Thermodynamic Considerations in Co-ordination. Part XVIII.[1] Formation Constants for Cadmium(II)–Amino-acid Complexes as determined by Glass and Solid-state Cadmium-electrode Potentiometry

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Potentiometrically determined formation constants are reported for the cadmium(II)–asparaginate,–aspartate,–cysteinate,–glutaminate,–histidinate,–phenylalanate,–serinate, and –tryptophanate systems at 25 °C and I = 3·00M-(Na)ClO_4. Computer-simulated models of blood-plasma conditions have been used to examine (*a*) complexing competition between cadmium(II) and manganese(II)–,iron(II)–,cobalt(II)–,copper(II)–, and zinc(II)–amino-acid anion complexes, and (*b*) to assess selectivities of ethylenediaminetetra-acetic acid (H_4edta) for cadmium(II) and the *in vivo* essential amino-acids and metal ions listed. Criteria for producing a drug more specific than those currently in use are discussed.

When man started to refine zinc he simultaneously started to pollute the hydrosphere with another Group IIB metal, cadmium. Zinc ores can contain as much as 0·5% cadmium and since 1940 there has been a dramatic increase in the number of both industrial and general-population cases of cadmium poisoning.[2] Indeed, cadmium is one of the five most toxic metals in the environment to which industrialised civilisation has exposed itself. Zinc has a complex homeostatic mechanism which controls its concentration throughout the body; cadmium has none and so accumulations occur in kidneys, liver, and blood vessels. Furthermore, cadmium has a chemistry similar to that of zinc and so the normal processes of zinc metabolism can be blocked and distorted by cadmium. It is possible that *low* concentrations of cadmium may have a beneficial role *in vivo*. Human renal metallothionein contains a higher concentration of cadmium than does any other metalloprotein of its constituent metal, the total (zinc + cadmium) content being fixed.[3–7] The metabolism of cadmium depends on relative intakes of iron, copper, and zinc and, in turn, dietary cadmium can stimulate or antagonise the metabolism of these essential transition-series metals. Cadmium concentrations in blood and the organs it supplies can be reduced by ligand therapy, but, in order to obtain optimal dosage, formation constants for cadmium–therapeuticals and –biological ligands ought to be available for computer-model calculations, and the specificity of the administered drugs for cadmium compared to the essential metals must be known (again this is formation-constant dependent).

This paper reports glass and solid-state cadmium-electrode potentiometry of cadmium solutions (including an improved technique for calibrating these electrodes); formation constants for some representative blood-plasma ligands, asparaginate, aspartate, cysteinate, glutaminate, histidinate, phenylalanate, serinate, and tryptophanate; cadmium(II) complexing reactions; computer models of cadmium-polluted plasma before, and during, ligand therapy; and, finally, some suggestions for ligands biologically more cadmium specific than drugs currently in use.

EXPERIMENTAL

Amino-acids were as described in refs. 8—13. Perchloric acid and sodium perchlorate were as described in ref. 14. Water was purified as in ref. 13. Cadmium(II) perchlorate was prepared by dissolving cadmium oxide in perchloric acid (60%) and filtering. Analysis for cadmium(II) was by titration against ethylenediaminetetra-acetic acid, H_4edta (Xylenyl Orange as indicator). The mineral acid content of the stock solution was determined by Gran titrations.[15] Lanthanum(III) perchlorate was prepared by dissolving dilanthanum trioxide (American Potash Chemical Corporation) in perchloric acid (60%). Analysis for lanthanum(III) was by titration against H_4edta (Eriochrome Black T). Solutions of H_4edta were prepared from Fisons Analytical Reagent without further purification.

The glass and reference electrodes and potentiometric equipment were as described in ref. 9. The solid-state cadmium electrode was a Radiometer Ruzicka Selectrode F3003Cd.[16] All potentiometric experiments were carried out at 25·0 °C and I = 3·00M-(Na)ClO_4 using the approach described in ref. 9.

Data Analysis.—SCOGS and ZPLOT Treatment of glass-electrode e.m.f. values were as described in ref. 9, the ranges of ligand and cadmium concentrations being 2—120 and 1—21mM respectively. β Values emerging from the SCOGS treatment were introduced into the HALTAFALL [17] program to calculate theoretical $\log(B/b)_{B,h}$

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against log $A_{B,h}$ curves similar to those described in ref. 11 [A and B = total concentrations of ligand and metal ions, and b and h = free concentrations of metal and hydrogen ions]. The COMICS program [18] was modified to permit an IBM 360/44 CIL6011 plotter output of concentration or −log (concentration) against −log h.

