SHORT COMMUNICATIONS

INTERACTION BETWEEN BERYLLIUM AND TRANSFUSION GELATIN

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OWING to several biological implications of beryllium[1-3], its interactions with proteins have been extensively investigated[4-8]. However, a literature survey indicates that although transfusion gelatin is a well-characterized protein[9,10], its interaction with beryllium has not been studied so far. The present paper describes the results on the binding of beryllium ions with transfusion gelatin using pH-metric and equilibrium dialysis methods. The effect of pH and temperature on the binding constants has been discussed.

A solution of beryllium sulphate (BDH) was prepared in double-distilled water and analyzed gravimetrically. Transfusion gelatin (TG) supplied by the National Chemical Laboratory, Poona was used in these studies. pH measurements of the beryllium-transfusion gelatin mixtures and TG alone with varying amounts of hydrochloric acid or potassium hydroxide were carried out at 30°C on a Systronic pH-meter. Total ionic strength of the solutions was adjusted to 0.15 by adding requisite amounts of 1 M potassium chloride solution. For equilibrium dialysis cellophane bags were filled with 5 ml of 0.6 % protein (0.15 M in KCl) solution and immersed in a 5 ml solution of beryllium sulphate (0.15 M in KCl). These experiments were arranged at four different pH values (3.5, 4.6, 5.0 and 5.5) and three temperatures (10, 30 and 45°C). The solutions were shaken for a period just sufficient to attain the equilibrium. The external metal solutions were analyzed colorimetrically[11]. Controlled studies indicated negligible binding with the dialysis bags. The values of $n_{M'}$ (average mole of beryllium ion bound per mole of TG, mol. wt. 75000), were calculated as described earlier[12,13].

The value of hydrogen ions dissociated per mole of TG (γ) was determined in the presence and absence of beryllium ions using Tanford method[14] and the results are shown in figure 1. Hydrogen ion equilibria curves of figure 1 were used for determining the number of hydrogen ions displaced by the beryllium ions. This is equal to $n_{M'}$, the number of beryllium ions bound per mole of protein, based on the concept of one-to-one

