SULFHYDRYL GROUPS AND TRANSKETOLASE ACTIVITY

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Received December 5, 1965

Transketolase, an enzyme of pentose cycle, catalyzes the reaction of glycolaldehyde transport from the xylulose-5-phosphate to the ribose-5-phosphate with the formation of sedoheptulose-7-phosphate. There is not much known about this enzyme, especially in respect to its functional groups. In the previous work (Kochetov, Kobylyanskaya, being published) it was shown that one of the functional transketolase groups is imidazole group (or groups) of histidine, intactness of which is required for transketolase activity.

In the present report data are presented indicating that a sulfhydryl group (or groups) is the other functional group of the enzyme. In this case silver ions served as a specific reagent for these groups.

Methods

Crystalline transketolase preparation was obtained from baker's yeast (Cooper,J.R. et al, 1958). Specific activity of the enzyme, expressed in the units suggested by Racker with associates (De la Haba et al, 1955) was about 15,000. Pentose phosphate mixture, obtained from ribose-5-phosphate (De la Haba et al, 1955) served as a substrate of transketolase reaction. The enzyme activity was determined by the quantity of sedohepulose-7-phosphate formed in the transketolase reaction. The composition of incubation samples (where it is not especially specified) was as follows: 0.28 ml of 0.12M tris-buffer, pH 7.5; 0.05 ml of 0.1M magnesium chloride; 0.05 mg of thiaminpyrophosphate (TPP): 7 mg of the barium salt of

pentose phosphates (converted to ammonium salt before the experiment); 0.1 ml of transketolase solution. Total volume - 0.7 ml. The incubation was carried out at room temperature. 0.1 ml samples taken from the incubation mixtures at 0,5 10 and 20 minutes, were poured into 0.7 ml of 5% trichloracetic acid; from this 0.5 ml was taken for determination of sedoheptuloso-7-phosphate according to Dische (1953).

Sulfhydryl groups in the enzyme were determined by amperometric titration with silver nitrate with a rotating platinum electrode, after Kolthoff with associates (Kolthoff et al, 1946); the method was somewhat modified.

In studying the effect of sulfhydryl group binding by the silver ions on transketolase activity, sulfhydryl group binding was carried out in the following conditions: 0.5 ml of 0.07M glycyl-glycine buffer, pH 8.4, containing NH_4OH in a final concentration of 0.2M; 0.15 ml of the enzyme solution and a corresponding amount of 10^{-4}M solution of silver nitrate.

Results and Discussion

By the method of amperometric titration there were about 2 sulfhydryl groups per enzyme molecule (transketolase molecular weight was accepted as 140,000, Datta et al, 1961). If one of these groups be bound by silver, the activity of the enzyme during the initial incubation period was not detected; however, it later returned to the initial level (curves 1 and 2, fig.1). When two sulfhydryl groups were bound the activity was also completely restored; however more time was required for this (curve 3, fig.1).

The data obtained may be explained by assuming that sulfhydryl groups are important for activity and when they are bound by silver the enzyme loses its enzymatic activity. During the incubation the silver ions are displaced (possibly by the added transketolase cofactors)

and the activity is restored. The following experiments were carried out to check this hypothesis. After two sulfhydryl groups were bound by silver, the enzyme was incubated for 15 minutes with magnesium and TPP (in the concentrations usually applied in determining the transketolase activity), then the activity was determined by the usual method. It appeared that in this case there was no delay in appearance of activity and from the very beginning the activity was equal to such of the refined enzyme (curve 4, fig.1). These experiments confirm our suggestion that during the incubation there occurs a displacement of silver ions from the sulfhydryl groups of the enzyme. As demonstrated by further experiments, the capacity to displace silver ions in this case was characteristic of thiaminpyrophosphate, not of magnesium, since preincubation with magnesium alone had no effect, whereas preincubation with TPP completely eliminated the initial lag in activity.

