the figure. On close examination, the curves for sample 1 reveal a 1-to-2 mg. uniform weight increase up to approximately 450°. Sample 2 shows a loss of the same magnitude. Above 450° the samples gain weight at a lowly increasing rate, and at about 515° a rapid weight gain begins. The onset of rapid oxidation occurs at a higher temperature for the coarser sample.

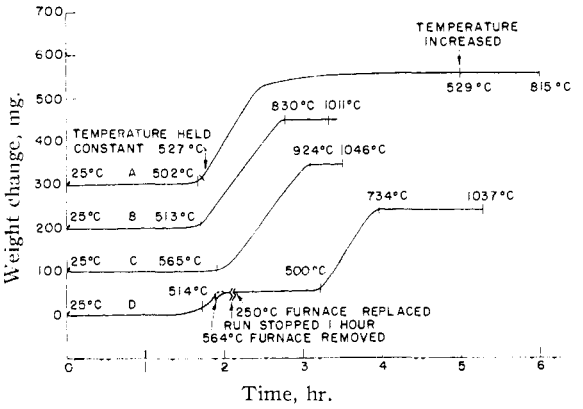

Fig. 1.—A, B and D, sample 1, carbon = 6.15, 6.26, 6.24%; C, sample 2, carbon = 6.20%.

In run A, the weight gain during uniform rapid burning at constant temperature was 4.8 mg. per minute. In run D the weight change after partial combustion indicated that 22% of the sample had burned. The per cent. carbon for each run was computed from the final weight change assuming the residue to be WO_3.

I wish to acknowledge helpful discussions with Dr. E. I. Simons, and assistance from Miss I. Aliferis in measuring some of the curves.

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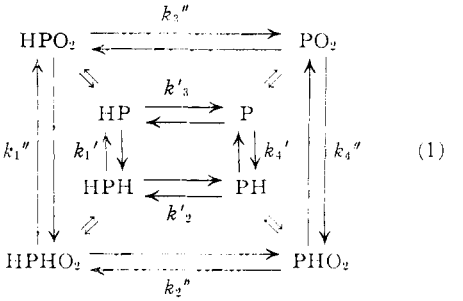
The Ionization Constants of the Heme-Linked Groups of Hemoglobin[1]

BY ROBERT A. ALBERTY

RECEIVED MARCH 17, 1955

Wyman[2] has shown how the equilibria of hemoglobin involving protons and molecular oxygen may be treated from a unified point of view by means of the theory for linked functions. It was shown that the differential titration data of Wyman and co-workers[3,4] and the *p*H variation of the oxygen tension as measured by Ferry and Green[5] could be interpreted quantitatively in terms of the ionization constants of two heme-linked groups. Upon oxygenation one of these groups is rendered stronger and the other weaker. It is the purpose of this note to show that one of the assumptions made by Wyman—namely, that the ionizations of these two groups are independent of each other—is unnecessary for a quantitative treatment.

Theory.—The part of hemoglobin (P) which affects the oxygen binding may be represented as a dibasic acid HPH, where one proton has been written to the left, and the other to the right, to distinguish between the two acidic groups. Since the first proton may dissociate from either position the resulting equilibria[6] may be represented by



(1)

where the k's are the microscopic acid dissociation constants which are not subject to direct measurement in the absence of means for distinguishing between isomers such as HP and PH. For example, $k_1' = (H^+)(HP)/(HPH)$, $k_3' = (H^+)(P)/(HP)$. These microscopic constants are not all independent since $k_1'k_3' = k_2'k_4'$ and $k_1''k_3'' = k_2''k_4''$.

