Thiamine, Pyrimidine and Thiazole as Bios Factors

BY ALFRED S. SCHULTZ, LAWRENCE ATKIN AND CHARLES N. FREY

In view of our discovery of the fermentation stimulating effect of thiamine,[1,2] studies were instituted relative to thiamine as a bios factor. Thiamine is a growth-active substance for various yeasts. Amongst strains of *Sacch. cerevisiae* the response to thiamine as a bios factor differs.

TABLE I

Total volume in each case 30 ml. seeded with 1 mg. of moist yeast and rocked at 30° for 24 hours. Crop × 4.54 gives mg. of moist yeast. Supplements: inositol [I] 1 mg., β-alamine [IIA] 0.005 mg., bios II B 0.13 mg., thiamine 0.01 mg., thiazole 0.01 mg., aminopyrimidine 0.01 mg., bios VI 1 cc. of a concentrate.

Ingredients of bios test	Crop Type A	 Type B
Sugar, salts, buffer, I, IIA, IIB	30	220
Sugar plus thiamine	120	120
Sugar plus thiamine and bios VI	220	220
Sugar plus thiazole	70	220
Sugar plus aminopyrimidine	80	200
Sugar plus aminopyrimidine + thiazole	120	150

Thus type A is stimulated by thiamine and type B is inhibited by it. This is true when the growth medium contains bios I (inositol), bios IIA (beta-alanine) and bios IIB. As may be seen it is possible to add another factor (bios VI, we have named it), which has not been freed of the other bioses but which may be found in many places. This factor further stimulates type A while removing the inhibition on type B. Examples of type A cultures are Luft II, and Rasse XII. Type B cultures are represented by *Sacch. cerevisiae* Toronto and Spc. 152.

Included in the table are results obtained with components of thiamine. "Thiazole" in the table is 4-methyl-5-beta-hydroxyethylthiazole and "aminopyrimidine" is 2-methyl-5-ethoxymethyl-6-aminopyrimidine. Thus type A yeast is partly stimulated by either fraction and completely activated by a combination. Type B yeast is not affected by the thiazole and is only slightly inhibited by the aminopyrimidine. The combination, however, inhibits type B to the same extent as thiamine.

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(1) Schultz, Atkin and Frey, THIS JOURNAL, **59**, 948 (1937).

(2) *Idem.*, p. 2457.

On the Calculation of the Dissociation Constants of Hypohalogenous Acids from Kinetic Data

BY EUGENE A. SHILOV

The author recently has determined the dissociation constant of hypobromous acid by electrochemical titration with glass electrode and found it to be 2.06×10^{-9} at 20°.

This value is in disagreement with that obtained by Chapin[1] by means of the kinetical method, namely, 2.5×10^{-8}, later adopted by Prutton and Maron[2] in the calculations of their measurements of the kinetics of hypobromite decomposition.

The kinetical method used first by Gallart[3] is based on the determination of the pH at which the velocity of a hypohalite decomposition is maximal. Under assumption that the kinetical equation of the reaction has the form

$$-\frac{d[\mathrm{OX}^-]}{dt} = k[\mathrm{HOX}]^2[\mathrm{OX}^-] \qquad (1)$$

$(p\mathrm{H})_{\mathrm{max.}}$ must correspond to one-third neutralization of the hypohalogenous acid present. From this relation in combination with the equation $K = [\mathrm{H}^+][\mathrm{OX}^-]/[\mathrm{HOX}]$ it follows

$$\log K = -(p\mathrm{H})_{\mathrm{max.}} - \log 2$$

The method involves two sources of errors: (1) experimental errors of the determination of $(p\mathrm{H})_{\mathrm{max.}}$; and (2) the inexactitude of the equation (1).

