

THE COMBINATION OF EDESTIN WITH HYDROCHLORIC ACID

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In a recent paper¹ it was shown that the amounts of the ions of HCl combined with gelatin could be calculated from electromotive force measurements of cells without liquid junction, of the type Ag, AgCl, HCl + protein, H₂. The maximum combining capacity of gelatin for H⁺, as calculated by this method, agreed with values obtained in other ways. The present paper records experiments in which this method has been applied to the protein edestin.

EXPERIMENTS²

A sample of edestin had been prepared from ground hemp seed by Osborne's method,³ slightly modified.⁴ It was freed from electrolytes by dialysis against running distilled water in a rocking apparatus of the type described by Kunitz and Simms.⁵ The liquid obtained by centrifuging the suspension after dialysis had a specific conductivity of 4.4×10^{-5} reciprocal ohms at 30°C., while the corresponding value for the water used was 2.6×10^{-6} . Solutions were prepared by mixing portions of the suspension with measured amounts of standard hydrochloric acid, made up from a distilled constant boiling mixture and redistilled water, and checked volumetrically by Na₂CO₃ and gravimetrically by AgCl. The concentrations of acid and of edestin were determined by weighing the whole solution containing a known volume of standard acid, neutralizing a weighed portion to methyl red with NaOH, and drying this neutralized portion to constant weight at 110°C. The weight of edestin was obtained by subtracting the weight of NaCl (calculated from the original HCl content of the solution) from the observed dry weight. The weight of water in a given weight of solution was obtained by sub-

¹ Hitchcock, D. I., *J. Gen. Physiol.*, 1928-29, **12**, 495.

² Most of the measurements were made by Esther R. Mason. The edestin was prepared by C. E. Heinrichs.

³ Osborne, T. B., *J. Am. Chem. Soc.*, 1902, **24**, 28.

⁴ Hitchcock, D. I., *J. Gen. Physiol.*, 1921-22, **4**, 597.

⁵ Kunitz, M., and Simms, H. S., *J. Gen. Physiol.*, 1927-28, **11**, 641.

tracting the sum of the weights of edestin and HCl from the total weight. The concentrations were expressed as molality of HCl and grams of protein per 1000 gm. H₂O. The measurements of E.M.F. were carried out at 30°C., as described in the previous paper.¹ The data are given in the first 3 columns of Table I. Each figure for the E.M.F. represents the mean of results with 3 or 4 cells not differing by more than 0.0003 volt, and each constant to ± 0.0001 volt for at least 1 hour.

TABLE I
Electromotive Force at 30°C. of the Cells
Ag, AgCl, HCl, H₂ and Ag, AgCl, HCl + edestin, H₂

| <i>m</i> | <i>g</i> | <i>E</i> (observed) | <i>E'</i> _o | <i>E</i> (calculated) | ΔE | pH (approx.) |
|----------|----------|------------------------|------------------------|--------------------------|------------|-----------------|
| 0.0100 | 0 | 0.4650 | 0.2244 | | | |
| 0.0200 | 0 | 0.4303 | 0.2260 | | | |
| 0.0301 | 0 | 0.4101 | 0.2271 | | | |
| 0.0401 | 0 | 0.3958 | 0.2278 | | | |
| 0.0501 | 0 | 0.3848 | 0.2284 | | | |
| 0.1004 | 0 | 0.3510 | 0.2309 | | | |
| 0.1007 | 13.05 | 0.3568 | 0.2307 | 0.3570 | +0.0002 | 1.08 |
| 0.1020 | 18.55 | 0.3592 | 0.2307 | 0.3592 | 0 | 1.11 |
| 0.1050 | 42.35 | 0.3732 | 0.2304 | 0.3730 | -0.0002 | 1.32 |
| 0.1055 | 62.70 | 0.3961 | 0.2301 | 0.3961 | 0 | 1.67 |

m = moles HCl per 1000 gm. H₂O.

g = gm. protein per 1000 gm. H₂O.

E (observed) = E.M.F. in volts, corrected to 1 atmosphere dry H₂.

*E'*_o = $E + 0.1203 \log m$, for cells containing HCl alone.

E (calculated) = $E'_o - 0.06015 \log (m - gx) (m - gy)$.

x = moles H⁺ combined with 1 gm. edestin = 13.4×10^{-4} .

y = moles Cl⁻ combined with 1 gm. edestin = 3.9×10^{-4} .

$\Delta E = E$ (calculated) - *E* (observed).

pH (approx.) = $-\log (m - gx)$.

Interpretation of Results

The agreement of the values in the third and fifth columns of Table I indicates that the data may be explained by assigning to 1 gm. of edestin a combining capacity of 13.4×10^{-4} equivalents of H⁺ and 3.9×10^{-4} equivalents of Cl⁻, both figures being constant in the range of these experiments, from about pH 1.1 to 1.7. The assumptions used in arriving at these values, and the method of calculation, are as fol-

lows. The relation between E.M.F. and ionic activity is given by the thermodynamic equation,

$$E = E_o - 0.06015 \log m_{\text{H}} m_{\text{Cl}} \gamma^2, \quad (1)$$

where E is the observed E.M.F. in volts, E_o is a constant, m_{H} and m_{Cl} are the molalities of the ions, and γ is the geometric mean activity coefficient of H^+ and Cl^- . Letting $E'_o = E_o - 0.1203 \log \gamma$, this becomes $E = E'_o - 0.06015 \log m_{\text{H}} m_{\text{Cl}}$, or

