THE ARGININE AND PREARGININE GROUPS IN EDESTIN

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I

INTRODUCTION

Evidence was given in 1928¹ to show that the combination of strong acid or alkali with a protein is produced by forming salts with the "extra groups" of those trivalent amino acids which can be isolated from the protein (namely, aspartic acid, glutamic acid, tyrosine, histidine, lysine, and arginine). The "titration curve" of a protein corresponds approximately to the amounts of these various groups, each with its typical titration index.²

Arginine, however, formed a marked exception. It appeared to contribute to the titration curve in much smaller amount than is found on hydrolysis. On the other hand, a group of unknown origin having a titration index of pH 4.6 was present in amounts equal to the deficiency in arginine.

The name *prearginine* was given to this group on the assumption that it is a chemical group which ionizes at pH 4.6 in proteins but yields *arginine* on hydrolysis of the protein.

It has subsequently developed that the index 8.1 assumed for the "extra group" of *arginine* was incorrect (being based on data obtained

¹ Simms, H. S., *J. Gen. Physiol.*, 1928, 11, 629; 1928, 12, 231.

² A "dissociation index" is: $pK' = -\log K'$ where K' is the apparent dissociation constant of a weak electrolyte (referred to the hydrogen ion). The dissociation index represents the pH value at which the group is half ionized.

The "titration indices" (pG') determine the titration curve and cannot be correctly called "dissociation indices" although there is little numerical difference between the titration constants (G') and the dissociation constants (K'). The use of these two sets of constants and indices is confusing but unavoidable. See Simms, H. S., *J. Am. Chem. Soc.*, 1926, 48, 1239.

FIG. 1. Titration curve of edestin. The solid symbols represent data from clear solutions; the open symbols, from cloudy solutions. The heavy dash line is drawn to fit the data in acid solution of Hitchcock and of Kodama.

Curve A is calculated from the amino acids found on hydrolysis.

Curve B (which branches off from C) represents the way edestin would ionize if it had the above groups (found on hydrolysis), but in addition had an *extra* group at pH 3.8.

Curve C corresponds with the data in Column 5 of Table I.

from impure material). Schmidt³ has obtained the value $pK'_s = 12.5$ which agrees with that of Hunter and Borsook ($pK'_s = 13.85$ at 18°C).

³ Schmidt, L. A., Kirk, P. L., and Appleman, W. K., *J. Biol. Chem.*, 1930, **88**, 285.

We have repeated our titration of arginine and corroborate the value of Schmidt.

In order to determine whether this would alter the above theory it was necessary to obtain more accurate data than were available, on a protein in very alkaline solution. Edestin was again chosen due to its high content in arginine.

II

RESULTS

A number of experiments were performed with edestin in solutions up to 14 per cent and at pH values up to 13.5. A method was adopted which as far as possible caused the unavoidable errors to cancel out. The data are given in Fig. 1 (and Table II).

An analysis of these data is given in Table I.

TABLE I

(1)	(2)	(3)	(4)	(5)	(6)
Type	Groups	Indices in edestin	Equivalents per 1000 gm.		
			On hydrolysis	Titration data	Difference
Acidic	Dicarboxylic acids	$pG_1' = 3.2$	0.72	0.55	-0.14
	Tyrosine	$pG_4' = 9.0$	.25	.15	-.10
	Sum		0.97	0.70	
Basic	PREARGININE	$pG_2' = 3.8$	0	0.53	+0.53
	Histidine	$pG_3' = 5.1$	0.25	.25	0
	Lysine	$pG_5' = 10.3$	.25	.25	0
	ARGININE	$pG_6' = 12.0$	.90	.25	-0.65
	Sum		1.40	1.28	
Sum of prearginine plus arginine			0.90	0.78	

Values in Column 4 are those listed by Mitchell and Hamilton,⁴ except for histidine and lysine in which case the values of Van Slyke⁵ are taken. The figure for dicarboxylic acids represents aspartic acid (0.76) plus glutamic acid (1.31) minus ammonia (1.35) and represents the carboxyl groups not bound by ammonia.

