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The Inactivation of Alcohol Dehydrogenase by Tyrosinase *

Inaktywacja dehydrogenazy alkoholowej przez tyrozynazę

Инактивирование спиртовой дегидрогеназы тирозиназой

INTRODUCTION

Plant tyrosinase catalyzes the oxidation of tyrosine to melanine. This enzymatic activity is connected with the presence of copper in the protein molecule. This enzyme can also oxidize mono-and diphenols, in some cases to a higher degree than tyrosine and DOPA. Plant tyrosinase is also able to oxidize tyrosine in some peptides and proteins. The influence of tyrosinase on proteins can be demonstrated by colorimetric determination of the increase in time of the extinction of these mixtures, or by measuring the oxigen uptake in the Warburg apparatus. Traces of DOPA and catechol can activate tyrosine in proteins and increase the degree of its oxidation.

So far the influence of tyrosinase has been studied with relation to the following proteins: thrombin, prothrombin, and fibrinogen (7). It has also been found that tyrosine present in the proteins of such animal enzymes as pepsin, trypsin, chymotrypsin (1, 5, 8), invertase (6, 8), and amine oxidase (2), can be oxidized under the influence of tyrosinase.

On the other hand, no studies can be found in the current literature on the inactivation of plant enzymes by tyrosinase.

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EXPERIMENTAL

The present paper reports the investigations on the influence of mushrooms tyrosinase on alcohol dehydrogenase from yeast. Tyrosinase from *Psalliota campestris* was purified according to the method given by K e i l i n and M a n n (3). The obtained preparation was characterized by $Q_{O_2} = 1350$, i.e. its activity was 22 times that of the homogenate; the preparation contained 2.5 mg of dry matter per 1 ml. Alcohol dehydrogenase was obtained from a twice crystalized suspension of the yeast enzyme suspended in the saturated solution of ammonium sulphate (produced by the firm Boehringer und Söhne, German Federal Republic). Before experimenting, alcohol dehydrogenase was dialyzed for 24 hours in the buffer solution KH_2PO_4/K_2HPO_4 0.01 M, pH = 7.5, at 0°C. After dialysis the preparation contained 2.85 mg of dry matter of the enzyme in 1 ml.

The activity of alcohol dehydrogenase was determined through using a Zeiss spectrophotometer, type VSU 1. The method developed by R a c k e r (4) was used. Alcohol dehydrogenase showed the activity of 680 units per ml. The spectrophotometric measurements of the inhibition of the activity of dehydrogenase by tyrosinase were carried out under the following circumstances:

	Tyrosinase ml	Alcohol dehydrogenase ml	Phosphate buffer 0.01 M pH = 7.5
Test	0.5	1.0	2.0
Control	0.5 (boiled enzyme)	1.0	2.0

The test and control samples were incubated at 25°C for 15 minutes, then the activity of alcohol dehydrogenase was determined spectrophotometrically. Under these circumstances alcohol dehydrogenase lost its activity completely.

The increase of the extinction of the mixture of tyrosinase and alcohol dehydrogenase solutions was determined colorimetrically. For this purpose a photocolorimeter of the type KF2 (produced by Warszawskie Zakłady Aparatury Laboratoryjnej) was used; the wave length was 425 mμ, the thickness of the layer 1 cm. The increase E was measured every 2 minutes during 30 minutes, according to the data contained in Table 1.

As appears from Table 1, the influence of trace amounts of DOPA and catechol on the oxidation of alcohol dehydrogenase in the presence

Table 1.

