[Jour. Indian Chem. Soc., Vol. 40, No. 12, 1963]

Studies on the Interaction of Metal Ions and their Hydrous Oxide Sols with Proteins. Part III. Interaction of Aluminium, Chromium, Beryllium and their Hydrous Oxide Sols with Haemoglobin

Hasan Arif, Salahuddin, and Wahid U. Malik

*p*H-metric titration of anionic haemoglobin has been carried out to ascertain the binding of Al^{3+}, Cr^{3+}, and Be^{2+} with the protein. From the shift in *p*H, when anionic protein is replaced by NaOH of the same *p*H, Cr^{3+} and Be^{2+} ions are assumed to be available both from the sols and the electrolytes to combine with the carboxyl groups of haemoglobin. Experiments, performed with equal volumes of the protein and electrolyte, each of *p*H 4.0, show decrease in *p*H of the protein, thereby confirming the above assumption. The combining powers are in the order: $Al^{3+} > Cr^{3+} > Be^{2+}$.

Earlier physico-chemical investigations[1,2], carried out in these laboratories, on the interaction of metals and their hydrous oxide sols with proteins had provided enough evidence regarding the binding of metal ions with the available sites in simple and transfusion gelatin. *p*H-metric and viscometric methods were employed to investigate the phenomenon and valuable information could be obtained from the *p*H shifts as well as from the inflection points in the viscosity-concentration curves[2]. In view of the prospective utility of these methods, it has been considered worthwhile to extend this investigation to proteins other than gelatin.

The present communication deals with the *p*H-metric studies on the interaction of anionic haemoglobin and hydrous oxide sols of aluminium, chromium, and beryllium.

EXPERIMENTAL

Haemoglobin (E. Merck, Technical) was dissolved in NaOH solution according to the method of Steinhardt and Zaiser[3,6] to prepare a 2% solution. The *p*H of the solution was adjusted to 11.0 by dialysing the protein solution for some time. The nitrogen content of the protein was determined by the micro-Kjeldahl method and was found to be 16.4%.

Electrolyte solutions (*p*H 2.0) of aluminium chloride, chromium chloride, and beryllium nitrate were prepared by dissolving the chemically pure samples in all-glass double distilled water. Respective concentrations of aluminium oxide and beryllium oxide (determined gravimetrically) were 0.5084 and 0.2343 g./litre. The chromium

1. Malik and Salahuddin, *J. Electro-anal. Chem.*, 1963, **5**, 68, 147.
2. Salahuddin and Malik, this *Journal*, 1962, **39**, 693, 699.
3. *J. Biol. Chem.*, 1951, **190**, 197.

content of the solution was determined colorimetrically according to Green and Ang[4] and was 1.0404 g./litre. Hydrochloric acid solution (*p*H 2.0) was prepared for comparison. Colloidal solutions of hydrous oxides of aluminium, chromium, and beryllium were prepared by following the methods of Weiser[5], Graham[6], and Krauskopf[7] respectively. Their *p*H's were adjusted to 2.0 by addition of requisite amounts of HCl solutions. The concentrations of alumina, chromium hydroxide, and beryllium hydroxide (as oxide) were found to be 1.3168, 0.2988, and 0.8112 g./litre respectively. The nature of the charge on the hydrophobic colloidal solutions was determined by Burton's type electrophoretic tube. All of them were found to be positively charged. Carbonate-free NaOH[8] was employed during these investigations. Potassium chloride solution was made from A.R. samples.

Measurements of *p*H were carried out with a Beckman *p*H-meter, Model G, using glass electrodes in an inert atmosphere. Following sets of solutions were prepared and their *p*H's determined after 12 hours.

Varying volumes, viz., 0,1,2,4,6,8,10,12,14, and 16 ml of either the electrolytes or their corresponding sols and HCl solution, each maintained at *p*H 2.0, were taken with 4ml of the haemoglobin solution (*p*H 11.0). The final volume was made 20 ml in each case by addition of the requisite amount of water. Similar sets of mixtures containing NaOH (*p*H11), instead of the protein, were also prepared. The *p*H-titration curves are shown in Figs. 1-3 and the shifts in *p*H, when the protein is replaced by NaOH, are shown in Table I.

