[CONTRIBUTION FROM THE BIOLOGICAL LABORATORY, COLD SPRING HARBOR]

The Calcium Binding Power of Egg Albumin

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Since Rona and Takahashi[1] demonstrated that the calcium in protein systems is for the most part non-ionic, the manner by which this calcium is bound has aroused much study. The modes of linkage which at first present themselves are through one or more of the common free groups of the protein molecule: probably the amino, carboxyl, aliphatic or phenyl hydroxyl. We therefore thought it of interest to make protein preparations which lack one or more of these functional groups and then to note their calcium binding power. We have not been able to block the carboxyl, but in view of the results obtained (see below) we did not feel that this was necessary.

Experimental

A. Preparations.—1. Crystalline egg albumin was prepared by the method of Kekwick and Cannan.[2] This was recrystallized three times, and was used as the parent material for the other preparations as well.

2. Deaminized egg albumin was made in the usual way with nitrous acid at 20° in very dilute solution in order to avoid premature precipitation. This was then dialyzed in cellophane bags against running tap water and distilled water, and left in the ice chest overnight, during which time a slight precipitate formed; this was dissolved by adjusting the *p*H of the solution to 8.4 and finally by the addition of hydrochloric acid the *p*H was lowered to 7.8. This protein solution was concentrated by ultrafiltration. Amino nitrogen (gasometric Van Slyke) on five such preparations showed that complete deamination had been accomplished.

3. Five separate preparations of acetylated albumin were made by the method of Freudenberg.[3] Ether-extracted albumin was dried and finely suspended in a mixture of equal volumes of glacial acetic acid and quinoline, and to this acetic anhydride and quinoline were added slowly at 5°, the *p*H of the system being kept at about 6.5. Aliquots were precipitated daily with 4 volumes of ether, filtered, washed with slightly acidified water, and suspended in water at *p*H 5.0. This was dialyzed overnight against running tap water and then distilled water with constant mechanical agitation; and finally dissolved in water at *p*H 8.2. Acetyl determinations were made on these solutions by the method of Herriott,[4] and when a constant value of 28 acetyl groups per molecule of albumin (molecular weight 34,500) had been obtained on the aliquots of three successive days, the main mass of the preparation was treated similarly and concentrated by ultrafiltration.

4. Gelatin, also employed in this study, was Eastman Kodak purified product. This, as well as the other proteins used, was calcium free.

B. Methods.—The method employed in determining the bound calcium was essentially that of R. Loeb[5] in his study of the effect of proteins on the diffusibility of the calcium ion. Ten to twenty cc. of protein solution was placed in a cellophane bag, and a rubber stopper was fitted into the filled bag to avoid subsequent dilution. The sac was then suspended in a large volume (300–400 cc.) of 0.80% sodium chloride brought to *p*H 7.6 with sodium bicarbonate and sodium carbonate, and to this latter solution was added calcium chloride in amounts from 1.0 to 12.0 mml./liter. The use of the sodium chloride in this instance serves to reduce the Donnan equilibrium. These systems were set aside for thirty hours at 20° (at 30° in the case of the gelatin experiments), after which time samples were withdrawn from both the protein solution and dialysate for calcium[6] and chloride[7] determinations.

Results

The results are perhaps best stated in a table of typical experiments.

The table is for the most part self-explanatory. From the amount of calcium in the dialysate (column 2) and from the ratio of chloride in dialysate to chloride in protein solution (3), the amount of calcium in the protein solution due to the Donnan effect may be calculated (4). When this value is subtracted from the observed amount of calcium in the protein solution (1), the remainder is that bound to some free group or groups of the protein molecule.