Variant No.	Tyrosinase ml	Alcohol dehydrogenase ml	DOPA 0.004 % ml	Catechol 0.004 % ml	Phosphate buffer 0.01 M pH = 7.5
I. Test	0.5	1.0	—	—	1.5
Ia. Control	0.5	—	—	—	2.5
Ib. Control	0.5 (boiled) enzyme)	1.0	—	—	1.5
II. Test	0.5	1.0	0.1	—	1.4
IIa. Control	0.5	—	0.1	—	2.4
IIb. Control	0.5 (boiled enzyme)	1.0	0.1	—	1.4
III. Test	0.5	1.0	—	0.1	1.4
IIIa. Control	0.5	—	—	0.1	2.4
IIIb. Control	0.5 (boiled enzyme)	1.0	—	0.1	1.4

of tyrosinase was also studied. The arithmetical means of the results of the three replications of the separate variants are shown in Fig. 1. The curve of the measurement of extinction of the controls Ib, IIb and IIIb had a linear course, in accordance with the control Ia in Fig. 1.

The increase of the extinction of a mixture of tyrosinase and alcohol dehydrogenase solutions suggested the possibility of an increase of the oxygen uptake of this system. In this connection manometric determinations in the apparatus of Warburg were undertaken. The measure-

Table 2.

Variant No.	Tyrosinase ml	Alcohol dehydrogenase ml	DOPA 0.004 % ml	Catechol 0.004 % ml	Phosphate buffer 0.01 M pH = 7.5
I. Test	0.5	1.0	—	—	1.3
Ia. Control	0.5	1.0	—	—	2.3
Ib. Control	0.5 (boiled) enzyme)	1.0	—	—	1.3
II. Test	0.5	1.0	0.1	—	1.2
IIa. Control	0.5	—	0.1	—	2.2
IIb. Control	0.5 (boiled enzyme)	1.0	0.1	—	1.2
III. Test	0.5	1.0	—	0.1	1.2
IIIa. Control	0.5	—	—	0.1	2.2
IIIb. Control	0.5 (boiled enzyme)	1.0	—	0.1	1.2
IV. Test	—	1.0	—	—	1.8
IVa. Control	—	—	—	—	2.8

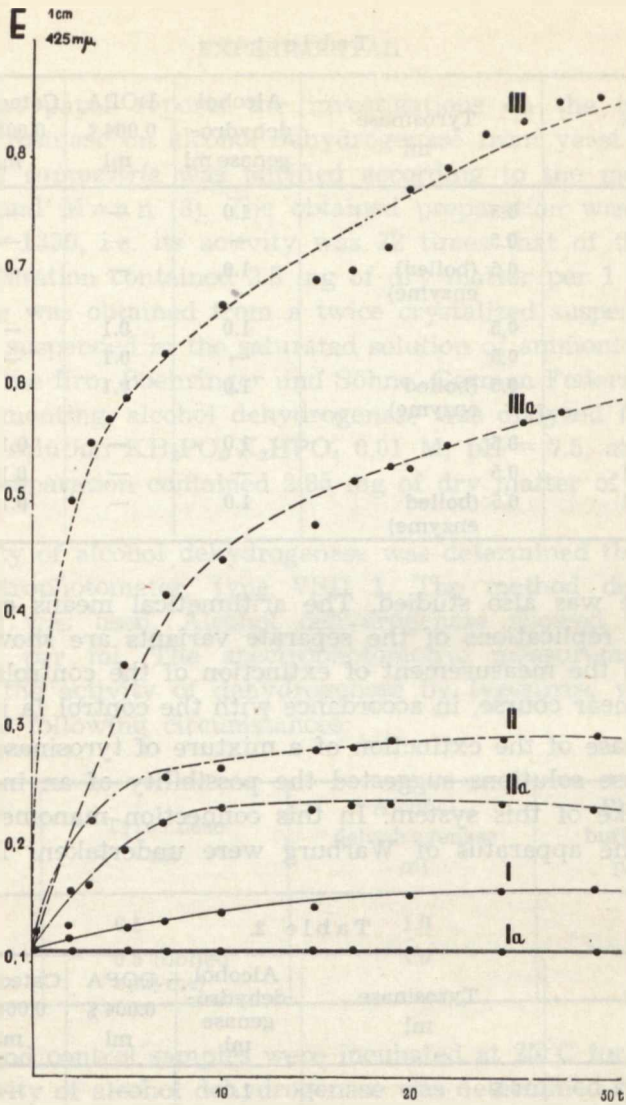

Diagram 1

Fig. 1. Colorimetric measurements of the extinction of a mixture of tyrosinase and alcohol dehydrogenase solutions in time min.;