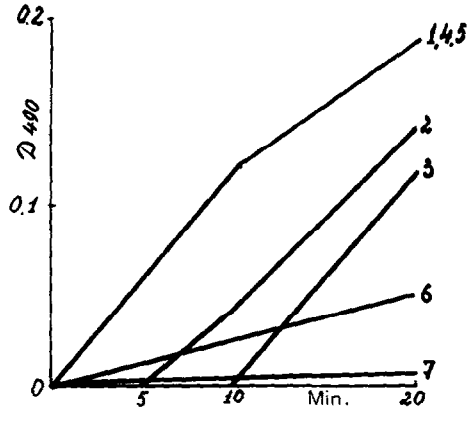

Fig.1. The effect of silver ions on the activity of transketolase.

Abscissa - incubation time in minutes.
Ordinate - enzyme activity, expressed in optic density units, obtained in measuring the colour intensity formed at the expense of sedoheptulose-7-phosphate, determined after Dische.
1 - purified enzyme;
2 - an enzyme in which 1 sulfhydryl group is blocked by silver;
3 - an enzyme in which 2 sulfhydryl groups are blocked by silver;
4 - refined enzyme preincubated for 15 minutes with magnesium and TPP for 15 minutes before activity determination;
5 - as 3, only before the activity was determined the enzyme was preincubated with magnesium and TPP for 15 minutes;
6 - the activity of refined enzyme, determined without any magnesium or TPP addition;
7 - as 3, only the activity was determined without any magnesium or TPP addition.

Sulfhydryl groups of the enzyme were titrated with silver ions in the presence of TPP at different periods after its addition. The results are presented in the table.

The number of sulfhydryl groups of transketolase in relation to the time of its preincubation with TPP

	Without TPP	TPP added		
Preincubation time (in min.):		0	5	20
The number of sulfhydryl groups (per mol of the enzyme)	2.1	2.1	0.9	0

As may be seen from the data presented in the table, immediately after TPP addition the number of sulfhydryl groups determined equal 2.1, i.e. it is the same as determined in the enzyme without any TPP addition. Five minutes after TPP addition the result is halved, and in 20 minutes it is zero.

These results correlate with the data presented on fig.1 (curves 2,3). At first the silver ions bind the

sulfhydryl groups and the enzyme activity is therefore absent. Then, during incubation with TPP, silver ions are displaced, and if only one sulfhydryl group is bound, the activity is restored already in 5 minutes (fig.1, curve 2). Silver ion displacement evidently occurs in such a way that TPP takes their place. This is particularly indicated by the fact that in 5 minutes only one sulfhydryl group is titrated (see table). If two sulfhydryl groups are bound, more time is required to displace the silver ions (fig.1, curve 3). This corresponds to the fact that with a sufficiently prolonged time of TPP-enzyme interaction the number of sulfhydryl groups determined is zero (see table), i.e. in these conditions silver is already incapable of interaction with the enzyme and cannot cause its inactivation.

Thus, TPP in the concentrations used in the experiments is capable of displacing silver from its bond with sulfhydryl groups of the enzyme. TPP concentrations used, as compared with the concentrations of silver ions, were so high that it was impossible to compare the silver and the TPP affinity to the enzyme.

Crystalline transketolase as prepared by us required the addition of magnesium and TPP ions for full activity. However some activity was manifested without addition of these cofactors (fig.1, curve 6), indicating that some TPP and magnesium remained bound to the enzyme and did not separate during extraction and purification. It appeared that if the activity of the enzyme in which 2 sulfhydryl groups are bound be determined without adding any cofactors, the activity is not manifested even after a prolonged incubation period (fig.1, curve 7). In other words, in the concentrations used silver ions are capable of displacing TPP from its bond with the sulfhydryl groups of the enzyme.

The data presented in this work permit the conclusion that sulfhydryl groups constitude a part of the active transketolase center. The fact that TPP eliminates the enzyme inhibition by the silver ions, as well as the fact that in the presence of TPP the number of sulfhydryl

groups determined is decreased indicate that one function of the sulfhydryl groups is to effect the binding of apoenzyme with the coenzyme, i.e. with TPP.

The authors are thankful to Professor S.E.Severin for his valuable advices and the interest in this work.

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