On examining the decomposition curves of hypochlorite in the article of Chapin[1] the probable error of determination of $(p\mathrm{H})_{\mathrm{max.}}$ on the curve appears to be 0.2–0.3 pH. The total experimental error of the determination is apparently much larger, as the values of different authors vary very much between themselves. Gallart[3] and Chapin[1] have obtained in the decomposition of hypochlorite $(p\mathrm{H})_{\mathrm{max.}} = 6.7$ and hence $K_{\mathrm{HOCl}} = 10^{-7}$, but Markuse[4] determined $(p\mathrm{H})_{\mathrm{max.}}$ as >7, and hence $K_{\mathrm{HOCl}} < 5 \times 10^{-8}$.[5]

It seems that the control of pH in the investigations of Chapin and particularly of Markuse did

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(1) Chapin, THIS JOURNAL, **56**, 2211 (1934).

(2) Prutton and Maron, *ibid.*, **57**, 1653 (1935).

(3) Gallart, *Anales Soc. españ. fís. quím.*, **31**, 422 (1933), cited from Chapin.[1]

(4) Markuse, *Reconstruction Text. Ind.* (Russ.), **5**, 43 (1935).

(5) Here may be noted also the earlier investigation of Giordani [*Gazz. chim. ital.*, **54**, 844 (1924)], who calculated the constant of hydrolysis of hypochlorite from the measurements of the kinetics of sodium hypochlorite decomposition in very alkaline solutions ($p\mathrm{H} > 13$). The value obtained by Giordani ($K_{\mathrm{Hydr.}} = 1.12 \times 10^{-6}$ at 30°, $K_{\mathrm{HOCl}} = 1.5 \times 10^{-8}$) is near to modern values, but perhaps only accidentally, as the method of Giordani is not exact enough.

not stay on a high level. Chapin has determined *p*H colorimetrically after the decomposition of hypochlorite by sulfite or hyposulfite; Markuse used the buffer mixtures of known composition without any further controlling.[6]

Values of K_{HOCl} of usual "static" methods of determination of dissociation constants are between 1×10^{-8} and 4×10^{-8}.[7] Particularly the method of the glass electrode gives very consistent values 3×10^{-8} to 4×10^{-8} at room temperature.

Even if equation (1) is quite correct for hypochlorites, the "static" value can today hardly be checked by the kinetical method, since the limits of the experimental errors of the latter are too large.[7a]

The results of the kinetic and static methods applied to hypobromous acid differ among themselves, as remarked above, very much (2.5×10^{-8} and 2.06×10^{-9}). The discrepancy is hardly to be explained as due to the experimental errors of the two methods.[8]

The more plausible explanation of the divergence is the invalidity of the kinetic equation (1) in the application to hypobromous acid. Indeed equation (1) is only one of many possible equations of transformation of active bromine. As Skrabal indicated,[9] a change of the law of decomposition of hypobromous acid between Φ^2 and Φ^3 takes place Φ (being summary concentration of HOBr, Br_2 and Br_3^-), if *p*H and bromide concentration varies.[10] At these conditions the calculation of the dissociation constant of hypobromous acid from $(p\mathrm{H})_{\mathrm{max.}}$ is very dubious.

On the contrary the determination of the dissociation constant of hypobromous acid enables one to calculate the composition of the reacting mixture at $(p\mathrm{H})_{\mathrm{max.}}$. For the value found by Chapin, *i. e.*, $(p\mathrm{H})_{\mathrm{max.}} = 7.3$, we calculate from the equation

$$[\mathrm{H^+}][\mathrm{OBr^-}]/[\mathrm{HOBr}] = 2.06 \times 10^{-9}$$

that

$$[\mathrm{OBr^-}] = 0.04\ [\mathrm{HOBr}]$$

Thus only 4% neutralization of hypobromous acid present suffices to make the velocity of decomposition maximal. It may be regarded as a demonstration that equation (1) is not valid in the region of *p*H near to 7.

Experimental

WITH J. N. GLADTCHIKOVA

The dissociation constant of hypobromous acid was determined by titration of free hypobromous acid with sodium hydroxide solution in presence of a glass electrode. The solutions of hypobromous acid were prepared by vacuum distillation of bromine water with silver phosphate. Contrary to the opinion of Pollak and Doktor,[11] shared by Liebhafsky and Makower,[12] who declared the preparation of pure hypobromous acid solution to be an insoluble problem, dilute solutions of hypobromous acid, containing practically no bromine and bromic acid, can be obtained easily by this method.

