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LETTER (S) TO THE EDITORS

A COENZYME FOR PHOSPHOGLUCOMUTASE

A study of the enzymatic transformation of galactose-1-phosphate revealed that extracts of *Saccharomyces fragilis* will transform this substance into a reducing ester only in the presence of a thermostable factor. The same factor has been found to be necessary for the conversion of glucose-1-phosphate into glucose-6-phosphate with extracts of *S. fragilis* or *S. cerevisiae*.

The action of this coenzyme can be revealed in crude maceration extracts, but these retain a considerable activity in the absence of added coenzyme. Results recorded in Fig. 1 were obtained with a brewers yeast enzyme partially purified by ammonium sulphate fractionation and dialysis. The coenzyme preparation was obtained from brewers' yeast, the ratio: activity/extinction at 260 mμ was 150 times higher than in the extract obtained by heating the yeast in one volume of water and filtering. The ratio activity/total phosphate was 20 times higher.

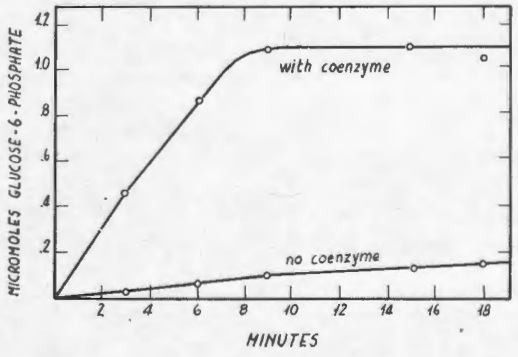

FIG. 1 Activation of phosphoglucomutase with the coenzyme. Reducing power measured with a copper reagent and a glucose-6-phosphate standard. Incubation at 30°C. of: partially purified enzyme, 0.005 μM Mg ++ synthetic glucose-1-phosphate 1.5 μM, purified coenzyme containing 0.1 μM total phosphate. Total volume 0.2 ml.

TABLE I

Changes in Phosphate Fractions

Results in micromoles. Conditions similar to those in Fig. 1. Half the amount of coenzyme.

Coenzyme	Time	P Inorg.	P Labile(*a*)	P Stable(*b*)	Reduction (*c*)
	min.				
With	0	0.04	0.89	0.07	0.00
With	15	0.08	0.09	0.83	0.86
Without	0	0.05	0.89	0.01	0.00
Without	15	0.08	0.85	0.07	0.01

a Phosphate liberated in 10 min, at 100°C. in 1*N* acid minus inorganic.
b Total phosphate minus labile and inorganic.
c Reducing power in terms of a glucose-6-phosphate standard.

In Table I are recorded the changes in the phosphate fractions and in reducing power. These correspond to those known to be brought about by phosphoglucomutase.

We have been unable to identify this factor with any of the known coenzymes. It can be precipitated from crude solutions with lead, mercury, silver, and barium salts. In general, it follows inorganic phosphate during fractionations with these reagents. Inorganic phosphate can be removed from it as magnesium ammonium or as uranyl salts, when the coenzyme will no longer precipitate with mercury salts.

Purified preparations are colorless and show ultraviolet absorption at 260 mμ, but purification will have to be carried on further to find out whether this absorption is due to the active compound.

Treatment with $N/2$ acid at 100°C. will destroy the activity in 15 min. It is more resistant to treatment with alkali under the same conditions.

Kendal and Stickland (1) obtained an activation of phosphoglucomutase by adding hexose diphosphate, but Cori *et al.* (2) were unable to obtain any effect. The hexosediphosphate preparation of Kendal and Sitckland may have been contaminated with the new coenzyme reported in this paper.

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2. Cori, G. T.; Colowick, S. P., and Cori, C. F.: *J. Biol. Chem.* **124**, 543 (1938).