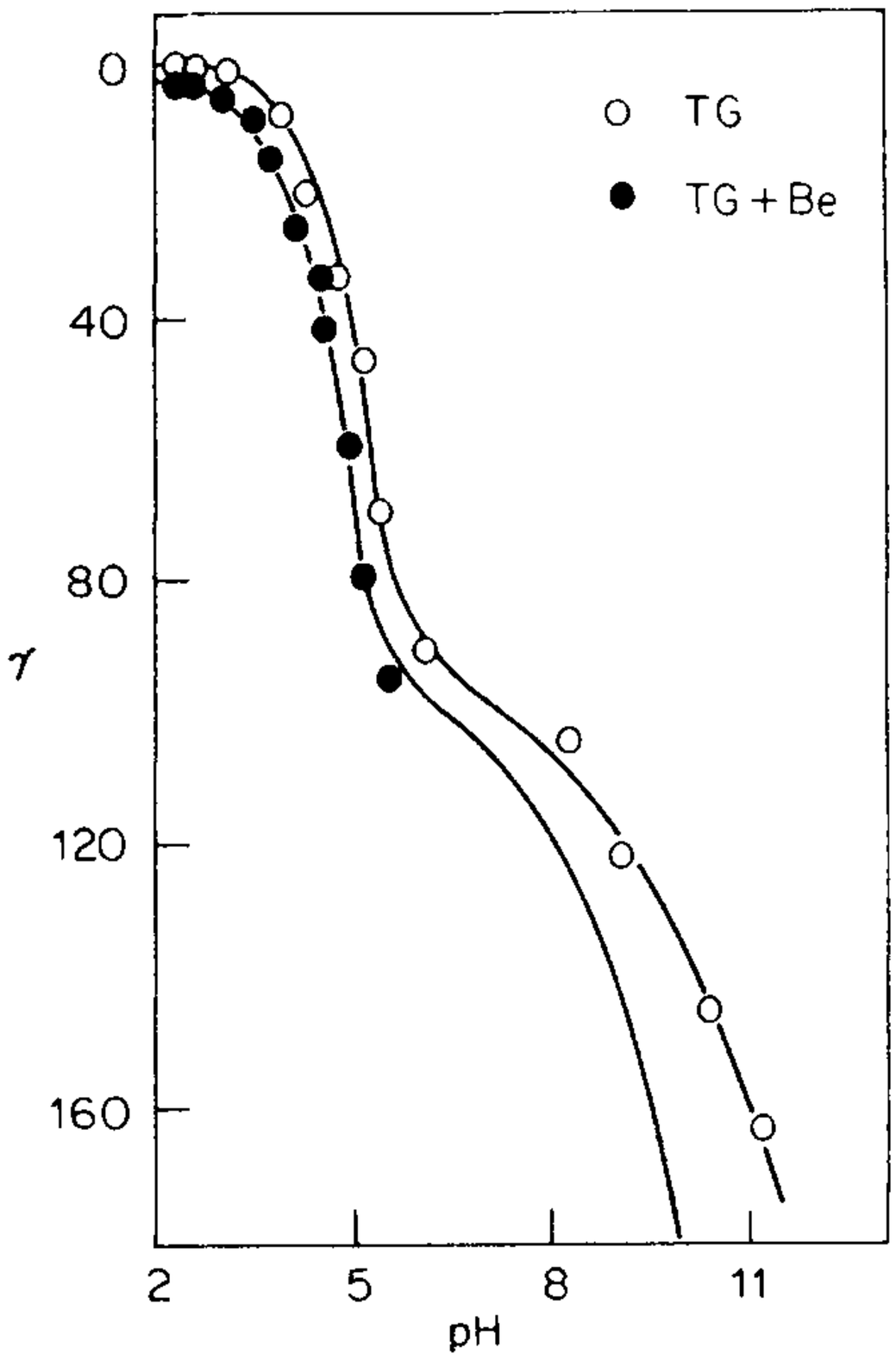

Figure 1. Titrations of transfusion gelatin in the presence and absence of beryllium ions at 30°C, u = 0.15, –o–o– (TG) and –●–●– (TG + Be).

binding[15] (one hydrogen ion for one metal ion). The $n_{M'}$ value in the pH range 3.0 to 5.5 would directly give the extent of beryllium-carboxyl interaction. The $n_{M'}$ value was not calculated above pH 5.5 owing to the precipitation of beryllium hydroxide. The value of intrinsic association constant (log K) computed from Scatchard's equation[16] comes out to be 1.735 at pH 5.5 for metal-carboxyl system. This value is in line with that reported for beryllium-bovine-serum albumin interaction[7]. The free energy change for beryllium-carboxyl interaction was found to be -2.422 Kcal/mol.

The results of equilibrium dialysis further provided sufficient evidence for the interaction of beryllium ions with carboxyl groups of the protein. The $n_{M'}$ value increased with increasing free equilibrium beryllium

concentrations at all pH values and temperatures. The binding constants were determined by Scatchard equation[16] in the form,

$$n_{M^+}/[\mathrm{Be}]_F = K_{n_{M^+}},$$

where $[\mathrm{Be}]_F$ is the free equilibrium beryllium concentration, n the number of binding sites, and K the intrinsic association constant for metal-carboxyl system. The above equation is based on the assumption that there are n binding sites with equal intrinsic association constant K, and that there is no interaction between the bound ions. Under these conditions a plot of n_{M^+}/Be_F vs n_{M^+} would be linear and the value of n and K can be calculated from the slope and intercepts. The results of dialysis experiments at pH 3.0 to 5.5 and three temperatures are plotted in figure 2. The value of n and $\log K$ are given in table 1. The values of n increase with increasing pH and temperature. The significance of the uniformity of $\log K$ is that a single class of site reacts with beryllium at all pHs and temperatures. The appearance of different sites (n) is therefore not responsible for the enhanced binding but may be due to the increased availability of the same class of sites. A similar pH dependence of binding sites was earlier reported[17] in the binding of oxovanadium(V) ions by trypsin. The pH and temperature dependence of binding sites may be attributed to successive deprotonation of carboxyl groups[10] as well as to structural changes in protein molecule.

Table 1 *Binding constants viz intrinsic association constants (log K), number of binding sites (n) and thermodynamic parameters viz free energy (ΔF° kcal/mol) entropy (ΔS° cal/mol/deg) and enthalpy (ΔH° kcal/mol), for the first binding sites in beryllium-transfusion gelatin reaction at different pHs and temperatures by equilibrium dialysis method*

	3.50	4.60	5.00	5.50
(a) 10°C				
n	3	5	8	12
log K	1.485	1.413	1.477	1.518
ΔF°	−1.936	−1.845	−1.926	−1.979
ΔS°	8.2	7.3	11.3	16.7
(b) 30°C				
n	6	15	25	35
log K	1.499	1.415	1.579	1.732
ΔF°	−2.088	−1.975	−2.204	−2.417
ΔS°	8.10	7.3	11.5	17.0
(c) 45°C				
n	7	17	30	40
log K	1.518	1.423	1.586	1.740
ΔF°	−2.223	−2.084	−2.323	−2.549
ΔS°	8.6	7.6	11.9	16.6
ΔH°	0.391	0.237	1.303	2.747

