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Catalase Activity in Thermal Blue-green Algae in Relation to Temperature

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Souhrn

Autor sledoval aktivitu katalasy termálních sinic v závislosti na teplotě. Jako materiálu bylo použito homogenátu z termálních sinic r. *Oscillatoria* (teplotní optimum 38° C). Manometrická metoda podle APPLEMANA (1951) byla poněkud modifikována, ale princip (stanovení uvolněného kyslíku v prvních fázích reakce) zůstal zachován. Na rozdíl od běžných manometrických metod se během stanovení nezachovává konstantní objem (V_0) a registruje se čas potřebný ke změně tlaku o určitý interval. Výsledky se přepočítávají na V_0 graficky.

Nebyly zjištěny žádné zvláštní vlastnosti katalasy termálních sinic, pokud jde o závislost její aktivity na teplotě, což potvrzuje předpoklad M. B. ALLENOVÉ (1950), že neexistují zvláštní bílkoviny resistentní k vysokým teplotám. Reakční rychlost se v závislosti na teplotě zvyšuje až do 55° C. Při teplotě 60° C je enzym z termálních sinic prakticky kvantitativně denaturován, stejně jako katalasa z mesofilních mořských řas nebo z býčích jater.

Summary

The author has studied the relation of the catalase activity in thermal blue-green algae to temperature. Experimental data were taken from the first phase of the reaction, so that oxidation of the enzyme by the substrate was eliminated. The relation of the activity of a homogenate of thermal blue-green algae to temperature gives a curve similar to that obtained by SIZER (1944) for pure enzyme preparations isolated from beef livers. The resistance of the enzyme to high temperatures is the same in thermal blue-green algae as in mesopholic seaweeds. The catalase of thermal blue-green algae does not exhibit any special characteristics differing from the catalase of mesophiles.

Introduction

The thermophily of some organisms still remains incompletely explained, although we know by now for certain that their metabolism is essentially the same as that of mesophiles, that no natural division with regard to temperature exists between thermophiles and mesophiles and that thermophily is a characteristic which organisms of the same species can gain and lose (*Sporovibrio desulfuricans*).

On the basis of her experiments ALLEN (1950) has come to the conclusion that the nature of thermophily is not to be sought in the specific resistance of plasmatic proteins to high temperatures. In her opinion the more intense metabolism at high temperatures is responsible for the higher resistance of thermophilic organisms, the synthesis of their enzymes and other plasmatic components taking place more rapidly than their denaturation. This assumption has been based on the direct relationship between consumption of nutrients from the environment and rising temperature. With insufficient nutrients thermophilic species or strains *(Bacteria)* perish at high temperatures as quickly as mesophilic species.

Blue-green algae are characterised by the wide temperature limits within which they can exist and the thermophily of many species can be regarded rather as facultative than strict. In connection with the study of the temperature dependence of photosynthesis and respiration in blue-green algae the author also observed the dependence of catalase activity on temperature.

Materials and methods

Thermal blue-green algae of the genus *Oscillatoria* (with optimum temperature for growth, photosynthesis and respiration at temperatures close to 35° C). Both intact algae filaments and homogenate were used. A pure 14-days-old culture of the species chosen cultivated in a small Roux flask (20 ml. medium) was homogenised for 3 minutes in an all-glass homogeniser after POTTER and ELVEHJEM (1936) with 10 ml. phosphate buffer pH = 7 (Na_2HPO_4 M/15 + KH_2PO_4 M/15). In each case 2·7 ml. of this suspension, corresponding to 0·25 mg. of total nitrogen, was pipetted into a manometric flask. By using cultures from eight cultivation dishes enough material was obtained for all tests. By preliminary experiments it was established that the catalase activity of the homogenate was not changed even after 24 hours if kept at 0° C in the dark. This period sufficed for the determination of enzyme activity at all temperatures chosen with a sufficient number of repetitions.

