On the occurrence of diphosphothiamine disulfide in baker's yeast

In a previous paper[1], the occurrence of diphosphothiamine disulphide in bakers' yeast, but not in brewer's yeast and in animal tissues, was demonstrated by an original method. The ratio of the contents of the disulphide to that of diphosphothiamine was found to be 1:9. Aeration with oxygen did not significantly affect this proportion. MYRBÄCK[2,3], who first provided indirect evidence for the occurrence of diphosphothiamine disulphide in yeast claimed, on the other hand, a dependence of the presence of this compound upon oxygen availability.

The possible function of the disulphide in yeast is not clear. The crystalline compound prepared according to ZIMA[4] had no coenzymic activity toward non-oxidative and oxidative pyruvate decarboxylase or toward transketolase. Only upon reduction with cysteine or glutathione to the thiol form did it become active in the above tests[1].

The disulphide, however, seems to have some affinity for the yeast apodecarboxylase. As shown in Table I, it combines with apoenzyme to give an inactive complex, which is only partially reactivated by a successive addition of equimolecular amounts of diphosphothiamine. On the other hand carboxylase, resulting from preincubation of apocarboxylase with diphosphothiamine, is not affected by a successive addition of the disulphide.

TABLE I

Cocarboxylase activity of diphosphothiamine and diphosphothiamine disulphide for purified yeast carboxylase. Reaction mixture: yeast apocarboxylase; diphosphothiamine, 0.20 μg; diphosphothiamine disulphide, 0.40 μg; 0.20 ml of 0.1 *M* $MgCl_2$; 0.30 ml of 0.5 *M* citrate buffer (pH 6); 0.50 ml of 1 *M* pyruvate. Distilled water to 3.30 ml. Incubated at 30° for 40 min.

Apocarboxylase (ml)	$\mu l\ CO_2$			
	Diphosphothiamine disulphide	*Diphosphothiamine*	*Diphosphothiamine + diphosphothiamine disulphide**	*Diphosphothiaminedisulphide** + diphosphothiamine*
0.05	6	120	130	91
0.03	8	68	74	37
0.02	0	49	49	18

* Disulphide added to the reaction mixture 5 min after the addition of diphosphothiamine.
** Disulphide added to the reaction mixture 5 min before the addition of diphosphothiamine.

The disulphide does not exhibit any affinity for apotransketolase.

A chromatographic analysis of thiamine derivatives in 20 g of wet baker's yeast, incubated for 48 h at 24° with 1% glucose and 10 mg [^{35}S]thiamine* (specific activity 1.94 μC/mg), is shown in Fig. 1.

All thiamine and thiamine disulphide compounds were found to be labelled; the relative radioactivity of each compound, expressed as a percentage of total radioactivity, closely parallels the relative amount of each compound found by us in baker's yeast[1].

Since no labeled thiamine disulphide derivatives appear when [^{35}S]thiamine is incubated in the same conditions with brewer's yeast or animal tissues, it seems

* [^{35}S]Thiamine was a gift from Hoffmann-La Roche, Basel.

reasonable to suppose that an enzyme, which reversibly oxidizes diphosphothiamine to the disulphide, is present in baker's yeast.

The biological significance of the diphosphothiamine disulphide–diphosphothiamine equilibrium, which has also been studied polarographically[5] and the meaning of the occurrence of the disulphide in baker's yeast deserve further delucidation.

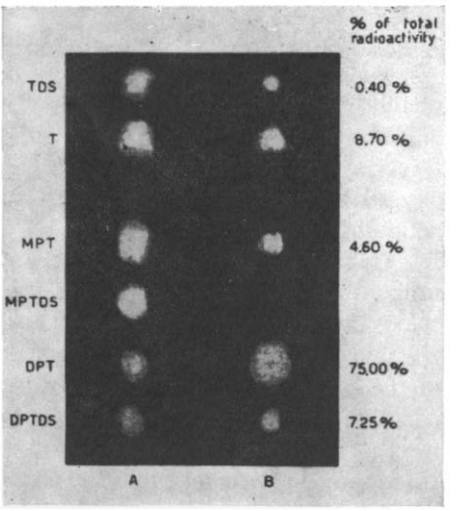

Fig. 1. Chromatogram of baker's yeast thiamine and thiamine disulphide compounds according to GREGOLIN *et al.*[1]. (A), reference mixture. (B), thiamine and thiamine disulphide derivatives from baker's yeast. T, thiamine; TDS, thiamine disulphide; MPT, monophosphothiamine; MPTDS, monophosphothiamine disulphide; DPT, diphosphothiamine; DPTDS, diphosphothiamine disulphide. The radioactivity of each spot has been measured by direct scanning of the paper using an end-window Geiger counter. The photograph of the chromatogram was taken in ultraviolet light at 254 mμ wavelength.

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The influence of some sulfur compounds on the catalysis by o-iodophenolate of ascorbate oxidation by oxygen

The bulk of information concerning the kinetics of ascorbate oxidation by molecular oxygen is based on catalysis of this process by heavy metal ions. In such systems many sulfur compounds of biochemical interest function as inhibitors by inactivating the heavy-metal ions by formation of a complex or an insoluble salt. In a preceding paper[1] we described another system with 7-iodo-8-hydroxychinoline-5-sulfonic acid (Ferron)