INTERACTIONS BETWEEN CADMIUM ION AND VARIOUS HYDROGEN ION BUFFERS

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The present study originated from efforts to determine the free cadmium ion activity in experiments in which the inhibitory effect of cadmium on Na/K ATPase activity was being investigated. In the course of this work, it was discovered that several commonly-used physiological hydrogen ion buffers had the effect of reducing cadmium activity by up to several orders of magnitude when present in concentrations employed in the ATPase experiments. Results suggested that the buffers were binding cadmium ion, and apparent binding constants for several of the buffers were determined. In addition, it was revealed that the apparent binding of cadmium ion was dependent on the pH in a manner which suggested that cadmium competed with hydrogen ion for a binding site on the buffer molecules.

Cadmium solutions were prepared by serial dilution of a concentrated (1 or 0.1 M) stock solution of cadmium nitrate using deionized water. Buffers, when present, were at a concentration of 50mM, and the pH was 7.6 unless otherwise noted. Cadmium activity was detected with a solid-state ion-selective electrode (Orion # 94-48). Electrode potentials were measured with a millivoltmeter (Orion #701A) and are reported as the potential of the cadmium electrode relative to a Ag/AgCl reference electrode (Orion # 90-01). At least one hour was allowed for equilibration of solution temperatures prior to measurement, and all measurements were made while solutions were vigorously stirred.

Figure 1 shows electrode calibration curves generated in the presence (open circles) and absence (closed circles) of imidazole buffer. Here, E is the electrode potential, and $[Cd]_o$ is the total (free + bound) cadmium concentration (molar). Theoretical slope (dotted line) was calculated according to the Nernst Equation:

$$E = E^o + \frac{2.3RT}{ZF}\log[Cd] \qquad (1$$

where E^o is the standard electrode potential, (2.3RT/ZF) = 28mV at room temperature, and [Cd] is the cadmium activity. Comparison of the curves reveals two things: 1) measured potentials were more negative in the presence of imidazole, corresponding to a lower cadmium activity than in the control (no buffer); 2) although both curves showed expected deviations from linearity at low cadmium concentrations, the imidazole curve showed deviations at higher concentrations. Figure 2 shows that the latter deviations are consistent with binding of cadmium by the buffer. For this hypothetical curve, cadmium activity (circles) was calculated assuming a 1:1 stoichiometry of binding according to:

$$B + Cd \rightleftharpoons B{\cdot}Cd \qquad (2$$

where B is the buffer, and B-Cd is the cadmium-buffer complex. A stability constant for the complex was defined according to:

$$K = [B{\cdot}Cd] / ([B]_o - [B{\cdot}Cd])\,[Cd] \qquad (3$$

where $[B]_o$ is the total buffer concentration. (In Figure 2, K was arbitrarily

taken to be $1000M^{-1}$.) The term in parentheses represents the total concentration of free buffer (protonated and unprotonated). Hence, no assumptions were made regarding the preference of cadmium for the protonated vs the unprotonated form.

Figure 1

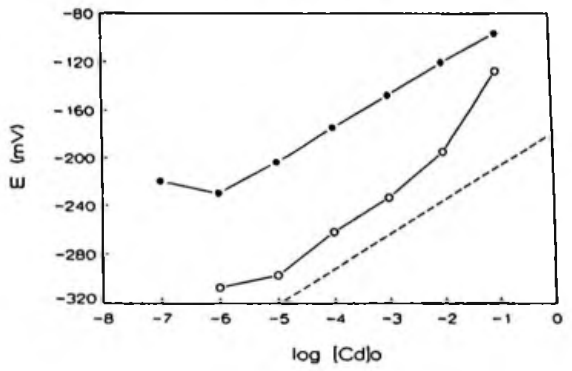
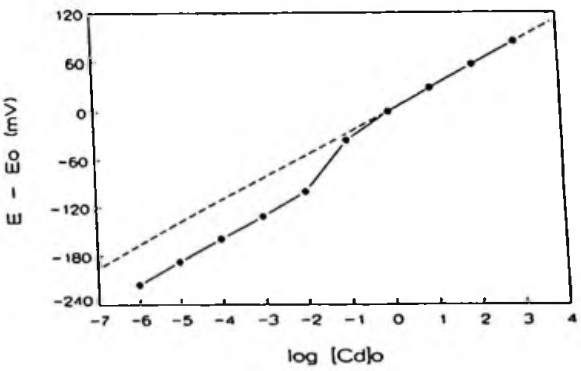


Figure 2



Because the results suggested that imidazole bound cadmium, sets of curves similar to Figure 1 were generated using other buffers with pK_a values in the physiological range. Total cadmium concentration was in the range 10^{-7} to 10^{-3}M, and 120mM salt (100mM $NaNO_3$ + 20mM KNO_3) was added to insure relative constancy of the ionic strength. (Variation of ionic strength from 0.1 to 0.5M was shown to cause not more than a 3mV negative shift over the linear portion of the curve in the absence of buffer.) Results showed that all buffers induced negative shifts in the calibration curve to varying degrees. Some buffers (histidine and phosphate) induced shifts that were so large that even the highest points gave values of E which lay below the linear portion of the control (no buffer) curve, so that the activity of free Cd could not be accurately estimated. The remainder yielded points which lay within the linear region, thus allowing estimation of apparent stability constants (defined by Equation 1). K was calculated for these buffers according to:

$$K = \frac{10^{-\Delta E/28} - 1}{[B]_o + [Cd]_o\{10^{\Delta E/28}-1\}} \qquad (4$$

where ΔE(mV) is the vertical shift induced by the buffer. Calculated K values are presented in Table 1. From these K values, the percent of total Cd which was bound was calculated for each of the buffers assuming $[B]_o$ = 50mM and $[Cd]_o$ << $[B]_o$. This is also shown in Table 1.