⁴ Mitchell and Hamilton, *The Biochemistry of the Amino Acids*, Chem. Cat. Co., 1929, 191.

⁵ Van Slyke, D. D., *J. Biol. Chem.*, 1911, 10, 15.

If edestin contained the free groups found on hydrolysis (Table I, Column 4) it would have a titration curve represented by Curve *A* in Fig. 1. This is obviously not the case.

If edestin had approximately the above free groups, and *in addition* a group with an index at pH 3.8, in proper amount, the titration curve would be that found experimentally up to pH 10.5; but above that it would have Curve *B*. This is also not the case.

The experimental data agree well with Curve *C* which corresponds to the values in Column 5 of Table I. *This supports the theory that the basic group in proteins having a titration index at pH 3.8 to 4.6 (depending on the protein) is due to a group which yields arginine on hydrolysis and may be properly called prearginine.*

III

EXPERIMENTAL

The two experiments, recorded in Tables II and III, were performed in water-jacketed hydrogen electrode cells⁶ with a saturated KCl liquid junction. A separate solution was made up, from the mother solution, for each determination. The cells were standardized against 0.100 *M* HCl (pH 1.075).

The four experiments in Tables IV to VII were performed with considerably higher concentrations of edestin (as high as 14 per cent) and it was necessary to use a titrating electrode with agar-KCl junction. In each experiment all the readings were taken on a single solution to which molar NaOH (or HCl) was added with a weighing burette.

Owing to the large correction for hydroxyl ion concentration to be made above pH 13, it was not found satisfactory to standardize the electrodes against HCl. Hence the electrodes were standardized against 0.05 *M* NaOH before (and sometimes after) each experiment. In Fig. 2 are the values of $\text{pH} - \log oh$ for NaOH⁷ as determined with the water-jacketed (liquid junction) cells. By using this chart as a standard of $\text{pH} - \log oh$ in correcting the edestin data, any errors due to the standard cell, the sodium hydroxide solution or the condition of the electrodes, would be eliminated in the calculation of $\frac{h - oh}{c}$.

⁶ Simms, H. S., *J. Am. Chem. Soc.*, 1923, **45**, 2503.

⁷ *oh* represents hydroxyl ion concentration in moles per liter. The value of $\text{pH} - \log oh$ is equal to $\text{pK}_w + \log f_{\text{OH}}$ where f_{OH} is the activity coefficient of the hydroxyl ion (namely, $f_{\text{OH}} \times oh = \text{OH} = \text{hydroxyl ion activity}$).

This method does assume, however, that solutions of the same ionic strength have the same liquid function potential and dielectric constant. The ionic strength of the protein solutions will be discussed below.

FIG. 2. Data of the titration of NaOH. Assuming complete dissociation, the concentration of hydroxyl ion (oh) is taken equal to the molarity of NaOH.⁷

IV

Calculations

A. Equivalents of Combined Base

The data in Tables II to VII were calculated as follows:

(1) The volumes of *strong base* (V_B) or *strong acid* (V_A) were calculated from the weight of the standard molar solutions, using the density at 25°C.

(2) The volume of *solution* (V) was taken as the sum of the volumes of water plus strong base (plus strong acid when present) and represents *protein-free* volume.

(3) The number of *millimoles* of strong base *minus* acid is:

$$1000 (B - A) = 1000 (V_B M_B - V_A M_A) \quad (1)$$

where M_B and M_A are the moles per liter in the standard base and acid solutions.

