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Active-Site Thiol Groups of Yeast Hexokinase

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Two forms of hexokinase (A and B) can be isolated from baker's yeast by a method that avoids proteolytic degradation of the enzymes (Lazarus, Ramel, Rustum & Barnard, 1966; Gazith, Schulze, Gooding, Womack & Colowick, 1968). Inactivation of hexokinase B with iodoacetate at low ionic strength was accompanied by the alkylation of all 8 thiol groups/molecule of mol.wt. 100000 (Lazarus, Derechin & Barnard, 1968). The present paper describes the alkylation of hexokinase B at high ionic strength.

Hexokinase B was reacted with iodoacetate (1–10mM) at 35° and pH 8.6 in glycine–NaCl–NaOH buffer, 1.0M, when the enzyme is known to exist as a dimer of mol.wt. approx. 50000 (Derechin, Ramel, Lazarus & Barnard, 1966). Inactivation of the enzyme is first-order with respect to the concentration of each reactant with a rate constant of $0.131 \text{ mole}^{-1} \text{ sec}^{-1}$. The rate of inactivation increases abnormally sharply at pH values above 8.5, probably owing to a progressive unfolding of the protein with increasing pH.

The enzyme is protected from this inactivation by the presence of glucose and, from these data, the dissociation constant for the enzyme–glucose complex has been calculated to be 0.46mM. This value compares favourably with that determined by equilibrium dialysis (Gazith *et al.* 1968).

Alkylation of hexokinase B, in the same glycine buffer, by iodo[^{14}C]acetate shows that inactivation follows closely the incorporation of 2 atoms of ^{14}C /molecule of enzyme. A further 2 atoms of ^{14}C /molecule are incorporated at a lower rate (k $0.011 \text{ mole}^{-1} \text{ sec}^{-1}$).

Analysis of the protein, after incorporation of 4 atoms of ^{14}C /molecule, identified over 90% of the radioactivity as *S*-carboxymethylcysteine; no free cysteine could be detected in hydrolysates of the alkylated protein.

The collective results indicate that 2 thiol groups/molecule (mol.wt. 50000) of hexokinase B are probably located at the active site. These essential thiol groups may be preferentially labelled with iodo[^{14}C]acetate and this technique may be a useful basis for investigating amino acid sequences in the active site of the enzyme.

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Enzyme Distributions in an Amoeba During Encystment

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The subcellular distributions of enzymes were studied in vegetative amoebae of *Hartmannella castellanii* and also during encystment in a replacement medium (Griffiths & Hughes, 1968). The cells were broken in an all-glass hand-operated homogenizer in 0.32M-sucrose–20mM-tris–10mM-EDTA medium. The cell-free homogenate was centrifuged at 35000 rev./min. for 2½ hr. ($\approx 100000g$ for 1 hr.) in an unbuffered sucrose gradient [15–60% (w/v) sucrose] in a BXIV zonal rotor.

In vegetative amoebae 44% of the acid phosphatase was sedimentable with a median buoyant density of 1.16g./ml., as was 76% of the catalase (median buoyant density 1.21) and all the recoverable cytochrome oxidase activity (median buoyant density 1.18). Of the other enzymes studied only 28% of the *N*-acetyl- β -glucosaminidase (1.15) and 27% of the NADPH–cytochrome *c* oxidoreductase (1.19) but 67% of the NADH–cytochrome *c* oxidoreductase were sedimentable.

After incubating the amoebae in the encystment medium acid phosphatase and *N*-acetyl- β -glucosaminidase showed increases in both their sedimentability (69% and 40% respectively) whereas the sedimentability and median buoyant density of catalase increased only after an initial decrease during the first 5 hr. of encystment. After 15 hr. encystment the sedimentable acid phosphatase and *N*-acetyl- β -glucosaminidase were both resolvable as double peaks (median buoyant densities 1.16 and 1.19 for acid phosphatase and 1.16 and 1.20 for 1.19 for acid phosphatase and 1.16 and 1.20 for *N*-acetyl- β -glucosaminidase).

An increase also occurred in the median buoyant density of cytochrome oxidase (1.20) after 15 hr. encystment, but its sedimentability remained