In interpreting the experimental data for hemoglobin Wyman assumed that the two ionizable groups are independent. This amounts to setting $k_3' = k_2'$, $k_4' = k_1'$, $k_3'' = k_2''$ and $k_4'' = k_1''$. On the basis of this assumption the number, $\Delta\bar{X}$, of equivalents of acid produced per heme upon complete oxygenation is

$$\Delta\bar{X} = \frac{k_1''}{(H^+) + k_1''} + \frac{k_2''}{(H^+) + k_2''} - \frac{k_1'}{(H^+) + k_1'} - \frac{k_2'}{(H^+) + k_2'} \quad (2)$$

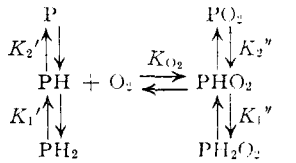
On the basis of the same assumption the oxygen pressure, $p_{1/2}$, required for half saturation is given by

$$p_{1/2} = (\text{const.})\frac{[(H^+) + k_1'][(H^+) + k_2']}{[(H^+) + k_1''][(H^+) + k_2'']} \quad (3)$$

The values of k_1', k_2', k_1'' and k_2'' obtained by Wyman from these two types of experimental data are listed in Table I under assumption (a).

The assumption of the independence of the ionizable groups upon which equations 2 and 3 are based is not required for a mathematical treatment and imposes a limitation which is undesirable since the two ionizable groups are presumably close to the heme and might be expected to have an effect on each other.

For a more general treatment the equilibria may be written as



(4)

(1) This research was supported by a grant from the National Science Foundation and a research grant-in-aid from the du Pont Company.

(2) J. Wyman, in M. L. Anson and J. T. Edsall, "Advances in Protein Chemistry," Vol. IV, Academic Press, Inc., New York, N. Y., 1948, p. 407.

(3) B. German and J. Wyman, *J. Biol. Chem.*, **117**, 533 (1937).

(4) J. Wyman and E. N. Ingalls, *ibid.*, **139**, 877 (1941).

(5) R. M. Ferry and A. A. Green, *ibid.*, **81**, 175 (1929).

(6) E. Q. Adams, THIS JOURNAL, **38**, 1503 (1916).

where

$$K_{O_2} = \frac{p_{O_2}(PH)}{(PHO_2)} \quad (5)$$

Here the symbol PH represents the sum of the isomers PH and HP and PHO_2 represents the sum of the isomers PHO_2 and HPO_2. The acid dissociation constants are of the usual type for a dibasic acid, that is, $K_1' = (H^+)[(PH) + (HP)]/(HPH)$, $K_2' = (H^+)(P)/[(PH) + (HP)]$, etc. It is readily shown[6] that these experimentally determinable ionization constants are related to the microscopic constants of mechanism 1 by

$$K_1' = k_1' + k_2' \qquad K_1'' = k_1'' + k_2''$$
$$K_2' = (1/k_3' + 1/k_4')^{-1} \qquad K_2'' = (1/k_3'' + 1/k_4'')^{-1} \quad (6)$$

The equation for the number of equivalents of acid produced per heme upon complete oxygenation is derived as follows from the equilibria expressed in 4

$$(P)_0\Delta\bar{X} = (PH)_i + 2(PH_2)_i - (PHO_2)_f - 2(PH_2O_2)_f = (PH)_i[1 + 2(H^+)/K_1'] - (PHO_2)_f[1 + 2(H^+)/K_1''] \quad (7)$$

where i stands for initial (before oxygenation), f stands for final (after complete oxygenation), and $(P)_0$ is the total molar concentration of heme groups in the hemoglobin solution. The concentrations $(PH)_i$, $(PHO_2)_f$ and $(P)_0$ are eliminated from equation 6 by use of

$$(P)_0 = (PH)_i + (PH_2)_f + (P)_i = (PH)_i[1 + (H^+)/K_1' + K_2'/(H^+)] \quad (8)$$

and

$$(P)_0 = (PHO_2)_f + (PH_2O_2)_f + (PO_2)_f = (PHO_2)_f[1 + (H^+)/K_1'' + K_2''/(H^+)]$$

to obtain

$$\Delta\bar{X} = \frac{1 + 2(H^+)/K_1'}{1 + (H^+)/K_1' + K_2'/(H^+)} - \frac{1 + 2(H^+)/K_1''}{1 + (H^+)/K_1'' + K_2''/(H^+)} \quad (9)$$