Glass-electrode Calibration.—The electrodes were calibrated with reference to a saturated sodium chloride–calomel electrode, the e.m.f. E_g (in mV) at 25 °C being given by equation (1).

$$E_g = E_{g0} - 59{\cdot}155 \log h \quad (1)$$

Solid-state Cadmium-electrode Calibration.—The electrode was calibrated with reference to a saturated sodium chloride–calomel electrode, the e.m.f. E_{Cd} (in mV) at 25 °C being given by equation (2). Thus, measuring E_{Cd} for a

$$E_{Cd} = E_{Cd0} - 29{\cdot}577 \log b_{Cd^{2+}} \quad (2)$$

series of solutions of known $b_{Cd^{2+}}$ and plotting E_{Cd} against log $b_{Cd^{2+}}$ ought to produce a line of intercept E_{Cd0} at log $b_{Cd^{2+}}$ = 0 and gradient = 29·577 mV. In practice the method was reliable for $b_{Cd^{2+}} = 10^{-1}$—10^{-3}M but, exactly analogous to problems encountered with glass-electrode calibrations, less reliable for unbuffered $b_{Cd^{2+}}$ values in the range of concentrations experienced in the amino-acid–cadmium complexing studies. In order to avoid this problem standard $b_{Cd^{2+}}$ solutions were prepared using a buffered system involving lanthanum (III) ions and edta in place of a diluent.

The theory is as follows.[19] If the total concentrations of components are $B_{Cd^{2+}} < A_{edta} < B_{La^{3+}}$ (*ca.* 2 : 3 : 4) and if $\beta_{Cd(edta)^{2-}} > \beta_{La(edta)^-}$, then $[Cd(edta)^{2-}] \simeq B_{Cd^{2+}}$ and $A_{edta} \simeq B_{La^{3+}} - b_{La^{3+}} + B_{Cd^{2+}}$. The overall formation constant $\beta_{Cd(etda)^{2-}} = [Cd(edta)^{2-}]/[Cd^{2+}][edta]$ and $\beta_{La(edta)^-} = [La(edta)^-]/[La^{3+}][edta]$. Now $[La(edta)^-] \simeq A_{edta} - B_{Cd^{2+}}$ and so $\beta_{La(edta)^-} \simeq (A_{edta} - B_{Cd^{2+}})/(B_{La^{3+}} - A_{edta} + B_{Cd^{2+}})[edta]$. Thus $[edta] \simeq (A_{edta} - B_{Cd^{2+}})/(B_{La^{3+}} - A_{edta} + B_{Cd^{2+}})\beta_{La(edta)^-}$, *i.e.* the free-ligand concentration is pH independent. Furthermore $b_{Cd^{2+}} = [Cd(edta)^{2-}]/\beta_{Cd(edta)^{2-}}[edta] \simeq B_{Cd^{2+}}/\beta_{Cd(edta)^{2-}}[edta]$ which is also pH independent. This approach provides a pH-independent, buffered, procedure for producing standard reference $b_{Cd^{2+}}$ solutions down to 10^{-7}M. The assumptions involved are valid and hydrolysis of the metal ions negligible according to COMICS [18] computations of log b against log h graphs. Such computations showed, for example, that E_{Cd0} as measured from a b = 730μM solution does not vary by greater than 0·1 mV between pH 3·0 and 7·0.

RESULTS AND DISCUSSION

The anions of asparagine, glutamine, phenylalanine, serine, and tryptophan form mono-, bis-, and tris-complexes with cadmium(II). Formation curves were established for each system for a range of differing total metal (B) and total ligand concentrations (A). All systems had superimposable curves (*i.e.* protonated and hydroxo-complexes could be assumed to be absent) and, apart from the cadmium(III)–cysteinate system, there was no curve straddling or persistence of the 1 : 1 complex. This can be attributed to cadmium being six-co-ordinate, unlike lead which can be either four- or eight-co-ordinate.[8] The cadmium(II)–cysteinate system, however, precipitated even at very low concentrations [1mM-cadmium(II), 2mM-cysteine].

In an attempt to check the β values obtained from glass-electrode potentiometry we studied the asparaginate and serinate systems using a solid-state cadmium-ion-selective electrode. This yielded log$(B/b)_{B,h}$–log$A_{B,h}$ values which were then compared with curves obtained from glass-electrode-potentiometry β values used in the HALTAFALL program to simulate solid-state-electrode titration curves. In practice it was difficult to obtain reliable results as the calculations were very sensitive to slight variations in E_0. Figure 1 demonstrates that the solid-state and glass-electrode approaches to quantifying the extent of cadmium complex formation are in