In determining the tempterature coefficient of enzymatic splitting the concentration of the substrate must be chosen with a view to the concentration of the enzyme so that the time dependence of the rate of reaction should be suited to the kinetic equation for "zero-order" reactions which are characteristic for heterogenous catalysis (FRUTON and SIMMONDS 1954). For this reason in the preliminary experiments hydrogen peroxide was added to the suspension of homogenised algae to give resultant concentrations of 0·033 M, 0·1 M and 0·3 M. Preliminary determinations were carried out at a temperature of 20° C. The amount of oxygen evolved in relation to time agreed in the time interval used with the kinetic equation of "zero-order" reactions

$$-dC/dt = kE$$

(where E is the amount of enzyme present) only with a resultant concentration of the substrate of 0·1 M. In this and all other experiments the material was incubated at the given temperature for 10 minutes. The concentration 0·3 M already caused a marked denaturation of the enzyme, while at the concentration of 0·033 M H_2O_2 the reaction took place clearly in accordance with the kinetic equation for a monomolecular reaction

$$-dC/dt = kC$$

In view of the strongly oxidising properties of the substrate it is not possible in the last case to decide to what extent the decrease in reaction rate in the course of time in the last phase is related to the concentration of the substrate or to the denaturation of the enzyme. Therefore, in further experiments, a resultant concentration of 0·1 M and a somewhat modified method after APPLEMAN (1951) were used which made it possible to determine rate constant during the first thirty seconds following the start of the reaction. Manometric determination forms the basis of this method, but the time corresponding to the change in pressure in a given interval is recorded.

With the experiments described this interval was 10 mm. of manometric liquid. The rate of reaction would make it difficult to maintain the volume of gas V_g at a constant level. In order to avoid this difficulty the values obtained were calculated according to the mathematically derived and experimentally confirmed relation

$$h = 2h_1 + \frac{P \cdot V_m}{V_g},$$

where $2h_1$ is the change in pressure in mm. of manometric liquid when the volume of gas V_g is not maintained at a constant level (h_1 is the change in level in one arm of the manometer) and V_m is the volume of gas formed in the capillary above the liquid in the closed arm of the manometer. The remaining symbols are common in manometry.

For the purpose followed Warburg's respirometer of usual type with a vessel lacking the central cup and with one side arm was used.

The above relation is only approximate, as follows from its derivation:

$$pV = (P + h) \cdot V_g \quad (1)$$

$$pV = (P + 2h_1) \cdot (V_g + V_m) \quad (2)$$

By comparison of the right sides of these equations the following expression is obtained

$$h = 2h_1 \frac{V_g + V_m}{V_g} + \frac{P \cdot V_m}{V_g}.$$

Since

$$V_g + V_m \cong V_g,$$

then

$$\frac{V_g + V_m}{V_g} \cong 1$$

and therefore this expression can be ignored in the formula. Within the pressure range of the Warburg manometer it is possible in most cases to ignore the error (less than 3%) so produced. For example, if $V_g = 16{,}850$ l., $\mu h_1 = 300$ mm. and $V_m = 800\ \mu$l. (the volume of gas corresponding to 1 mm. of manometric liquid must be known; in our case it is 2·67 μl.) Calculating h according to the original formula the resultant value is 1105 mm., when using the simplified formula h is 1075 mm.

Within the pressure range of the Warburg manometer the relation between h and h_1 is also practically linear, which can be usefully employed in the graphic evaluation of the results.

Time intervals corresponding to a change in pressure of 10 mm. of manometric liquid were registered photographically. A spring machine was used in which a constant speed was ensured