I — Test (0.5 ml tyrosinase solution, 1 ml alcohol dehydrogenase solution, 1.5 ml phosphate buffer 0.01 M, pH = 7.5), Ia — Control (0.5 ml tyrosinase solution, 2.5 ml phosphate buffer 0.01 M, pH = 7.5), II — Test (0.5 ml tyrosinase solution, 1.0 ml alcohol dehydrogenase solution, 0.1 ml 0.004% DOPA solution, 1.4 ml phosphate buffer 0.01 M, pH = 7.5), IIa — Control (0.5 ml tyrosinase solution, 0.1 ml 0.004% DOPA solution, 2.4 ml phosphate buffer 0.01 M, pH = 7.5), III — Test (0.5 ml tyrosinase solution, 1.0 ml alcohol dehydrogenase solution, 0.1 ml 0.004% catechol solution, 1.4 ml phosphate buffer 0.01 M, pH = 7.5), IIIa — Control (0.5 ml tyrosinase solution, 0.1 ml 0.004% catechol solution, 2.4 ml phosphate buffer 0.01 M, pH = 7.5).

ments were carried out at 25°C; the manometers were shaken about 80 times/min. 0.2 of 10% KOH was introduced into the centre well.

The variants were analogous to those in colorimetric test and were studied in the following arrangement (Table 2).