$$E = E'_o - 0.06015 \log (m - gx) (m - gy). \quad (2)$$

Here m is the total molality of HCl, x and y are the numbers of moles of H^+ and Cl^- , respectively, combined with 1 gm. of protein, and g is the number of grams of protein per 1000 gm. H_2O in the solution. As a first approximation, the value of E'_o for the protein cells was assumed to be constant and equal to that calculated for the cell containing 0.1004 M HCl without protein by the relation

$$E'_o = E + 0.1203 \log m, \quad (3)$$

which follows from equation (1) and the definition of E'_o . When the equations obtained by substituting the measured values of E , m , and g in equation (2) were solved simultaneously for x and y by the graphical method of the previous paper,¹ the curves did not intersect in a single point, but the provisional values $x = 13.4 \times 10^{-4}$ and $y = 3.5 \times 10^{-4}$ were obtained by averaging the different intersections. This relatively high value for y indicated a possible error in the assumption that E'_o (or γ) was constant for all the solutions. If all the protein is combined with H^+ , and a certain amount also combined with Cl^- , then the latter part should behave as un-ionized molecules. Hence the ionic strength of such a solution should not be equal to m , but to $m - gy$. Provisional values of $m - gy$ were calculated and values for E'_o corresponding to them were interpolated from a plot of the E'_o values given for pure HCl in the first part of Table I. These values for E'_o are given in the last part of Table I, and were used in re-calculating the edestin data. A second application of the graphical method again failed to give a single intersection of the curves for y as a function of x , but the values selected from this plot, $x = 13.4 \times 10^{-4}$ and $y = 3.9$

$\times 10^{-4}$, gave such agreement of the observed and calculated values for E that further repetitions of the procedure seemed unnecessary. The second approximation reduced the average deviation of the calculated values (average of ΔE) only from 0.00013 to 0.00010 volt.⁶

DISCUSSION

The value here obtained for the combining capacity of edestin for H^+ is slightly higher than any of the values compiled by Cohn,⁷ who selected 12.7×10^{-4} moles HCl per gram edestin as the probable maximum value. In an earlier paper⁴ curves were given by the writer which lead to the value $12.7 (\pm 0.6) \times 10^{-4}$ equivalents of HCl or H_2SO_4 , and the same number of moles of $(COOH)_2$ or H_3PO_4 , as the combining capacity of 1 gm. of edestin at pH 2.0 or below. Somewhat later⁸ another curve was obtained for HCl which points to the value 14.7×10^{-4} . These divergent values are probably to be explained by insufficient removal of combined acid or base from some of the protein preparations. Attempts to purify edestin are handicapped by the uncertainty which still exists as to the exact location of its isoelectric point. It should be added that, according to Osborne,³ edestin is changed by acid solutions into a substance insoluble in NaCl solutions. Hence any value for the maximum combining capacity with acids is probably a property of this denatured substance edestan rather than of the native protein edestin.

The present value, like all previous values obtained from experiments with edestin in solution in hydrochloric acid, is much lower than that recently reported by Bancroft and Barnett⁹ for the combining capacity of dry edestin with hydrogen chloride gas. Their figure of 110 mg. or 30.2×10^{-4} equivalents HCl per gram edestin is more than twice the present figure of 13.4×10^{-4} . The latter falls

⁶ A recalculation by this second approximation method of the values given for gelatin (Ref. 1) did not change them significantly. The difference between the values for E' for 0.1 M HCl in Table I and in the previous paper is probably due to differences in the AgCl electrodes, which were prepared by the electrolytic method and used repeatedly without replating.

⁷ Cohn, E. J., *Physiol. Rev.*, 1925, 5, 349.

⁸ Hitchcock, D. I., *J. Gen. Physiol.*, 1922-23, 5, 383.

⁹ Bancroft, W. D., and Barnett, C. E., *J. Phys. Chem.*, 1930, 34, 449.

on the horizontal part of their curve of pressure of HCl gas against weight of HCl taken up. If the present figure, which appears to result from the saturation with H^+ of certain groups of the protein in solution, had any significance in their experiments, it might be expected to show up as a step in their curve, such as they found with gliadin and HCl. The absence of any such break in their curve seems to indicate that the protein behaves quite differently in solution and in the dry state. Both figures cannot represent the maximum combining capacity of edestin for HCl, and it is quite possible that neither does. Further experiments would be required to determine whether the protein is more altered by solution in 0.1 M hydrochloric acid or by saturation with dry hydrogen chloride gas. The fact that in the present experiments the E.M.F. remained constant for some hours would seem to indicate that, if there is any alteration in the protein, it takes place quickly and the system reaches a steady state. The discoloration of the dry protein which they report may be indicative of some secondary chemical reaction between the protein and the gas, which may possibly open up other acid-combining groups not sharing in the reaction in solution.

SUMMARY

Electromotive force measurements of cells without liquid junction, of the type Ag, AgCl, HCl + protein, H_2 , lead to the conclusion that 1 gm. of edestin (or, more probably, edestan) combines with a maximum of 13.4×10^{-4} equivalents of H^+ and 3.9×10^{-4} equivalents of Cl^- , when the protein is dissolved in 0.1 M HCl.