Discussion

The following points which Loeb[5] has noted for egg albumin have been confirmed: that on the alkaline side of its isoelectric point there is always a binding of calcium, and as the protein concentration is increased the total amount of bound calcium is increased. The same has also been noted for gelatin. The values found for groups of calcium to egg albumin molecule (about 1:1) agree fundamentally with those noted by other workers using serum proteins and serum.[8,9]

It is apparent immediately that the calcium is not bound through a carbamino linkage, for, if

(1) Rona and Takahashi, *Biochem. Z.*, **31**, 336 (1911).
(2) Kekwick and Cannan, *Biochem. J.*, **30**, 227 (1936).
(3) Freudenberg, *Z. physiol. Chem.*, **213**, 226 (1932).
(4) Herriott, *J. Gen. Physiol.*, **19**, 283 (1935).
(5) Loeb, *ibid.*, **8**, 541 (1928).
(6) Kramer and Tisdall, *J. Biol. Chem.*, **47**, 475 (1921).
(7) G. Smith, "Quantitative Analysis," The Macmillan Company, New York, 1922, p. 55.
(8) Marrack and Thacker, *Biochem. J.*, **20**, 580 (1926).
(9) Greenberg and Gunther, *J. Biol. Chem.*, **85**, 491 (1929–1930).

Table I

	1 Observed Ca mml./l. in protein soln.	2 Ca in dialysate mml./l.	3 Cl_d/Cl_p	4 Calcd. Ca in protein soln. mml./l.	5 Bound Ca in protein soln. mml./l.	6 Protein mml./l.	7 Groups of Ca to protein
Egg albumin	5.05	3.20	1.06	3.60	1.45	1.18	1.23
	4.70	3.56	1.04	3.94	0.76	0.60	1.26
Deaminized albumin	4.95	2.75	1.05	3.10	1.85	0.60	3.10
	6.45	3.40	1.08	3.95	2.50	1.16	2.16
Acetylated albumin	4.25	3.80	1.06	4.22	0.03	0.67	0.04
	3.15	2.88	1.05	3.18	− .03	.26	Approx. 0
"Deacetylated" acetylated albumin	4.42	3.27	1.05	3.60	.82	.64	1.29
	4.30	3.05	1.06	3.42	.98	.72	1.36
Gelatin	4.82	3.51	1.05	3.88	.94	.70[a]	1.34
	3.54	2.53	1.04	2.70	.84	.60[a]	1.38

[a] 34,500 was chosen arbitrarily as the molecular weight of gelatin to provide comparison with the ratios obtained for the egg albumin.

this were so, deamination of the albumin should result in a decrease of bound calcium instead of in an increase. These higher ratios might be interpreted as being due to (a) an increased calcium binding power of the protein resulting from the splitting of the chain in the course of deamination; (b) the substitution of functional hydroxyl groups for the amino, which is the result of a deamination in aqueous solutions; or (c) to both.

The substitution of hydroxyl groups was tested by using the acetylated albumin. Such experiments show that these proteins no longer have the calcium linkage; and since the acetylation has blocked the amino and hydroxyl groups, and not the carboxyls, we may conclude that the functional group is very probably the hydroxyl. This is further shown by the restoration of the calcium binding power of the protein when the acetyl groups are removed by treating the protein solution with alkali to *p*H 11.0, for two minutes, bringing back to *p*H 8.2, and removing the acetate by dialysis. That the hydroxyl group is not necessarily a phenolic one is seen from the fact that gelatin, with no phenyl groups, binds calcium to the same extent as does albumin. It would be interesting, however, to determine whether or not the phenolic radical plays any role; this might be done by correlating tyrosine values of partially acetylated proteins with their ability to bind calcium.

Summary

A study of protein derivatives seems to indicate that calcium is bound to albumin through an hydroxyl linkage.

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The Spontaneous Stable Formation of Colloids from Crystals or from True Solution through the Presence of a Protective Colloid[1]

By J. W. McBain and M. E. Laing McBain

The subject of colloids has suffered greatly from the commonly accepted stigma that colloidal systems are inherently unstable and that they therefore show the effects of every chance incident in the history of each individual specimen. This discouraging view has probably arisen from the older habit of regarding colloids merely as fine dispersions without mention of any factor that should hold them in so unlikely a condition, much less cause them, when disturbed or destroyed, to revert spontaneously to that former state.

In 1926[2] one of us presented the argument that certain colloidal solutions, such as those of soap, must be regarded as stable in the strictest thermodynamic sense, because they exist in equilibrium with crystalloidal constituents and crystals, and hence must be as stable as these. This has been accepted by many leading colloid authori-

(1) Read at the Pittsburgh meeting, September, 1936.

(2) J. W. McBain, "Colloid Symposium Monograph," Cambridge, 1926, p. 1; *Kolloid-Z.*, **40**, 1 (1926).