TABLE I

0.4% Haemoglobin soln. used.

Vol. of sol or electrolyte.	HCl.	Alumina.	$AlCl_3$.	$Cr(OH)_3$ sol.	$CrCl_3$.	$Be(OH)_2$ sol.	$Be(NO_3)_2$.
0 ml	—0.10	—0.10	—0.10	—0.10	—0.10	—0.10	—0.10
1	0.60	1.95	5.10	1.35	4.12	3.20	2.80
2	4.70	5.70	3.90	3.58	1.95	3.70	1.95
4	5.60	5.90	2.15	5.00	1.80	3.10	1.75
6	5.10	4.88	1.50	4.72	1.70	2.50	1.60
8	4.18	3.80	1.40	4.20	1.45	2.05	1.40
10	3.22	2.80	1.38	3.60	1.30	1.80	1.30
12	2.45	2.00	1.30	3.28	1.20	1.70	1.20
14	2.00	1.75	1.19	2.05	1.20	1.50	1.10
16	1.85	1.50	1.15	1.62	1.00	1.18	1.00

N. B. Figures, excepting those in column 1, refer to $\triangle$ *p*H.

Since *p*H titrations provided evidence of the possible reaction between the metal and the protein in the *p*H range 2 to 5, it was felt necessary to determine precisely the

4. *J. Amer. Chem. Soc.*, 1955, **77**, 5482.
5. *J. Phys. Chem.*, 1920, **24**, 505.
6. *Phil. Trans.*, 1861, **151**, 183.
7. *J. Phys. Chem.*, 1931, **35**, 3237.
8. Kolthoff and Sandell, "Text Book of Quantitative Inorganic Analysis", 3rd ed., The Macmillan Co., N. Y., 1952.
9. Steinhardt and Zaiser, *J. Amer. Chem. Soc.*, 1953, **75**, 1599.

competing tendency of the hydrogen and the metal ions in this pH range. To achieve this Scatchard's method of finding out the pH shift was employed. Two sets were arranged, one containing equal volumes (5ml) of protein (pH 4.0) and the electrolyte of the same pH and other containing 5 ml of water instead of the electrolyte. The total volume was made to 20 ml. The ionic strengths in the first set were maintained at 0.066, 0.10, 0.14, and 0.20 by addition of a requisite amount of KCl solution. The pH of the mixtures was determined after 12 hours at 30°. The results are recorded in Table II.

TABLE II

0.5% Protein soln. was used. Conc. of the electrolytes $= 2.5 \times 10^{-3} M$.

Ionic strength.	$AlCl_3$.	$CrCl_3$.	*$Be(NO_3)_2$.
0.066	0.26	0.25	0.18
0.100	0.29	0.29	0.18
0.140	0.30	0.30	0.20
0.200	0.31	0.30	0.25

N. B. Figures in columns 2-4 refer to Δ pH.

* The conc. of $Be(NO_3)_2$ was 2.3×10^{-3}.

DISCUSSION

The interaction of positively charged alumina, chromic hydroxide, or beryllium hydroxide sol and haemoglobin (pH 11.0) can possibly lead to three different types of combinations, viz., (*i*) binding of hydrogen ions, (*ii*) binding of metal ions, and (*iii*) neutralisation of the free base in the protein by the free acid in the sol. The first two conditions exist when the hydrogen ions, or the metal ions, or the both are made available from the sol for reaction with the reactive groups of the protein. As far as realisation of the last condition is concerned, there appears to be a remote possibility of simple neutralisation reaction taking place, since ionic combinations are always possible with the protein, depending of course on the pK values of the reactive sites. Another factor, which might play quite an important role, is adsorption provided that repulsive forces between the two are not operative.