Logarithms of formation constants for cadmium(II)–amino-acid anion complexes at 25 °C and I = 3·00M-(Na)ClO_4

	log β_{pqr} [a]				
Amino-acid	110	210	310	n [b]	Literature data (pqr, log β, I/M, t/°C)
asn	4·071 ± 0·012	7·581 ± 0·007	9·610 ± 0·018	71	210, 7·1, 0·005 ($CdSO_4$), 15,[c] 210 6·8, 0·01, 20;[d] 310, 8·60, 1·0, 30,[e] 210, 6·9; 310, 8·58, 1·0 (KNO_3), 30 [f]
asp	5·013 ± 0·005	9·120 ± 0·011		108	210, 8·8, 0·005 ($CdSO_4$), 15;[c] 110, 4·37; 210, 7·48, 0·1 (KCl), 30;[g] 110, 4·39, 0·1 (KCl), 25;[h] 310, 10·30, 1·0, 30;[e] 210, 8·89; 310, 10·31, 1·0 (KNO_3), 30 [f]
cys	12·875 ± 0·057	19·627 ± 0·136		42	210, 9·89 (phosphate buffer), 0·2, 25 [i]
gln	4·099 ± 0·023	7·664 ± 0·012	9·999 ± 0·030	61	210, 7·4, 0·005 ($CdSO_4$), 15 [c]
his	6·484 ± 0·021	11·105 ± 0·029		86	210, 11·10, 0·0025 ($CdSO_4$), 20°;[c] 110, 5·65; 210, 9·79, 0·01, 25;[j] 210, 10·20, 0·1 (KNO_3), 25;[k] 210, 11·10, 0·15 (KNO_3), 25 [l]
phe	4·363 ± 0·025	7·935 ± 0·099	11·090 ± 0·093	85	210, 7·2, 0·005 ($CdSO_4$), 20 [c]
ser	4·154 ± 0·025	7·863 ± 0·012	10·221 ± 0·033	62	210, 7·4, 0·005 ($CdSO_4$), 20 [c]
trp	4·482 ± 0·008	8·582 ± 0·014	12·028 ± 0·023	126	210, 7·0, 0·005 ($CdSO_4$), 20;[c] log K_2 = 8·1, 0·01, 20° [d]

asn = Asparagine, asp = aspartic acid, cys = cysteine, gln = glutamine, his = histidine, phe = phenylalanine, ser = serine, and trp = tryptophan.

[a] β_{pqr} Refers to the complexes $[(ligand)_p(metal\ ion)_q(proton)_r]$. [b] n = Number of experimental observations. [c] D. J. Perkins, *Biochem. J.*, 1953, **55**, 649. [d] A. Albert, *Biochem. J.*, 1950, **47**, 531. [e] G. N. Rao and R. S. Subrahmanya, *Current Sci.*, 1962, **31**, 55. [f] G. N. Rao and R. S. Subrahmanya, *Proc. Indian Acad. Sci.*, 1964, **60**, 165, 185. [g] S. Chaberek, jun., and A. E. Martell, *J. Amer. Chem. Soc.*, 1952, **74**, 602. [h] R. Thumb and A. E. Martell, *J. Phys. Chem.*, 1953, **57**, 690. [i] I. H. Suffet and W. C. Purdy, *J. Electroanalyt. Chem.*, 966, **11**, 302. [j] S. Vallasdas-Dubois, *Compt. rend.*, 1953, **237**, 1408. [k] A. C. Andrews and J. K. Romary, *J. Chem. Soc.*, 1964, 405. [l] N. C. Li and R. A. Manning, *J. Amer. Chem. Soc.*, 1955, **77**, 5225.

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reasonable agreement. However, it is felt that glass-electrode potentiometry is still the more reliable, and accurate, method of establishing log β values.

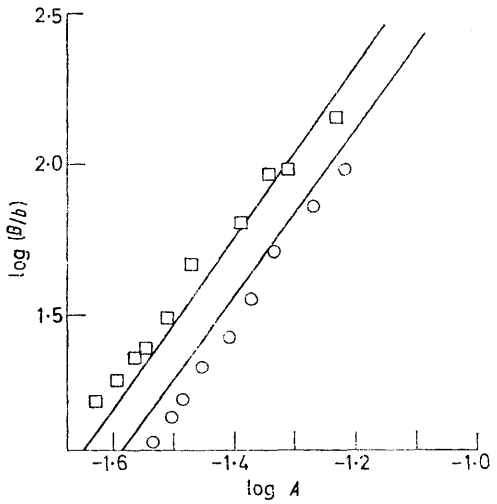

FIGURE 1 Examples of the relative positions of HALTAFALL simulated log (B/b) against log A curves and experimental solid-state cadmium(II) electrode measurements: (□), asparaginate; (○), serinate