The present study indicates that ionized carboxyls interact with beryllium ions at pH 5.5 and below[10]. The thermodynamic parameters, viz free energy ($\Delta F°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$), were calculated by the usual methods and are given in table 1. The free binding energy is mostly due to changes in entropy with the apparent enthalpy contribution being small

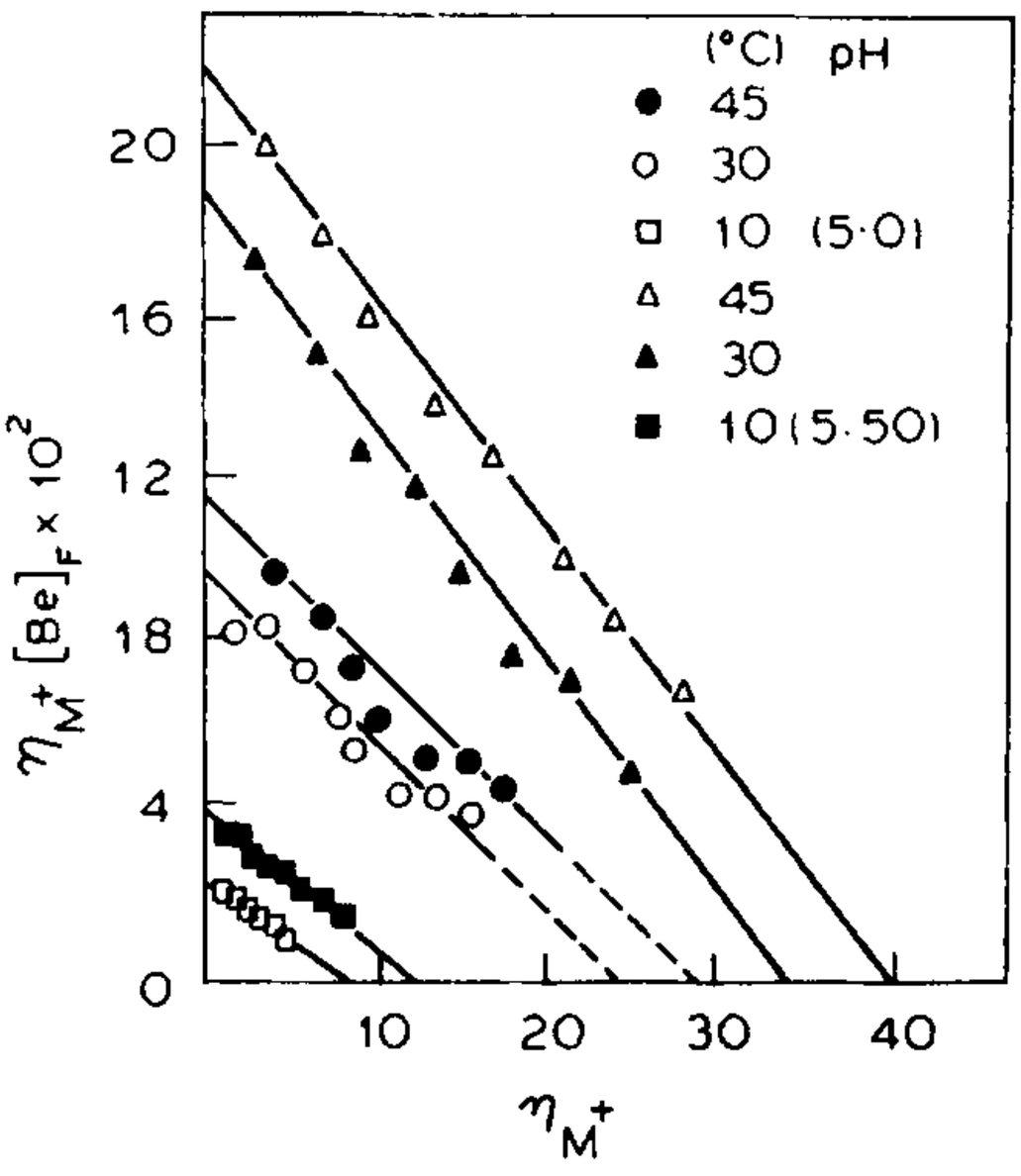

Figure 2. Scatchard plots for Be(II)-TG at pH 5.0 and 5.57 at different temperature; –●–● (45°C), –o–o– (30°C), –□–□– (10°C) (pH 5.0). –△–△– (45°C), –▲–▲– (30°C), □–□ (10°C) (5.50).