Comparing the curves for alumina (pH 2.0) and haemoglobin pH (11.0), on the one hand, and that of the hydrochloric acid (pH 2.0) and the protein (of the same pH), on the other, it will be seen that the aluminium ions from the sol do not make themselves available for binding with the protein. Instead, the hydrogen ions are quite reactive and this can easily be seen from the proximity in the two curves (Fig. 1, curves A and B). From these results it could not, however, be concluded that aluminium ions binding to the protein was not feasible. That it is not so, is confirmed on comparing curves C and E (Fig. 1) which provide ample indication as to the protein competing with its free base in combining with the metal ions. The binding of hydrogen ion by the protein differs from that of aluminium ion since the latter appears to be bound exclusively through the carboxyl groups in the vicinity of pH 4.5 (pK values of the group in different proteins lie in the

neighbourhood of *p*H 4.0)[10], whereas the former enters into combination also with other groups, e.g., imidazole, amino, etc. Similar reasoning can be offered while considering the binding of chromium from chromic chloride where the curve for electrolyte lies above that for the corresponding curves for NaOH (Fig. 2, curves C and E), again pointing towards the fact that protein competes with the base in combining with the metal ions in the *p*H range 3.5-4.5 where carboxyl groups are available for the reaction. The nature of the curves (Fig. 2, curves A and B) for the chromic hydroxide sol and hydrochloric acid is almost the same as for alumina, with the only difference that the chromic hydroxide sol provides larger number of hydrogen ions for combination than the acid. That the interaction of chromium and the carboxyl group of haemoglobin is quite probable may also be concluded on the basis of the systematic researches of Gustavson[11] on the fixation of the metal through the carboxyl group of gelatin.

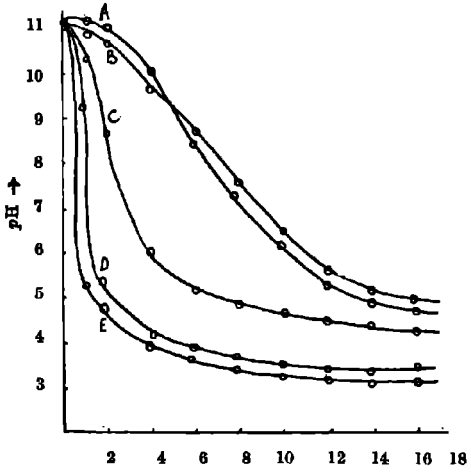

FIG. 1.

Curve A for alumina and haemoglobin.
B ,, HCl ,, ,,
C ,, $AlCl_3$,, ,,
D ,, alumina ,, NaOH.
E ,, $AlCl_3$,, ,,

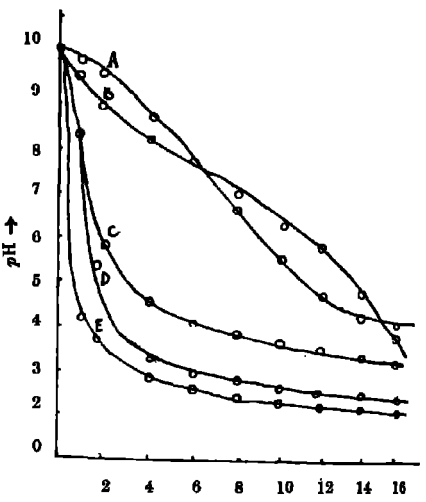

FIG. 2

Curve A for chromium hydroxide and protein.
B ,, HCl ,, ,,
C ,, $CrCl_3$,, ,,
D ,, chromium hydroxide ,, NaOH.
E ,, $CrCl_3$,, ,,

The behaviour of berryllium hydroxide sol towards the protein differs from the two hydrophobic sols discussed above. Here the curves for the sol-protein as well as for the electrolyte—protein lie below that for HCl—protein (Fig. 3, curves A, B, and C),

10. Gurd and Wilcox, "Advances in Protein Chemistry", Vol. XI, Academic Press, N.Y., 1956, p. 324.
11. *J. Amer. Chem. Soc.*, 1952, 74, 4608.

indicating the possible availability of metal ions from sol and also from the electrolyte for binding to the carboxyl groups of the protein.