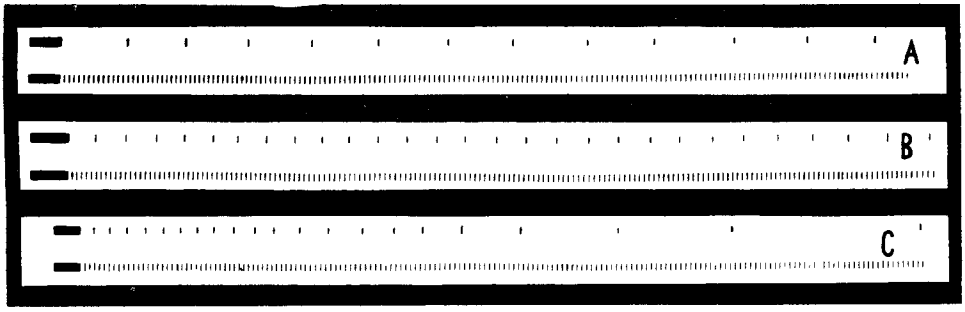

Fig. 1. Photograph of original time records from which the rate of decomposition of hydrogen peroxide by the catalase was worked out.
A) 0° C, B) 30° C, C) 50° C.

by a centrifugal regulator. To the machine there was attached a drum with grooves for the insertion of polarographic paper and with a signalling apparatus to announce the beginning and the end of the record paper. The speed of rotation was one turn in two and a half minutes (r = = 8 cm.). Opposite the drum there was a slit with two signal electric bulbs one above the other. The contacts of metronome were connected to the circuit of one bulb recording the interval of one second. The circuit of the other bulb was connected by the pressing of a switch at the moment

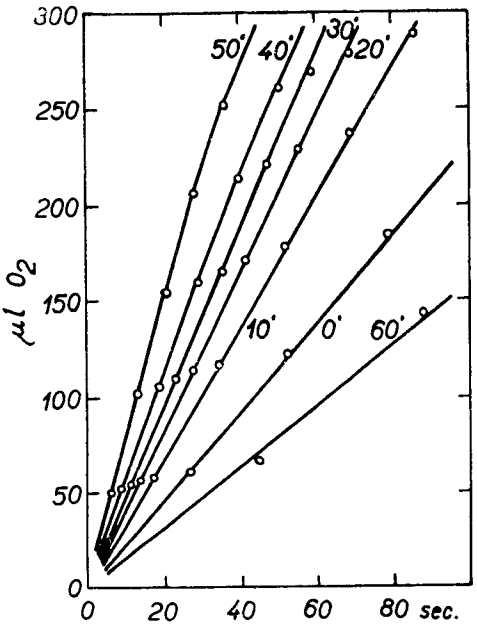

Fig. 2. Rate of liberation of oxygen from 0·1 M solution of hydrogen peroxide at various temperatures. The reaction was catalysed by a homogenate of the alga *Oscillatoria* at pH = 7.

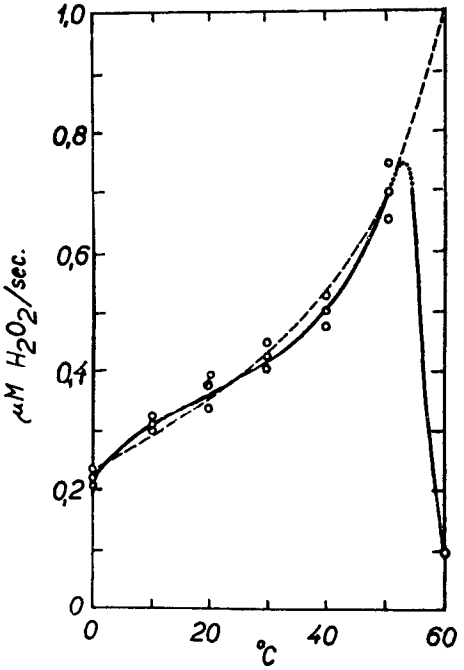

Fig. 3. Relation of the catalytic activity of the *Oscillatoria* homogenate to temperature. The measure of activity of the homogenate is the original rate of decomposition of hydrogen peroxide expressed in micromols of H_2O_2 distributed in seconds.

when a change in pressure of 10 mm. of manometric liquid took place. In this way, after developing the records, two rows of signs were obtained, of which one was a measure of the time in seconds and the other indicated—according to this scale—the time necessary for a change in pressure of the degree chosen to occur (fig. 1). It is possible to read the pressure with sufficient accuracy even during shaking (100 oscillations to the minute). It is best to read it at the moment when the moving meniscus first reaches each fifth graduation of the scale.