Due to the cationic nature of cadmium, it seemed reasonable to expect that the metal would bind preferentially to the unprotonated form of the buffer. In keeping with this possibility, it was noted that acidification of the medium (by adding a drop of concentrated HNO_3 to a 10ml volume) caused electrode potentials to shift upward. (There was a negligible effect in the absence of buffer). In the limiting case (no Cd binding to the protonated form), Cd and hydrogen ion would compete according to:

$$BH \underset{K_a}{\rightleftharpoons} B + H \; ; \; B + Cd \underset{K'}{\rightleftharpoons} B{\cdot}Cd \qquad (5$$

where K_a and K' are the equilibrium constants of their respective reactions. Figure 3 shows calibration curves for imidazole at pH 5.6 (open circles) and pH 7.6 (closed circles) and in the absence of buffer (triangles). In the presence

of buffer, decreasing pH caused the curve to shift upwards to higher Cd activities, as would be predicted by Equation 5. Using these equations as a model, apparent stability constants, K', were estimated according to:

$$K' = K\{1 + 10^{pKa - pH}\} \qquad (6$$

These are presented in Table 1.

Another prediction of Equation 5 is that Cd should shift titration curves of buffers in the direction of lower pH. To test this, titration curves were determined for buffers (25mM) in the presence and absence of 50mM cadmium nitrate. Titrants were added in concentrated form, so that the total change in volume did not exceed 2%. Solutions were stirred continuously, and pH was measured with a standard Corning glass electrode. Figure 4 shows titration curves for imidazole in the presence (open circles) and absence (closed circles) of Cd and for Cd alone (triangles). Similar results were obtained with TRIS, HEPES and MOPS buffers. In principle, it should be possible to use shifts in titration curves to estimate stability constants for metal-buffer complexes (see Good, N.E. et al, Biochem. 5:467-477, 1966), but this was not done in the present study, since the formation of Cd-hydroxides placed a practical upper limit of around 7.8 (dotted line, Figure 4) on the range of pH values. (Calculations using known values of pKa of hydrolysis showed that less that 1% of Cd was present as hydroxide complex in experiments conducted at pH 7.6, however). Nevertheless, Cd appeared to cause roughly parallel shifts for all buffer curves, thus allowing us to rank them in order of increasing magnitude: HEPES=MOPS<TRIS<imidazole.

Figure 3

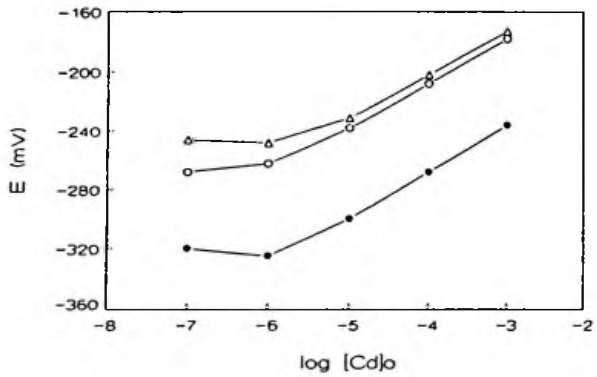


Figure 4

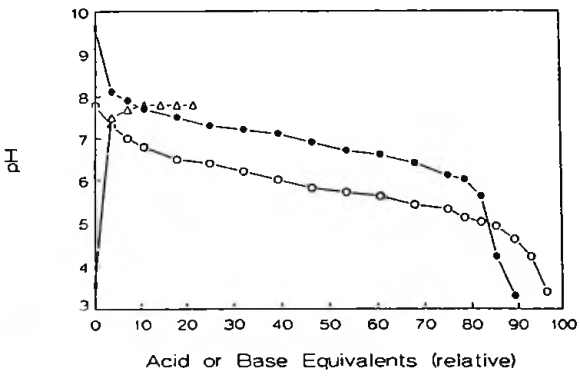


Table 1

Buffer (pH = 7.6)	K (M^{-1})	K'(M^{-1})	% Cd bound*
Imidazole	2.8×10^3	3.4×10^3	99.3
TRIS	2.2×10^1	9.1×10^1	52.4
HEPES	7.1×10^0	1.4×10^1	26.2
MOPS	4.9×10^0	6.9×10^0	19.7

* total buffer concentration $[B]_o$ = 50mM and $[B]_o \gg [Cd]_o$

Although the results of the present study suggest that Cd and protons effectively compete for buffer molecules, we have no evidence that the binding of one of these cations necessarily excludes binding of the other. Protonation of the buffer may affect charge distribution at sites somewhat removed from the proton binding site and may thus alter the molecule's affinity for Cd. Regardless of the molecular details of Cd-buffer interactions, the results show that of all buffers tested, HEPES and MOPS exhibit the least apparent tendency to bind Cd and should be the buffers of choice for use in experiments where minimal Cd binding is desired.

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