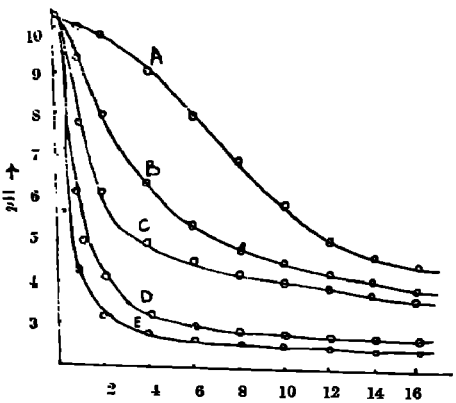

FIG. 3

Curve A for hydrochloric acid and protein.
B ,, $Be(OH)_2$ sol ,, ,,
C ,, $Be(NO_3)_2$,, ,,
D ,, $Be(OH)_2$ sol ,, NaOH.
E ,, $Be(NO_3)$,, ,,

While considering the interaction of the metal ions and protein, two important operative factors should be taken into account. It may be argued that physical adsorption may also be responsible for the pronounced shift in *p*H. Such an effect can have its significance in the higher *p*H range, but should not be of any value in the lower *p*H range (3-5) where repulsive forces between two positive particles would be operative. Further more, this seems to be quite improbable in view of the reactivity of the ionisable groups of the protein in the *p*H range under consideration. Secondly, the influence of the buffering capacity of the protein may play a major role during the course of the reaction. Had it been so, the curves for sols, electrolyte, and hydrochloric acid, each having equal amount of hydrogen ions, would have almost coincided, which is not the case. On the other hand, the fact that the mixtures, in which the chemical combination is indicated by *p*H-metry, remain suspended, strongly goes to prove that chemical forces[12] are involved during the interaction.

The shift in *p*H, viz., $\triangle p$H, when the anionic protein is replaced by NaOH of the same *p*H, shows the extent to which the protein competes with the hydroxyl ion in combining with the metal ion; hence it may be taken as a qualitative measure of the extent of metal-protein interaction. From Table I it appears that the extent of metal binding follows the order: $Al^{3+} > Cr^{3+} > Be^{2+}$ ($\triangle p$H for beryllium being least). Furthermore, a definite decrease in $\triangle p$H values, with decrease in *p*H, shows comparatively small metal-binding, a behaviour quite understandable in view of the availability of lesser number of free carboxyl groups due to protonation ($-COO^- + H^+ \rightleftharpoons COOH$).

Experiments performed with the mixtures containing equal volumes of the electrolyte and protein, each of *p*H 4.0, reveal that the *p*H of the protein shows small but detectable decrease ($\triangle p$H) as a result of the metal-protein combination. This can very well be understood from the Scatchard and Black[13] equation,

$$r = W\ (\triangle p\mathrm{H})$$

(where $\triangle p$H is the shift towards lower *p*H due to the binding of r moles of metal/protein molecule, and W depends upon ionic strength) which also predicts such a decrease in

12. Weiser, "Text Book of Colloid Chemistry", John Wiley & Sons, N.Y., 1949, p. 297.
13. *J. Phys. Coll. Chem.*, 1949, 53, 88.

pH. The values of ($\triangle p$H) for different metal ions show that the order of metal binding is $Al^{3+} > Cr^{3+} > Be^{2+}$, same as that obtained from the above results. Moreover, it is interesting to note that (pH) values are not significantly altered by the increase in ionic strength from 0.066 to 0.200, ($\triangle p$H) being invariably the same, leading to the conclusion that the uptake of the metal ions by haemoglobin is almost independent of the concentration of anion, chloride ion in the present case.

Thanks of the authors are due to Prof. A. R. Kidwai, Head of the Department of Chemistry, Aligarh Muslim University, for providing facilities and to C.S.I.R., New Delhi, for the award of a followship to one of them (S). One of the authors (H.A.) is grateful to the U.P. Scientific Research Committee for awarding him a research assistantship.

CHEMICAL LABORATORIES,
MUSLIM UNIVERSITY,
ALIGARH.

Received November 23, 1962.