Sodium hydroxide solutions were prepared from metallic sodium and carbon dioxide-free water.

The apparatus, based on the use of an electrometer valve, was constructed by the scheme of Sokolov and Ptchelin.[13] The glass electrodes were calibrated by means of buffer solutions of which the *p*H had been determined with a hydrogen electrode.

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(6) The original article of Gallart is not available to me.

(7) From the solubility of carbon dioxide in sodium hypochlorite solutions: 3.7×10^{-8} at 25° [Sand, *Z. physik. Chem.*, **48**, 614 (1904)] from the partition of hypochlorous acid between the aqeous solution and a vapor phase: 1×10^{-8} at 25° [Soper, *J. Chem. Soc.*, **125**, 2227 (1924)]; from the electrical conductivity of hypochlorous acid solution: $\sim 10^{-8}$ at room temperature (Nernst, cited from Sand. *l. c.*); 1.9×10^{-8} at 0° [Shilov and Kaniaev, *J. Phys. Chem.* (U. S. S. R.), **5**, 654 (1934)]; by titration with glass electrode: 4×10^{-8} [Yorston, *Pulp Paper Mag. Can.*, **31**, 374 (1931)]; 3.7×10^{-8} at 18–20° [Davidson, *J. Text. Inst.*, **24**, T185 (1933)]; 3.2×10^{-8} at 15° [Britton and Dodd, *Trans. Faraday Soc.*, **29**, 537 (1933)]; 3.7×10^{-8} at 18°; the "true" constant is calculated to be 2.95×10^{-8} [Ingham and Morrison, *J. Chem. Soc.*, 1200 (1933)]; last value excepted, all others are the apparent dissociation constants. Two values fall out from this series: (1) that of Noyes and Wilson, 6.7×10^{-10} [THIS JOURNAL, **44**, 1630 (1922)], the fallibility of which is demonstrated by Soper (*l. c.*), and (2) that of Ríus and Arnal, 1.46×10^{-7} at 25° [*Anales soc. españ. fís. quím.*, **31**, 497 (1933); *C. A.*, **27**, 4989 (1933)] obtained by means of an unproved method (electrochemical titration with chlorine electrode).

(7a) During the printing of this article the writer obtained the knowledge of a paper of A. Skrabal and A. Berger [*Monatsh.* **70**, 168 (1937)], treating the determination of the dissociation constant of hypochlorous acid from kinetic data. The results of this thoughtful and thorough investigation are in accordance with the views evolved in this note. The mean kinetic value of K_{HOCl}, obtained by Skrabal and Berger, is 5.6×10^{-8} at 25°.

(8) Our value of the dissociation constant of hypobromous acid is at present unique, but it may be confirmed to a certain degree by our determinations of the specific conductance of hypobromous acid. The observed specific conductance of 0.01 molal solution of hypobromous acid was $1.7–2.0 \times 10^{-6}$ rec. ohms at 0° (without any correction, *i. e.*, maximal). By calculating the specific conductance by means of the dilution law (under assumption that Λ_∞ is 250) one finds for our value of K_{HOBr} $\lambda = 1.1 \times 10^{-6}$, for Chapin's value $\lambda = 4.10^{-6}$.

(9) A. Skrabal, *Z. Elektrochem.*, **40**, 237 (1934).

(10) Certainly Prutton and Maron [THIS JOURNAL, **57**, 1653 (1933)] have found that ordinary reaction of decomposition of hypobromite at *p*H 6.4–7.8, *i. e.*, in the region of the maximal velocity, obeys equation (1). However, this conclusion rests on the dissociation constant of hypobromous acid calculated by Chapin by kinetical method based on equation (1). We have, therefore, a *circulus vitiosus* here.

(11) Pollak and Doktor, *Z. anorg. allgem. Chem.*, **196**, 89 (1931).

(12) Liebhafsky and Makower, *J. Phys. Chem.*, **37**, 1037 (1933); Makower and Liebhafsky, *Trans. Faraday Soc.*, **29**, 597 (1933).

(13) Sokolov and Ptchelin, *J. Applied Chem.* (U. S. S. R.), **7**, 1316 (1934).