The functional dependence on hydrogen ion concentration is the same as in equation 2, as may be seen by clearing fractions

$$\Delta\bar{X} = \frac{(H^+)^3[K_1'' - K_1'] + 2(H^+)^2[K_1''K_2'' - K_1'K_2'] + (H^+)[K_1'K_1''(K_2'' - K_2')]}{[(H^+)^2 + K_1'(H^+) + K_1'K_2'][(H^+)^2 + K_1''(H^+) + K_1''K_2'']} \quad (10)$$

and comparing with the rearranged form of equation 2.

$$\Delta\bar{X} = \frac{(H^+)^3[k_1'' + k_2'' - (k_1' + k_2')] + 2(H^+)^2[k_1''k_2'' - k_1'k_2'] + (H^+)[k_1''k_2''(k_1' + k_2') - k_1'k_2'(k_1'' + k_2'')]}{[(H^+)^2 + (k_1' + k_2')(H^+) + k_1'k_2'][(H^+)^2 + (k_1'' + k_2'')(H^+) + k_1''k_2'']} \quad (11)$$

The experimental data may be represented equally well by equation 10 or 11, but the values and significance of the constants is different.

If it is assumed that the groups are independent, that is, $k_3' = k_2'$, $k_4' = k_1'$, $k_3'' = k_2''$ and $k_4'' = k_1''$, equations 6 may be rewritten

$$K_1' = k_1' + k_2' \qquad K_1'' = k_1'' + k_2''$$
$$K_2' = \frac{k_1'k_2'}{k_1' + k_2'} \qquad K_2'' = \frac{k_1''k_2''}{k_1'' + k_2''} \quad (12)$$

Substitution of equations 12 into equation 10 yields equation 11 as it should.

If the oxygen pressure, $p_{1/2}$, required to half saturate hemoglobin is considered to be an apparent equilibrium constant, the variation of this pressure with pH may be derived as follows from the equilibria in 4

$$p_{1/2} = \frac{p_{O_2}[(PH) + (PH_2) + (P)]}{[(PHO_2) + (PH_2O_2) + (PO_2)]} = K_{O_2}\frac{[1 + (H^+)/K_1' + K_2'/(H^+)]}{[1 + (H^+)/K_1'' + K_2''/(H^+)]} \quad (13)$$

where K_{O_2} is defined by equation 5. Rearranging leads to

$$p_{1/2} = \frac{K_{O_2}K_1''}{K_1'}\frac{[(H^+)^2 + K_1'(H^+) + K_1'K_2']}{[(H^+)^2 + K_1''(H^+) + K_1''K_2'']} \quad (14)$$

which may be seen to give the same dependence on hydrogen ion concentration as equation 3 which may be rewritten as

$$p_{1/2} = (\text{const.})\frac{[(H^+)^2 + (k_1' + k_2')(H^+) + k_1'k_2']}{[(H^+)^2 + (k_1' + k_2'')(H^+) + k_1''k_2'']} \quad (15)$$

Substitution of equations 12 into 14 yields equation 15, as it should.

Discussion.—It has been shown that the equations for the acid production upon oxygenation of hemoglobin and the pH variation of $p_{1/2}$ may be expressed in terms of the usual type of acid dissociation constants (capital K's) for a dibasic acid as well as in terms of the microscopic constants (lower case k's) for the two groups assuming they are independent. The values of K_1', K_2', K_1'' and K_2'' are conveniently calculated from Wyman's values of k_1', k_2', k_1'' and k_2'' by use of equations 12. The values given in Table I show that the pK values for hemoglobin are not detectably different from the pk values given by Wyman and that the values for oxygenated hemoglobin are only slightly changed. The changes would not affect the interpretation of the change in ionization constant given by Coryell and Pauling.[7]