(4) The number of millimoles *per liter* is:

$$b - a = \frac{1000 (B - A)}{V} \quad (2)$$

(5) The *concentration* in moles per liter of a substance of molecular weight M is:

$$c = \frac{1000 W}{M V} \quad (3)$$

where W is the weight. But in dealing with a protein or any other substance of uncertain molecular weight it is more convenient to substitute 1000 for the molecular weight (giving us a final value of equivalents per 1000 gm., or milli-equivalents per gram):

$$c' = \frac{W}{V} \text{ gm. per ml.} = \frac{c M}{1000} \quad (3a)$$

(6) The number of *equivalents* of strong base minus acid is:

$$\frac{b - a}{c} = \frac{M (B - A)}{W} \text{ equivalents added per mole} \quad (4)$$

Or, for a protein:

$$\frac{b - a}{c'} = \frac{1000 (B - A)}{W} \text{ equivalents added per 1000 gm.} \quad (4a)$$

(7) The ionic strength (μ) was calculated as explained below and used with the curve in Fig. 2 to obtain the value of $\text{pH} - \log oh$. The experimental pH value was subtracted from this and the value of $\frac{h - oh}{c'}$ was calculated (where h may be neglected in alkaline solutions).

(8) The "corrected equivalents of base," or the "combined base," is:

$$b' = \frac{b - a}{c} + \frac{h - oh}{c} \text{ equivalents combined per mole} \quad (5)$$

Or, for a protein:

$$b'' = \frac{b-a}{c'} + \frac{h-oh}{c'} \text{ equivalents combined per 1000 gm.} \quad (5a)$$

$$b'' = b' \text{ when } M = 1000$$

B. Calculation of Ionic Strength

If we take the experimental data for the effect of ionic strength on protein ionization and assume a point charge, we can calculate an "apparent valence" (ν_A) which is not much greater than unity.⁸ This is because the charges on the ionized groups in a protein molecule are so distant from each other that they act almost like separate monovalent ions (*i.e.*, *separate* point charges). The value $\nu_A = 1.8$ was found in acid solutions of gelatin, and $\nu_A = 2.4$ in alkaline solutions.

By using the same assumptions (of point charge) we can use this apparent valence to calculate the approximate contribution of the protein to the ionic strength of its solution. In alkaline solution the contribution of the protein and its bound cation will be:

$$\mu_0 = \frac{\nu_A^2 + \nu_A}{2 \nu_A} b'' c' = \frac{\nu_A + 1}{2} b'' c' = 1.7 b'' c'$$

while the contribution of the unbound NaOH will be given by the hydroxyl ion *concentration*, hence:

$$\begin{aligned} \mu &= 1.7 b'' c' + oh + a \\ &= 1.7 (b - a - oh) + oh + a \\ &= 1.7 b - 0.7 oh - 0.7 a \end{aligned}$$

The data of edestin were calculated in this manner and confirmed the findings with gelatin that the contribution of protein to the ionic strength is even less than that given by this approximation. The results in Tables II to VII and Fig. 1 were calculated by assuming $\nu_A = 1$ (*i.e.*, infinitely distant point charges), hence:

$$\mu = b'' c' + oh + a = b$$

(where the amount of strong base b , being greater than the strong acid a , includes all free inorganic salt which may be present).

⁸ Simms, H. S., *J. Gen. Physiol.*, 1928, 11, 613.

TABLE II

Titration of edestin in jacketed electrodes with saturated KCl liquid junction at 25°C. (pH standard: 0.100 M HCl has pH 1.075).

Each solution contained 0.025 gm. edestin, plus 0.015 millimole of NaOH, plus the indicated volume of 0.0200 M HCl and was made up to 10.0 ml. $c' = 0.0025$ and $\mu = 0.0015$. All the solutions were cloudy.

V_A	pH	$\frac{b-a}{c'}$	b''
0.40	9.848	0.280	0.243
.50	9.135	.200	.193
.60	8.181	.120	.119
.70	7.993	.040	.039

TABLE III

Titration of edestin in jacketed electrodes with saturated KCl liquid junction at 25°C. (pH standard: 0.100 M HCl has pH = 1.075).

Each solution contained 0.0625 gm. edestin plus 0.0375 millimole of NaOH, plus the indicated volume of 0.0200 M HCl and was made up to 10.0 ml. $c' = 0.00625$ and $\mu = 0.00375$ in each solution.

