INFLUENCE OF POTASSIUM AND AMMONIUM IONS ON YEAST FERMENTATION

by

TH. J. M. MAESEN

Laboratory for Physiological Chemistry, The University, Utrecht (Netherlands)

The anaerobic fermentation of fresh bakers' yeast* in glucose-sodium acetate medium of pH 3.5 is strongly inhibited by the undissociated acetic acid which predominates in this medium[1,2].

According to CONWAY AND DOWNEY the average intracellular pH of yeast is 6.35 when glucose is fermented[3]. Hence, when acetic acid penetrates into the cells it will dissociate almost completely, and thus may cause a shift of the internal pH to more acid values. Now the fermentation of dried yeast is very sensitive to the hydrogen ion concentration; the pH-optimum is 6.3, and the rate of fermentation at pH 3.0 is negligible (see e.g. NILSSON[4]). Thus it does not seem unreasonable to presume that the inhibiting action of acetic acid on yeast fermentation is due to a shift of the pH at some locus within the cell.

If the inhibition by acetic acid is not too strong the rate of fermentation may be restored by adding a small amount of potassium ions to the suspension. Sodium ions exert a corresponding though weaker action, and ammonium ions are still less effective in this respect (Tables I and II). This interaction between acetic acid and these cations may easily be understood if it is considered to be connected with the phenomenon of "cation exchange" as described by CONWAY *et al.*[5] and ROTHSTEIN *et al.*[6].

TABLE I

INFLUENCE OF POTASSIUM AND AMMONIUM IONS ON FERMENTATION IN ACETATE MEDIA AT pH 3.5

3.4 mg fresh yeast per Warburg vessel (= 2 mg/ml); 2.5% glucose; various concentrations of sodium acetate buffer, pH 3.5; KCl and NH_4Cl added after 2 h of fermentation; temp. 27° C; gas phase: N_2. Buffers prepared by mixing 1 volume of 0.1 *M* sodium acetate and 16 volumes of 0.1 *M* acetic acid, and diluting this mixture until the desired concentration was obtained. Data: average rates in mm^3 CO_2/20 min/3.4 mg fresh yeast after development of the effects. Controls: means of 8 expts with s.d. Example of an experiment in Fig. 1.

Conc. of sodium acetate buffer		*0.017 M*	*0.034 M*	*0.068 M*
Controls		54 ± 1.7	27 ± 2.5	10 ± 2.3
KCl	0.0017 *M*	81	72	12
	0.017 *M*	76	84	18
	0.17 *M*	85	89	25
NH_4Cl	0.0017 *M*	57	28	5
	0.017 *M*	53	41	13
	0.17 *M*	68	52	17

TABLE II

INFLUENCE OF POTASSIUM AND SODIUM SALTS ON FERMENTATION IN ACETATE MEDIA AT pH 3.5

0.034 *M* sodium acetate buffer, pH 3.5; additions of salts at time zero. Conditions of fermentation as in Table I. Fermentation followed for about 5 h. Data: average rates in mm^3 CO_2/20 min/3.4 mg fresh yeast.

Exp. No.	*0.017 M KCl* *0.017 M NaCl*	*0.017 M* Na_2SO_4	*0.017 M* NaH_2PO_4 *0.017 M NaCl*	*0.034 M NaCl*	*No salt added*
1	96	65	72	70	—
2	91	63	70	62	30

* "Koningsgist" from the "Nederlandse Gist- en Spiritusfabriek", Delft (Netherlands).

In a previous paper[7] investigations were reported on the stimulating influence of ammonium ions on fermentation at pH 5.6. This effect, which was readily observed with ammonium concentrations as low as 0.0003 M (0.002% ammonium sulphate), gradually develops in the course of several hours. From Fig. 1 it may be seen that at pH 3.5 the addition of ammonium ions is followed by an immediate and steep rise of the rate of fermentation. Moreover, this effect of ammonium ions at pH 3.5 is only observed with higher concentrations (Table I). This leads us to the conclusion that the action of ammonium ions at pH 3.5 is quite different from that at pH 5.6. The latter has been attributed to the function of these ions as a source of nitrogen for protein synthesis[7].

