Inorganica Chimica Acta, 46 (1980) L51–L53

Potentiometric Studies on Nickel(II)–Glutathionate Interactions

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Received January 16, 1980

Previous spectroscopic studies on the Ni(II)–glutathionate system in aqueous solution have established that both octahedral and square planar complexes exist in an equilibrium that is strongly dependent upon pH [1–3]. At least two octahedral and two square planar complexes occur in the region between pH = 4 and pH = 12. These investigations concluded that the octahedral species formed at the lowest pH involves co-ordination by the carboxyl and amine groups of the glutamic acid residue but conclusions were uncertain concerning the bonding in the other complexes. Similarly, the involvement of the sulphur atom has been the subject of controversy [1–5]. Rabenstein *et al.* have recently concluded that the sulphydryl group is a minor binding site for Ni(II) under most solution conditions [5]. The spectroscopic evidence was similarly interpreted: sulphur was thought to be involved in Ni(II) co-ordination only above pH = 9.

The lack of thermodynamic formation constants for Ni(II)–glutathionate prompted us to undertake a detailed potentiometric study of this system over a wide pH range. In order that the data would be applicable to biological conditions of temperature and ionic strength, it was decided to work at 37 °C and in background electrolyte solutions of sodium chloride (150 mmol dm^{-3}). Glutathione occurs at relatively high concentrations in cells [6] and is probably involved in the way they respond to heavy metal insult [5]. It is, therefore, of interest to know how this naturally-occurring tripeptide interacts with transition metal ions *in vivo*.

Such information can be obtained by computer simulation once the formation constants have been determined [7–11]. For example, the potentiometric investigation of glutathione with zinc(II), cadmium(II) and lead(II) has suggested that the ligand will selectively complex the latter toxic metal ions [12, 13]. A knowledge of the Ni(II)–glutathione constants would thus be very useful in considering the biochemistry and treatment of nickel poisoning.

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Experimental

Reduced glutathione (BDH Chemicals) was recrystallized from ethanol and dried at 100 °C *in vacuo* (C, H, N *Analysis:* Found, C = 39.20%, H = 5.70%, and N = 13.50%; Calcd. for $C_{10}H_{17}N_3O_6S$: C = 39.10%, H = 5.58%, and N = 13.70%).

Solutions of glutathione were freshly prepared each day. $NiCl_2$ (Analar) solutions were analysed for metal ion concentration by EDTA titration and for hydrogen ion concentration by Gran's plots. The potentiometric approach has been previously described [9]. All titrations were carried out at 37 °C and I = 150 mmol dm^{-3} (NaCl).

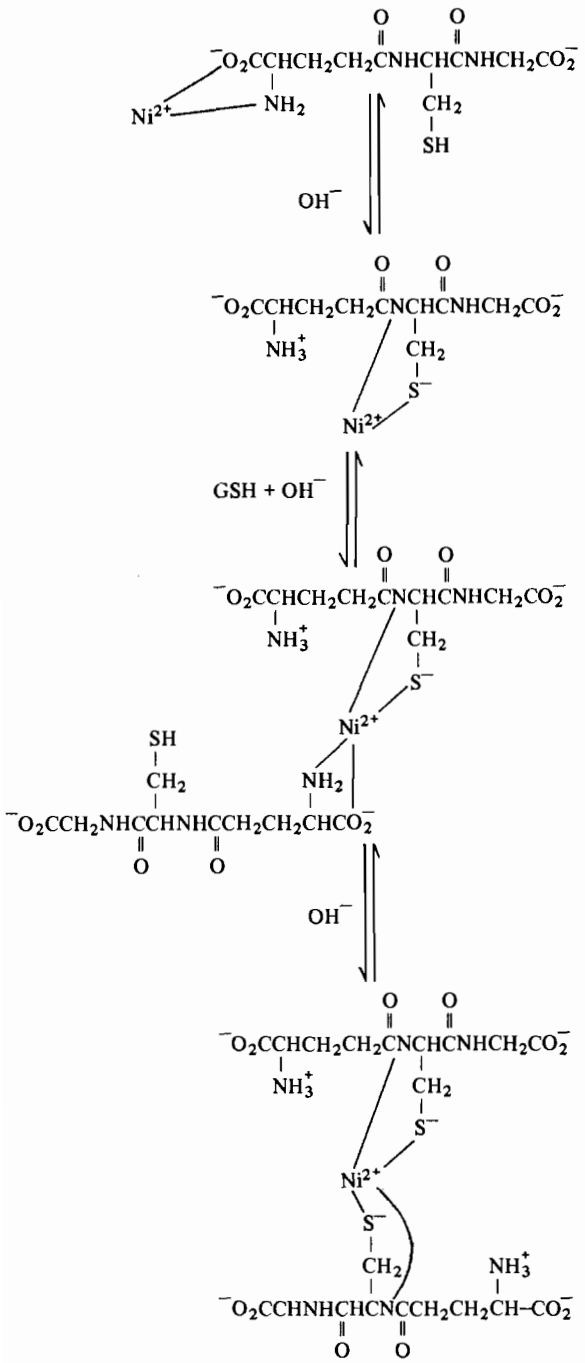
Formation constants were computed from the potentiometric data using the MAGEC [14], MINIQUAD [15] and ZPLOT [16] computer programs. The computer program PSEUDOPLOT was used to check selected sets of formation constants, following our usual practice [16, 17].