TABLE I

pK VALUES FOR HEME-LINKED GROUPS IN HORSE HEMOGLOBIN AT 25° AND 0.16 IONIC STRENGTH

	Hemoglobin	Oxygenated hemoglobin
pK_1	5.25	5.70
pK_2	7.93	6.73
Assumption (a): pk_1	5.25	5.75
pk_2	7.93	6.68
Assumption (b): pk_1	5.55	6.00
pk_3	7.63	6.43

Once the values of K_1', K_2', K_1'' and K_2'' have been obtained it is possible to calculate the microscopic ionization constants on the basis of any of the following special assumptions: (a) the two groups are independent, *i.e.*, $k_1' = k_4'$, $k_2' = k_3'$, $k_1'' = k_4''$ and $k_2'' = k_3''$; (b) the two groups are identical but not independent, *i.e.*, $k_1' = k_2'$, $k_3' = k_4'$, $k_1'' = k_2''$ and $k_3'' = k_4''$; or (c) the intermediate ionized form is predominately one isomer (say HP), *i.e.*, $k_1'/k_2' \gg 1$ and $k_1''/k_2'' \gg 1$. The pk values calculated on assumption (a) are those of Wyman's which are given in Table I

(7) C. D. Coryell and L. Pauling, *J. Biol. Chem.*, **132**, 769 (1940).

under (a).[8] For assumption (b), $k_1' = K_1'/2$, $k_3' = 2K_2'$, $k_1'' = K_1''/2$, and $k_3'' = 2K_2''$, and the pk values are given in Table I. For assumption (c), $k_1' = K_1'$, $k_3' = K_2'$, $k_1'' = K_1''$ and $k_3'' = K_2''$ if HP is the predominant isomer. The pk values obtained by this assumption are the same as the pK values.

This analysis of the hemoglobin data is closely related to that which has been applied in the interpretation of the pH dependence of the forward and reverse reactions catalyzed by fumarase.[9] The values of pK_1 and pK_2 for fumarase in 0.01 M acetate buffer at 25° are 6.2 and 6.8. When fumarate forms a complex with the enzyme pK_1 is reduced to 6.3 and pK_2 is raised to 7.3. When l-malate forms a complex with the enzyme pK_1 is raised to 6.6 and pK_2 is raised to 8.4.

It is not to be expected that the values of pK_1', pK_2', pK_1'' and pK_2'' will be constant over a wide range of pH. They are not thermodynamic constants for the hemoglobin molecule, but are rather group constants which will vary with the net charge on the hemoglobin molecule.[10,11] The result of the electrostatic effect of the other charged groups is to increase the apparent pK values as the pH is increased above the isoelectric point and to decrease them as the pH is decreased below the isoelectric point. Estimates of the electrostatic effect calculated in the usual way[12,13] show that they are large enough to be taken into consideration for hemoglobin and indicate the need for further research on the pK values of the heme-linked groups of hemoglobin.

(8) The designation of pk_1 and pk_2 used here is the reverse of Wyman's. The change is made so that pk_1 refers to the more strongly acidic group.

(9) C. Frieden and R. A. Alberty, *J. Biol. Chem.*, **212**, 859 (1955).

(10) K. Linderstrøm-Lang, *Rec. Trav. Lab. Carlsberg*, **15**, No. 7 (1924).

(11) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

(12) C. Tanford, THIS JOURNAL, **72**, 441 (1950).

(13) C. Tanford and M. L. Wagner, *ibid.*, **76**, 331 (1954).

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The Heats of Formation of Lithium, Sodium and Potassium Hydrides

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RECEIVED MARCH 21, 1955

As a part of a general study of the physical and thermodynamic properties of light metal hydrides, we have reinvestigated the heats of formation of lithium and sodium hydrides, and have measured the heat of formation of potassium hydride calorimetrically for the first time.[2] The uncertain purity of many of the earlier hydride preparations makes such reinvestigation desirable.

The thermochemical method used was the standard one, involving the determination of the difference between the heat of hydrolysis of the hydride and that of the corresponding metal, both measured in the same calorimeter. The calorimeter was of the bomb type, the evolved hydrogen from the hydrolysis being confined. Ketchen and Wallace[3] and Rengade[4] discuss the objections to the open type of calorimeter for this type of reaction.