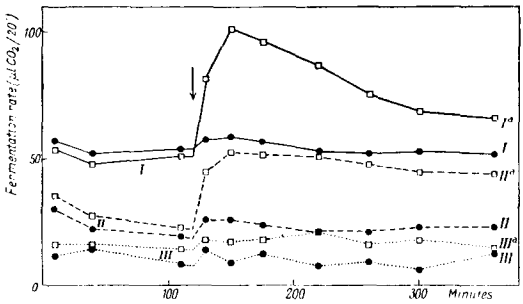

Fig. 1. Influence of ammonium ions on established fermentation in acetate media at pH 3.5. Conditions of fermentation as in Table I. Concentration of sodium acetate buffer in curve I: 0.017 M; curve II: 0.034 M; curve III: 0.068 M. Arrow: 0.17 M NH_4Cl added (curves Ia, IIa and IIIa).

The author is indebted to Professor H. G. K. WESTENBRINK for his kind interest and stimulating criticism during this work, and to the Netherlands Organisation for Pure Scientific Research (ZWO) for grants which have supported it financially.

REFERENCES

1 F. E. SAMSON, A. M. KATZ, D. L. HARRIS AND J. Z. HEARON, *Fed. Proc.*, 11 (1952) 136.
2 TH. J. M. MAESEN AND E. LAKO, *Biochim. Biophys. Acta*, 9 (1952) 106.
3 E. J. CONWAY AND MARY DOWNEY, *Biochem. J.*, 47 (1950) 347, 355.
4 R. NILSSON in E. BAMANN AND K. MYRBÄCK, *Methoden der Fermentforschung*, Leipzig (1941) p. 2150.
5 See e.g. E. J. CONWAY AND E. O'MALLEY, *Biochem. J.*, 40 (1946) 59; E. J. CONWAY AND T. G. BRADY, *ibid.*, 47 (1950) 360.
6 A. ROTHSTEIN AND L. ENNS, *J. Cell. Comp. Physiol.*, 28 (1946) 231.
7 TH. J. M. MAESEN, *Biochim. Biophys. Acta*, 12 (1953) 445.

Received September 8th, 1954

DPNH OXIDASE

by

D. E. GREEN*, BRUCE MACKLER**, R. REPASKE*** AND H. R. MAHLER§

Institute for Enzyme Research, University of Wisconsin, Madison, Wis. (U.S.A.)

In 1937 GREEN, DEWAN AND LELOIR[1] described in heart tissue a particulate enzyme system which catalyzes the oxidation of DPNH by molecular oxygen. This particulate system which has been extensively studied by SLATER[2] and by CHANCE[3] has now been prepared from beef heart mitochondria in highly purified state by application of the methods for separating particulate enzymes referred to previously[4]. DPNH oxidase while capable of catalyzing reaction (1) in absence of any additions shows only slight activity as a catalyst for reactions (2) and (3):

$$\text{DPNH} + \tfrac{1}{2}\,O_2 + H^+ \longrightarrow \text{DPN}^+ + H_2O \qquad (1)$$

$$\text{DPNH} + 2 \text{ cytochrome } c + H^+ \longrightarrow \text{DPN}^+ + 2 \text{ reduced cytochrome } c \qquad (2)$$

$$2 \text{ reduced cytochrome } c + 2\,H^+ + \tfrac{1}{2}\,O_2 \longrightarrow 2 \text{ cytochrome } c + H_2O \qquad (3)$$

DPNH oxidase contains no detectable amount of cytochrome c. Reaction (1) is completely inhibited by low levels of antimycin A[5].

* This investigation was supported by a grant from the National Heart Institute of the National Institutes of Health.

** Postdoctoral trainee of the National Heart Institute.

*** Present adress: Indiana University.

§ Supported by a grant-in-aid of the American Cancer Society, on recommendation by the committee on Growth.