Studies of Transphosphorylating Enzymes. The Incorporation Rate of ^{32}P into Trichloroacetic Acid Soluble Nucleotides, Protein-Phosphoserine and Phosphothreonine of Baker's Yeast

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In a recent paper [1] it was demonstrated that radioactive phosphoserine could be isolated from a yeast hexokinase preparation incubated with AT^{32}P or glucose-6-^{32}P. The results were interpreted in the way that the transfer of phosphorus in the hexokinase system is at least a two-step reaction with the formation of an enzyme-phosphate as an intermediate. It seemed to be of considerable interest if similar types of transphosphorylations could be demonstrated in later steps of the carbohydrate metabolism.

As in animal cells [2] the only phosphorylated amino acids present in partial hydrolysates of the Schneider protein residue from yeast cells were phosphoserine and phosphothreonine [3]. Recently Schmitz [4] also found that the perchloric acid soluble mononucleotides from yeast were the same as present in the corresponding extracts from animal cells [5]. Yeast cells were accordingly cultivated in a glucose medium and the rate of phosphorus incorporation into the phosphorylated amino acids and some of the metabolically active nucleotides were determined and compared.

In preliminary experiments the cells were preincubated in a 1 % glucose medium for 30 min followed by the addition of ^{32}P-phosphate at the beginning of each series. The specific activity values of the trichloroacetic acid soluble mononucleotides were determined at 0.5, 2, 5, 10, and 20 min of cultivation, mainly in the same way as Brumm *et al.*[6]. The values were compared with the specific activity values of the phosphoserine and phosphothreonine fractions isolated from the Schneider protein residues of each batch of cells [1,2]. With the increase in time there was a parallel increase of the phosphorus incorporation into the different mononucleotides and phosphoamino acids.

In several experiments the yeast cells were grown in the presence of enzyme inhibitors acting on enzymes in metabolic steps after the initial hexokinase system. In each series one of the inhibitors was added after 20 min of preincubation in a 1 % glucose medium. 10 min later the radioactive phosphate was added. The specific activities of the different compounds were determined after 2 and 20 min of cultivation. A control without inhibitor was always run parallel. The inhibitors, iodoacetic acid, potassium fluorid, sodium azide [7] or phlorhizin [8] were added to a final concentration of 2×10^{-3} M in the different series.

As compared with the controls there was always a decrease of the specific activity values of the trichloroacetic acid soluble nucleotides in the different inhibitor series. A parallel decrease in the specific activity values of the phosphorylated amino acids from the Schneider protein residues was also observed. This is taken as further evidence that several phosphoproteins in the later steps of the carbohydrate metabolism may also function as transphorylating enzymes in the same way as has previously been demonstrated for the hexokinase system.

1. Ågren, G. and Engström, L. *Acta Chem. Scand.* **10** (1956) 489.
2. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 1570.
3. Ågren, G. and Engström, L. *Acta Chem. Scand.* **10** (1956) 877.
4. Schmitz, H. *Biochem. Z.* **325** (1954) 555.
5. Schmitz, H., Hurlbert, R. B., Brumm, A. F. and Potter, V. R. *J. Biol. Chem.* **209** (1954) 41.
6. Brumm, A. F., Potter, V. R. and Siekevitz, P. *J. Biol. Chem.* **220** (1956) 713.
7. Spiegelman, S., Kamen, M. D. and Sussman, M. *Arch. Biochem.* **18** (1948) 409.
8. Meyerhof, O. and Wilson, J. R. *Arch. Biochem.* **17** (1948) 153.

Yeast Lactic Dehydrogenase

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The enzyme has been isolated from bakers yeast after mechanical disruption of the cells. Preparations of the enzyme are able to reduce cytochrome c to a remarkable extent without substrate added. Enzymatic properties will be discussed.