Experimental

The apparatus and technique closely resemble those employed in measuring heats of combustion of organic compounds. The bomb was surrounded by 2000 ml. of water inside the Daniels adiabatic jacket of an Emerson bomb calorimetric apparatus. Temperature measurements were made to a precision of 0.001° by means of a Leeds and Northrup platinum resistance thermometer, Bureau of Standards calibrated, and a Leeds and Northrup Type G-2 Mueller bridge. The temperature difference between jacket and bomb was controlled manually, and indicated to 0.03° by means of a thermocouple and galvanometer.

The bomb was of Monel metal, with a volume of 625 ml. Its cover was pressed leaktight against the bomb by means of a threaded collar and a neoprene gasket, and was provided with a crushing rod system enabling sample capsules within the bomb to be crushed from the outside. Baffle plates with irregularly spaced holes prevented the reactants from being thrown to the top of the bomb by the violence of the reaction. A valve in the cover enabled preliminary evacuation of air and later release of evolved hydrogen.

The sample capsule, made by sealing off the material in a Pyrex test-tube of 150 mm. by 15 mm., was placed in the bomb with 150 ml. of water. Sample size was about 0.1 gram atom of metal, or 0.05 mole of hydride. It was established that the necessary evacuation of air before crushing removed 1.0 ± 0.5 g. of water. Final equilibration resulted 30–40 minutes after crushing. Corrections were made for heat losses of about 0.0005 to 0.001° per minute during the equilibrations.

Calibration and Units.—The water equivalent of the calorimeter was determined electrically by potential and current measurements on a Leeds and Northrup Type K potentiometer. The total energy equivalent of the calorimeter was 2626 ± 3 (standard deviation of the mean) cal. per degree, including 149 g. of water inside the bomb. The defined calorie, equal to 4.1840 absolute joules, was used.

Atomic weights employed were: Li 6.940, Na 22.997, K 39.100, and H 1.0080.

Materials.—Lithium metal from the Metalloy Corporation was trimmed of its coating under CO_2, and subjected to vacuum fusion in a stainless steel retort for four hours at 400° and 3 μ final pressure, to remove sodium. Sample capsules were filled and weighed under dry argon.

The sodium metal was J. T. Baker reagent grade, used without further purification. The sample capsules were prepared *in vacuo* using a technique which removed the oxide coating by fusion and led to almost mirror-like samples.

The potassium metal, furnished by the Callery Chemical Company, contained about 0.8% sodium by spectrographic analysis. It was used without further purification, correction being made for its sodium content. Capsules were filled by the same technique used for sodium.

Hydrogen for hydride synthesis was purified by passage through a Deoxo purifier, Drierite and a uranium metal getter at 500°.

Lithium hydride was synthesized by passing hydrogen over molten lithium at 720° and one atmosphere for 24 hours. The well-developed crystals were 1–3 mm. in size varying from white to light gray in color.

Sodium and potassium hydrides were made by passing hydrogen over the molten metal at one atmosphere and 650–750° and 600°, respectively. The products condensed as fine white to gray cottony masses on the cold part of the apparatus. The gray portions were rejected. Considerable difficulty was encountered in obtaining suitable samples of sodium hydride. Several preparations which either showed globules of metallic sodium or had abnormally low contents of active hydrogen were rejected. Since batch size was limited to 3–6 g., final measurements were performed on five batches

(1) Based in part upon a thesis submitted by Ludwig G. Fasolino in partial fulfillment of the requirements for the degree of Master of Science in Chemistry at Tufts College.

(2) I. Kasarnowsky and M. Proskurnin, *Z. Physik*, **43**, 512 (1927), report a very rough unpublished value of −9 kcal./mole.

(3) E. E. Ketchen and W. R. Wallace, THIS JOURNAL, **73**, 5810 (1951).

(4) E. Rengade, *Bull. soc. chim.*, [4] **3**, 188, 190 (1907).