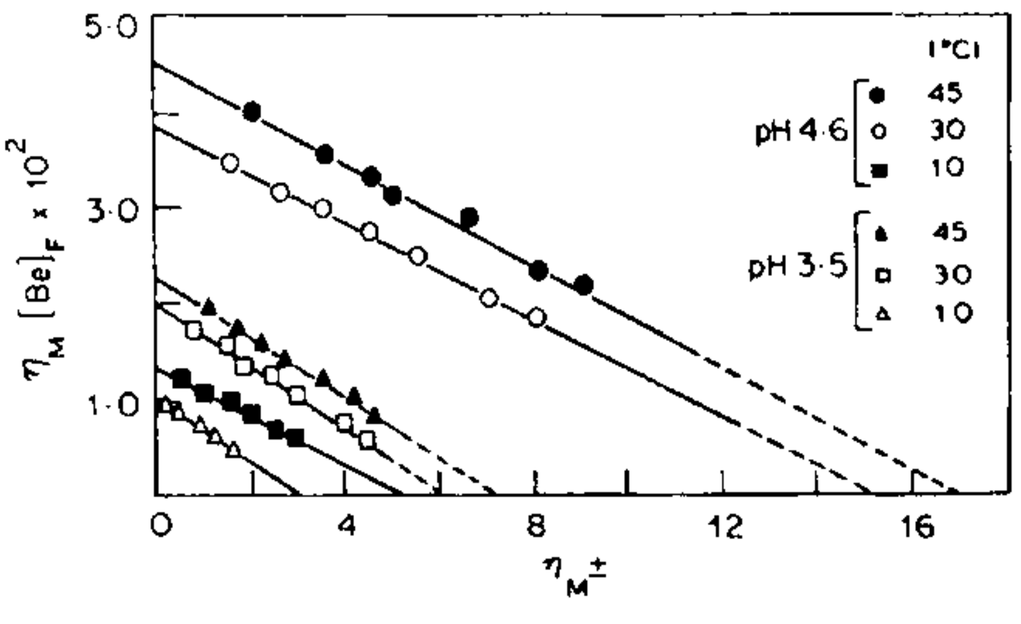

Figure 3. Scatchard plots Be(II)-TG at pH 3.50 and 4.60 (u = 0.15), –●–● (45°C), –o–o– (30°C), –■–■– (10°C) (pH 4.60), –△–△– (10°C), –□–□– (30°C), –▲–▲–(45°C) (pH 3.50).

and sometimes in the range of 0.273 to 0.391 K cal/mole at pH-values 4.6 and 3.5 respectively. The entropy changes are in the range of 7–17 cal/mole/deg within which the entropy values of beryllium-TG reaction lie. The thermodynamics of this interaction is comparable to that of other ion-protein interactions[18–20].

The positive values of entropy probably indicate that the transfer of beryllium ion from the solvent to protein is accompanied by the release of water of hydration from macromolecule and the ions of buffer, and that the configuration of folded molecule changes into an unfolded one[21]. The decreased enthalpy at lower pH-values may be attributed to larger electrostatic repulsion between cationic protein and the beryllium ions and therefore lesser release of water of hydration[22]. The temperature dependence of the present interaction indicates that all the 84 carboxyl groups[10] are not equally available for interaction with beryllium ions; presumably, a large fraction of these groups remains buried in the interior of folded regions of polypeptide chain. However, with a rise of temperature the folded polypeptide chain is perturbed and the masked carboxyls exposed for binding with beryllium ions. The results discussed suggest that any environmental effect altered the small ion binding affinity of the macromolecular system.

21 October 1985

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HAEMOCYTE RESPONSE AS A POSSIBLE AND NEW PARAMETER IN THE PRELIMINARY SAFETY/TOXICITY EVALUATION OF BIOMATERIALS

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HAEMOCYTES (blood cells) of insects do respond to foreign bodies that enter into their haemocoele[1–3]. In the light of the above observation and in our search for simple and less costly alternative test systems, haemocyte response of insects was investigated as a possible new parameter in the toxicity/safety screening of biomaterials.

Eight different formulations of polyvinylchloride sheets, on which routine toxicological data available (4 nontoxic and 4 toxic samples) were selected as test samples for the present study. Tygon tubings (PVC, Norton Company, USA) were used as control samples. Test and control samples were cut into small strips and were sterilized by gamma radiation. The common house pest, *Periplaneta americana* was chosen as the experimental animal. These animals were immobilized by ether and the samples were implanted into the haemocoel through an incision made between the second and third abdominal sternite of the animal.