Results

The course of the decomposition of hydrogen peroxide in relation to time is given in fig. 2. The relation of the rate constant determined (M H_2O_2/sec.) to temperature is illustrated in fig. 3. Deviations from the theoretically pre-

sumed exponential curve cannot be fully explained since the work was not carried out with the pure enzyme but with a suspension of homogenised material. Therefore, not even the logarithmic expression of the constant in relation to 1/T gives a straight line (fig. 4).

Activation energies for individual temperature intervals were calculated from Arrhenius's equation $= 2{\cdot}303 \cdot R \frac{\log k_2 - \log k_1}{1/T_1 - 1/T_2}$ (where R is the gas constant expressed as 1.987 cal./mol.). They are set out in the following table:

0 to 50° C	4100 cal.
0 to 10° C	5500 cal.
10 to 40° C	2900 cal.
40 to 50° C	6400 cal.
50 to 60° C	59,000 cal.

Experiments with whole, non-homogenised material gave similar results. For obvious reasons their dispersion was, however, rather greater.

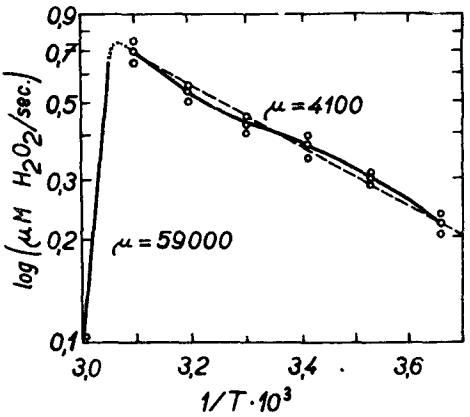

Fig. 4. Relation of the logarithm of the rate constant of decomposition of hydrogen peroxide (catalysed by homogenate of the alga *Oscillatoria*) to the inversion value of absolute temperature at which the reaction took place.

The results obtained do not show any essential differences from these of SIZER (1944), using catalase isolated from beef liver, or from those of the Japanese author TAKAGI (1953) who worked with five species of seaweeds: *Ulva pertusa*, *Enteromorpha linza* var. *crispata*, *Scytosiphon lomentaria*, *Polysiphonia venticulosa* and *Porphyra pseudolinearis*. As far as the resistance of the catalase to high temperatures is concerned, the results are also numerically in complete agreement both for marine mesophiles and for thermal blue-green algae. BONNICHSEN and co-workers (1947) obtained, on the basis of their experiments with very pure preparation, a considerably lower value of activation energy (1700 cal.) and they stated that higher values were obtained only when working with imperfectly pure preparations. For denaturation SIZER has quoted a lower activation energy (51,000 cal.), but this can be explained by differences in method (different incubation period, etc.). It seems, then, that the catalase of thermal blue-green algae does not exhibit any special characteristics as regards the relation of its activity to temperature and resistance to high temperatures.

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Активность каталазы термофильных синезеленых водорослей в зависимости от температуры

ШТЕПАН КУБИН

Резюме

Автор исследовал активность каталазы термофильных синезеленых водорослей в зависимости от температуры. В качестве материала применялся гомогенат из термофильных синезеленых водорослей *Oscillatoria* (оптимум температуры 38° С). Манометрический метод Appleman-a (1951) был несколько модифицирован, но его принцип (определение освобождающегося кислорода в первых фазах реакции) оставался неизменным. В отличие от обычных манометрических методов, в течение определений не сохраняется постоянный объем (V_o) и регистрируется время, необходимое для изменения давления на определенную величину. Результаты перечисляются на V_o графически.

Зависимости активности каталазы термофильных синезеленых водорослей от температуры не были отмечены. Это подтверждает предположение M. B. Allen-a (1950), что не существуют особые белки, устойчивые к высоким температурам. Скорость протекания реакции повышается пропорционально повышению температуры вплоть до 55° С. При 60° С энзим из термофильных синезеленных водорослей практически полностью денатурируется, как и каталаза из мезофильных морских водорослей или из бычьей печени.