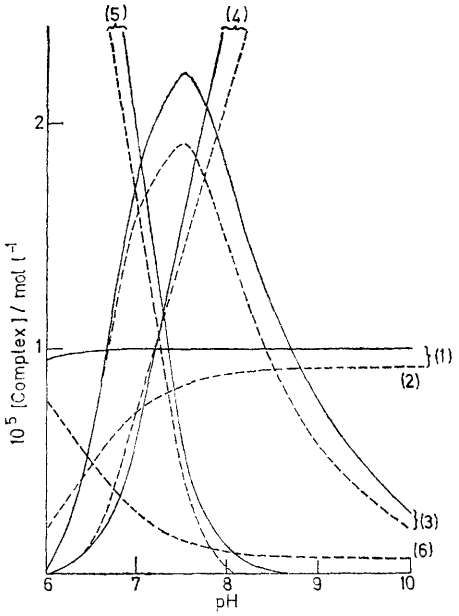

FIGURE 2 HALTAFALL model of zinc(II)- and cadmium(II)-cysteinate, -histidinate, and -ethylenediaminetetra-acetate complexes present for total $B_{Cd^{2+}} = 10$ and $A_{edta} = 0$ (——) and 10 μM (- - - -): (1), cadium(II)-cysteinate; (2) zinc(II)-ethylenediaminetetra-acetate; (3), zinc(II)-histidinate; (4) zinc(II)-cysteinate; (5), zinc(II); and (6), cadmium(II)-ethylenediaminetetra-acetate [Curve (2) is actually two dotted curves, (2) being superimposed on (1).]

Conclusions.—2,3-Dimercaptopropan-1-ol, nitrilotriacetic acid, and H_4edta (and its derivatives 2-hydroxyethylethylenediaminetriacetic acid and diethylenetriaminepenta-acetic acid) have all been used for treating cadmium poisoning. In general they all reduce the cadmium mortality initially but eventually increase the nephrotoxicity.[7] Our computer-model calculations have shown that these drugs are not specific for cadmium (Figure 2). The similarity between the atomic structures of cadmium and zinc means that cadmium metabolism is intimately connected with that of zinc. Thus, some of the cadmium toxicity observed may arise from cadmium replacing zinc in metalloenzymes such as metallothionein, and some of the drug toxicity may occur because the drugs sequester, and hence cause depletion of, essential manganese, iron, cobalt, copper, and zinc ions.

Previously [8] we have used computer models to suggest that lead *in vivo* complexes wholly at the expense of zinc present, but this is not true for cadmium as the pollutant; direct competition of cadmium for zinc is far less than that of lead and the essential metal complexes most likely to be depleted during cadmium poisoning are those of iron(II) and manganese(II). Further, the increase in concentration of zinc–amino-acid anion complexes observed for lead-poisoning models is less marked in those for cadmium poisoning. Nevertheless, when H_4edta is introduced into the system concentration patterns parallel the lead–D-penicillamine system in that zinc complexes with the drug at the expense of histidinate and cysteinate ligands.

The next phase of this project will involve a potentiometric search for ligands more specific for cadmium ions. The principles involved will encompass as many of the following points as is practicable. (*i*) The specificity is to be judged from $\log \beta_{Cd} - \log \beta_{Zn}$. (*ii*) Metalloenzymes involving zinc have predominantly amine–zinc bonds (and some sulphur–zinc). From metallothionein (which is 11% sulphur) cadmium appears to prefer sulphur to nitrogen (*i.e.* Cd^{2+} is softer in the hard–soft acid–base sense). Thus, the new ligand ought to involve sulphur, or even selenium, donor atoms.[6] (*iii*) Zinc(II) ions prefer to be bonded tetrahedrally whereas cadmium(II) is usually octahedral or planar *in vivo*.[20] This stereospecificity can most easily be induced through a polydentate ligand. (*iv*) If this ligand is of the cryptate variety the central cavity must clearly have a size ideal for cadmium(II) ions. Although this is a possible means of excluding zinc(II) ions we might note that cadmium(II) has an ionic radius similar to that of calcium(II) (Cd^{2+}, 103; Zn^{2+}, 69; Ca^{2+}, 106 pm).[21] However, calcium ions can be excluded by the careful selection of soft donor atoms as in (*ii*), calcium preferring hard donors such as oxygen. (*v*) The aim of ligand therapy is to excrete a cadmium complex, but we ought also to consider the quantity of ligand drug that does not become successfully complexed to Cd^{2+}; it ought to be non-toxic, excretable, or composed of components that can be reconstituted into a naturally occurring compound. Peptides of

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L-amino-acids would appear to satisfy all these requirements. In the first instance, one ought to avoid aromatic amino-acids (since metallothionein has none) and incline towards many SH-containing components. (*vi*) Computer models of blood plasma containing the chosen ligand will indicate the extent of ' topping-up ' with zinc, calcium, *etc.*, required during ligand therapy.

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