibited by cyanogen bromide in a biphasic reaction. A fast reaction is pH-dependent and a slower reaction is pH-independent.

It is concluded that the iodine reaction is the reversible oxidation of a methionine sulphur atom and that this reaction leads to an approximate halving of the number of effective active sites. It is postulated that the active site of yeast invertase contains a carboxylate anion because of the difference in reactivity between cysteine and mercaptoethylamine, and between iodoacetamide and iodoacetic acid. On the basis of the observations a mechanism of action of yeast invertase is postulated. An imidazolium cation protonates the glycosidic oxygen atom. Departure of the neutral alcohol group will leave behind an unstable intermediate carbonium ion in which the electron deficiency is spread over the C-2 atom as well as the ring oxygen atom. The active-site carboxylate anion will function during this and the previous stage by

stabilizing the electron-deficient species. The next stage is the attack on the C-2 cation by a nucleophilic oxygen atom of an alcohol or water to yield a fructoside or fructose.

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Some Aspects of the Effects of Electrostatic Attraction and of Water-Entropy Changes on the Helical Stability of Deoxyribonucleic Acid

By S. LEWIN. (*Physical-Biochemistry Laboratory, Department of Postgraduate Molecular Biology, North-East London Polytechnic, Forest Road, London E17 4JB, U.K.*)

It is currently considered that there exists a major inter-strand electrostatic repulsion, due to the negatively charged phosphate groups, in DNA, thereby adversely affecting helical stability. This repulsion has been generally considered to be overridden by pro-helix forces that include Watson-Crick hydrogen-bonding, base-stacking hydrophobic interactions and hydrogen-water-bridging (Watson & Crick, 1953; Crothers & Zimm, 1964; for extensive references see Zimm & Kallenbach, 1962; Marmur, Rownd & Schildkraut, 1963; Lewin, 1967*a,b*). Since the phosphate groups are 'neutralized' by their hydrated counter-ions, residual electrostatic repulsions would arise only if the latter were intra-strand sited, whether situated in intra-phosphate or inter-phosphate locations (the latter in accord with Rice & Geiduschek, 1958). However, the hydrated Na^+ ion in the sodium salt of DNA can bridge the two strands when situated inter-strandwise between the respective phosphate groups (Lewin, 1967*a*, 1971).

Stereochemical considerations, based on the construction of several correctly proportioned molecular models of the B-form of the DNA double helix (using Corey-Pauling-Koltun atomic models and Courtauld atomic models) following those of

Wilkins, Stokes & Wilson (1953) and energy considerations lead to the conclusion that the hydrated Na^+ ion bridgings enforce powerful electrostatic attractions. Therefore, non-electrostatic anti-helix forces must exist that are able to override the pro-helix forces under suitable conditions. I have computed that several water displacements can be involved (one of which is concerned with the rupture of water attachments to the amino, imino, ternary nitrogen and carbonyl groups to enable Watson-Crick hydrogen-bonded base-pairing in the formation of the double strand) as well as mechanical strains, one of which occurs along the phosphate-ribose chain, can adversely affect helical stability (Lewin, 1971).

A temperature-rise enforced bulk-water-entropy increase (TS) can offer major anti-helix contributions, one of which is a decreased trend to draw away water molecules from their attachments to the above-listed nucleotide groups, thereby adversely affecting base-pairing. For example, a temperature rise from 10 to 25°C can withhold approximately 1.9 kcal from 1 mol of adenine-thymine and approximately 2.9 kcal from 1 mol of guanine-cytosine base-pairings. Thus the bulk-water-entropy-temperature factor can contribute effectively more to the latter than to the former base-pairings; and this differential contribution may be of paramount importance in ensuring the experimentally noted increased helical stability with increasing guanine-cytosine content of DNA (e.g. Marmur & Doty, 1959).

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Is Synthesis of Glucose 6-Phosphate Dehydrogenase Induced by a Metabolite of Glucose?

By C. D. GREEN, J. SKARDA and J. M. BARRY. (*Department of Agricultural Science, University of Oxford, Oxford OX1 3PF, U.K.*)

Leader & Barry (1969) showed that when mammary tissue from pregnant C3H mice is cultured