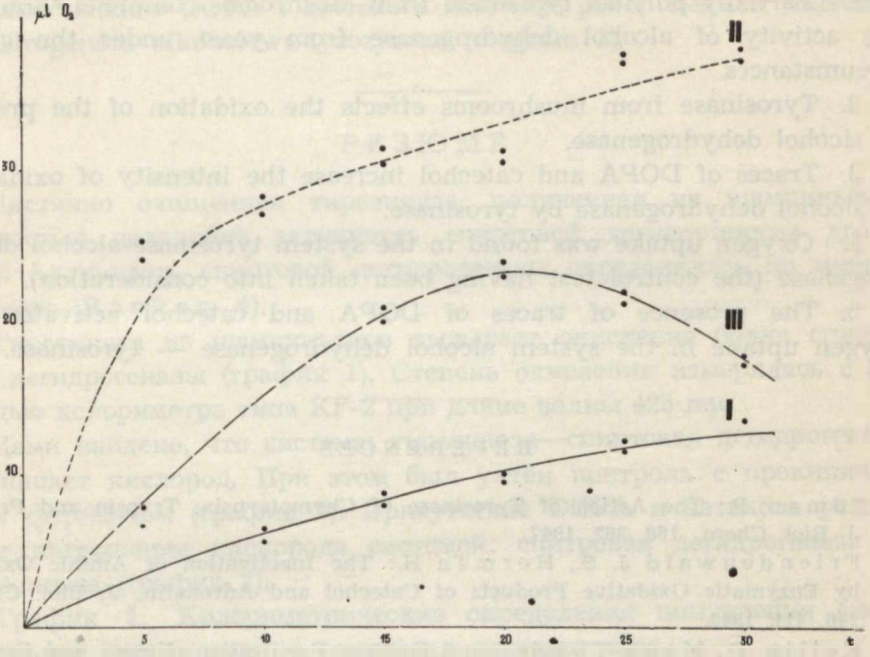

Diagram 2

Fig. 2. The results of the measurements of oxygen uptake shown in this figure were obtained from the differences in the amount of oxygen (in μ litres) between test and controls of the following mixtures of solutions;
I — Test (0.5 ml tyrosinase solution, 1.0 ml alcohol dehydrogenase, 1.3 ml phosphate buffer 0.01 M, pH = 7.5), Ia — Control (0.5 ml tyrosinase solution, 2.3 ml phosphate buffer 0.01 M, pH = 7.5), II — Test (0.5 ml tyrosinase solution, 1.0 ml alcohol dehydrogenase solution, 0.1 ml 0.004% DOPA solution, 1.2 ml phosphate buffer 0.01 M, pH = 7.5), IIa — Control (0.5 ml tyrosinase solution, 0.1 ml 0.004% DOPA solution, 2.2 ml phosphate buffer 0.01 M, pH = 7.5), III — Test (0.5 ml tyrosinase solution, 1.0 ml alcohol dehydrogenase 0.1 ml 0.004% catechol solution, 1.2 ml phosphate buffer 0.01 M, pH = 7.5), IIIa — Control (0.5 ml tyrosinase solution, 0.1 ml 0.004% catechol solution, 2.2 ml phosphate buffer 0.01 M, pH = 7.5).

The experiments were carried out in two replications. The results were interpolated and arranged in Fig. 2, which presents them as differences between the test and the controls without alcohol dehydrogenase. Control determination of the separate variants with the boiled tyrosinase solution showed no uptake of oxygen; the same applies to variant IV.

I wish to thank Professor Heller for his suggesting the topic, and Doc. Dr Trojanowski for some remarks concerning the method.

CONCLUSIONS

1. Partially purified tyrosinase from mushrooms completely inhibits the activity of alcohol dehydrogenase from yeast under the given circumstances.
2. Tyrosinase from mushrooms effects the oxidation of the protein of alcohol dehydrogenase.
3. Traces of DOPA and catechol increase the intensity of oxidation of alcohol dehydrogenase by tyrosinase.
4. Oxygen uptake was found in the system tyrosinase-alcohol dehydrogenase (the control test having been taken into consideration).
5. The presence of traces of DOPA and catechol activates the oxygen uptake in the system alcohol dehydrogenase — tyrosinase.

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STRESZCZENIE

Częściowo oczyszczona tyrozynaza z pieczarek hamuje w 100% aktywność dehydrogenazy alkoholowej z drożdży. Aktywność dehydrogenazy alkoholowej oznaczano wg metody Rackera (4).

Tyrozynaza z pieczarek powoduje utlenianie białka dehydrogenazy alkoholowej (diagram 1). Ślady DOPA i katecholu zwiększają intensyw-

ność utleniania dehydrogenazy alkoholowej przez tyrozynazę (diagram 1). Pomiar utleniania wykonywano na kolorymetrze typu KF-2 przy długości fali 425 mu.

Stwierdzono pobór tlenu przez układ tyrozynaza-dehydrogenaza alkoholowa. Uwzględniono próbę kontrolną z zagotowanym enzymem. Obecność śladów DOPA i katecholu aktywuje pobór tlenu w układzie: dehydrogenaza alkoholowa-tyrozynaza (diagram 2).

РЕЗЮМЕ

Частично очищенная тирозиназа, полученная из шампиньонов полностью подавляет активность спиртовой дегидрогеназы дрожжей. Активность спиртовой дегидрогеназы определялась по методу Раккера (R a c k e r, 4).

Тирозиназа из шампиньонов вызывает окисление белка спиртовой дегидрогеназы (график 1). Степень окисления измерялась с помощью колориметра типа KF-2 при длине волны 425 mμ.

Нами найдено, что система: тирозиааза—спиртовая дегидрогеназа поглощает кислород. При этом был учтён контроль с прокипячённым ферментом (график 2). Присутствие DOPA и катехола усиливает поглощение кислорода системой: спитровая дегидрогеназа — тирозиназа (график 2).

График 1. Колориметрические определения помутнения смеси растворов тирозиназа — спиртовая дегидрогеназа.

График 2. Результаты поглощения кислорода исследованной пробой и контролью следующих смесей растворов.

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