Results and Discussion

Compared with systems in which glutathione interacts with other transition metals such as zinc(II), both the experimental work and its elucidation with nickel(II) proved unexpectedly difficult. Chiefly, this was because of an observed drifting of electrode potential in solutions at high pH. In alkaline media, glutathione is prone to decompose and this appears to be accelerated in the presence of nickel(II). Furthermore, it soon became clear that an especially wide variety of complexes were formed as the pH was raised.

Accordingly, the data for the metal–ligand titrations was treated in three stages: an initial set of 224 data points which were considered completely reliable was successively extended to 266 and then to 288 points by including readings from the more alkaline solutions which were reproducible and reasonably stable. The set of 266 points included all data for solutions up to $-\log[H^+] = 10$. The best results from a total of over 70 models which were tried as a basis for the computer calculations are shown in Table I.

The main species in solutions of intermediate pH are undoubtedly the 111 and 110 complexes. The latter predominates over the former in solutions where $-\log[H^+] > 6.5$. Two other species, the 211 and 210 emerge as the most important complexes around $-\log[H^+] = 9$. Under these conditions, at least two but probably three further species co-exist at low but significant concentrations. Moderate decreases in S, the sum of squared residuals, were obtained when either deprotonated (111) or polynuclear (320, 430)



complexes were considered but in view of the high standard deviations of their calculated formation constants, the improvement was insufficient to justify positive stoichiometric identification.

These results permit a re-interpretation of the spectroscopic evidence referred to earlier [2, 3]. The low pH, octahedral complex with carboxyl and amine bonding is obviously the 111 species but the square planar complex, present between pH = 5 and pH = 9 is the 110 species and not the 210 as previously suggested. Both the magnetic moment [3] and potentiometric measurements indicate that over this pH range the 110 complex increases its share of nickel(II) from a negligible amount to over 90%. The most likely donor sites to the metal ion in this planar complex are the sulphur atom and the peptide nitrogen between the glutaminate and cysteine residues. The loss of two protons which this requires would be partly compensated by the protonation of the terminal amine group on the glutamate moiety below

TABLE I. Formation Constants for the Proton- and Nickel(II) glutathionate Equilibria at 37 °C and in Sodium Chloride Background Electrolyte (150 mmol dm^{-3}).

pqr	$\log \beta_{pqr}$	n	S	R
101	9.289 ± 0.003	399		
102	17.669 ± 0.003			
103	21.105 ± 0.005			
104	23.139 ± 0.007			
110	7.38 ± 0.02	224	1.8×10^{-6}	0.004
111	13.85 ± 0.04			
110	7.37 ± 0.02	266	2.5×10^{-6}	0.005
111	13.91 ± 0.04			
210	10.44 ± 0.06			
211	19.34 ± 0.08			
110	7.36 ± 0.03	288	5.7×10^{-6}	0.008
111	13.91 ± 0.06			
210	10.42 ± 0.08			
211	19.41 ± 0.11			

$$\beta_{pqr} = \frac{[L_pM_qH_r]}{[L]^p[M]^q[H]^r}$$

n = number of data points
S = sum of squared residuals
R = MINIQUAD crystallographic factor

pH = 8.5. The only other possibility, which seems very unlikely, is to assume deprotonation of both peptide linkage nitrogens. whilst the sulphydryl and amine groups remain protonated.

The scheme shows how these conclusions can be extended to the modes of co-ordination for the 211 and 210 species at higher pH. The initial bonding of a second ligand molecule to form the 211 species again involves the amino acid groups of the glutaminate residue. This would be expected to destabilise the planar configuration of the nickel(II) bound between the sulphur and the peptide nitrogen and thus give rise to the second octahedral complex observed spectroscopically. Finally, the 210 species would be formed by a rearrangement of the coordination site to a symmetrical structure in which the sulphur atom and the peptide nitrogen from both ligands act as donors. It is reasonable to think that this would be accompanied by a change from octahedral to square planar configuration.

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