V_A	pH	$\frac{b-a}{c'}$	b''
0	10.886	0.600	0.431
0.25	10.710	.520	.408
.50	10.482	.440	.374
.75	10.208	.360	.325
.87	10.078	.320	.294
1.00	9.898	.280	.263*
1.12	9.396	.240	.235*
1.25	9.195	.200	.196*

* Cloudy.

TABLE IV

Titration of edestin in a titrating electrode with agar-KCl bridge at 25°C. (pH standard: 0.0494 M NaOH has pH = 12.562).

4.996 gm. (W) of edestin in an initial non-protein volume of 35.00 ml. containing 2.420 millimole NaOH. The amount of 0.991 M NaOH is indicated by V_B (determined by weight; $d = 1.040$ at 25°C.).

V_B	$c' = \frac{W}{V}$	μ	pH	$\frac{b-a}{c'}$	b''
2.442	0.1428	0.069	11.438	0.484	0.457
2.774	.1414	.078	11.672	.550	.503
2.960	.1407	.083	11.807	.587	.523
3.246	.1396	.090	11.979	.644	.547
3.859	.1372	.105	12.216	.765	.591
4.457	.1350	.119	12.372	.884	.627

TABLE V

Titration of edestin in a titrating electrode with agar-KCl bridge at 25°C. (pH standard: 0.0494 M NaOH has pH = 12.562).

2.500 gm. (W) of edestin in an initial non-protein volume of 17.72 ml. containing 1.708 millimole NaOH. The volume of 0.991 M NaOH is indicated by V_B (determined by weight; $d = 1.040$ at 25°C.).

V_B	$c' = \frac{W}{V}$	μ	pH	$\frac{b-a}{c'}$	b''
1.724	0.1411	0.0964	11.966	0.683	0.569
5.523	.1161	.254	13.040	2.19	0.65
8.619	.1015	.346	13.217	3.41	.72

TABLE VI

Titration of edestin in a titrating electrode with agar-KCl bridge at 25°C. (pH standard: 0.0494 M NaOH has pH = 12.562).

2.500 gm. (*W*) of edestin in an initial non-protein volume of 18.14 ml. containing 2.168 millimole NaOH. The volume of 0.991 M NaOH is indicated by V_B (determined by weight; $d = 1.040$ at 25°C.).

V_B	$c' = \frac{W}{V}$	μ	pH	$\frac{b-a}{c'}$	b''
2.188	0.1371	0.109	12.376	0.867	0.615
4.583	.1218	.221	12.936	1.817	.692

TABLE VII

Titration of edestin in a titrating electrode with agar-KCl bridge at 25°C. (pH standard: 0.0492 M NaOH has pH = 12.560).

0.600 gm. (*W*) of edestin in an initial non-protein volume of 30.45 ml. containing 0.413 millimole NaOH. To this was added 0.991 M HCl in volumes indicated by V_A (determined by weight; $d = 1.016$ at 25°C.).

V_A	$c' = \frac{W}{V}$	μ	pH	$\frac{b-a}{c'}$	b''
0	0.01971	0.0136	11.423	0.688	0.518
0.072	.01967	.0159	11.144	.570	.481
.139	.01962	.0180	10.841	.458	.414
.196	.01957	.0198	10.383	.365	.349
.230	.01956	.0209	10.083	.308	.300*

* The last solution was cloudy. The other solutions were clear.

V

SUMMARY

The author corroborates the data of Schmidt showing that the dissociation index of the third group of arginine is $pK_s' = 12.5$.

New titration data of edestin have been obtained in very alkaline solutions and show that there is a corresponding group with a titration index of $pG' = 12.0$, but present in much less quantity than can account for the arginine found on hydrolysis. The data support the theory that the combination of strong base or strong acid with proteins is produced by the formation of salts with the "extra groups" of those trivalent amino acids which can be isolated from the protein, with the exception of arginine. Arginine contributes to the titration curve in much smaller amount than is found on hydrolysis. This deficiency in the arginine group may be accounted for by the basic group in proteins having a titration index of $pG' = 3.8$ to 4.6 (depending on the protein), which apparently yields arginine on hydrolysis, and may properly be called *prearginine*.