

2.2: Acid-base Equilibria

It is convenient to discuss the cobalt-substituted carbonic anhydrase enzyme, since its electronic spectra are markedly pH-dependent and easy to measure (Figures 2.7 and 2.8).^{56,57} The spectra are well-shaped, and a sharp absorption at 640 nm is present at high pH and absent at low pH. Whereas CoHCA I is almost entirely in the low-pH form at pH 5.7, this is not true for the CoBCA II isoenzyme. The acid-base equilibrium for Co-substituted carbonic anhydrase (deprotonation of the metal-coordinated water) involves three species:

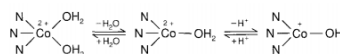


Figure 2.8).

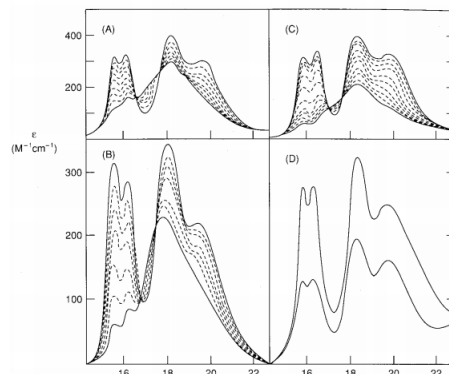


Figure 2.7 - pH-variation of the electronic spectra of cobalt(II)-substituted BCA II (A), HCA II (B), HCA I (C), and BCA III (D). The pH values, in order of increasing ϵ_{15-6} , are (A) 5.8, 6.0, 6.3, 6.7, 7.3, 7.7, 7.9, 8.2, 8.8; (B) 6.1, 6.6, 7.1, 7.8, 8.3, 8.6, 9.5; (C) 5.3, 6.1, 6.6, 7.0, 7.3, 7.5, 7.9, 8.4, 8.6, 9.1, 9.6.⁵⁶

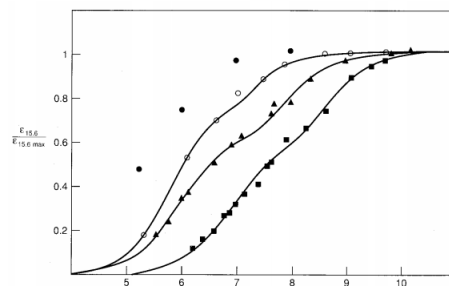


Figure 2.8 - pH dependence of ϵ_{15-6} for cobalt(II)-substituted BCA III (●), HCA II (○), BCA II (▲), and HCA I (■) isoenzymes. The high pH limit value of ϵ_{15-6} is normalized to 1 for each isoenzyme.⁵⁷

* An isosbestic point is a value of frequency where the two species in an $A \rightleftharpoons B$ equilibrium have the same absorption. As a consequence, all mixtures of A and B also show the same absorption at that frequency, and all the spectra along, e.g., a pH titration from A to B, plotted one on top of the other, cross at the isosbestic point. The presence of isosbestic points thus indicates the presence of only two species in equilibrium.

These kinds of isoenzymes contain at least another histidine in the cavity, which represents another acidic group, with a pK_a of about 6.5 in its free state. The interaction between such an acidic group and metal-coordinated water, for example, via a network of hydrogen bonds, provides a physical picture that can account for the observed experimental data.⁴⁹ Two apparent acid dissociation constants K_a can be obtained from the fitting of the curves of Figure 2.8. They are called apparent, because they do not represent actual acid dissociations at the microscopic level. When there are two acidic groups interacting with each other, the system must be described in terms of four constants, also called microconstants, because the dissociation of each of the two groups is described by two different pK_a 's, depending on the ionization state of the other group (Figure 2.9); so the two apparent constants can be expressed in terms of four microconstants describing two interacting acidic groups.

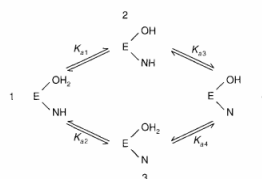


Figure 2.9 - General scheme for two coupled acid-base equilibria applied to carbonic anhydrase. The two acid-base groups are the metal-coordinated water molecule and a histidine residue present in the active-site cavity.⁵⁸

It is again a general feature of these systems that the four microconstants can be obtained only by making some assumptions. In one analysis the molar absorbances of species (1) and (3), and of species (2) and (4), were assumed to be equal.⁵⁸ In other words, it is assumed that the changes in the electronic spectra of cobalt(II) (Figure 2.7) are due entirely to the ionization of the coordinated water, not at all to the ionization state of the other group. This assumption accounts for the observation of approximate isosbestic points, even though there is an equilibrium between more than two species. With this assumption the four microconstants could be obtained (Table 2.5).

Table 2.5 - Values of microconstants associated with acid-base equilibria^a in cobalt(II)-substituted carbonic anhydrases.⁵⁸

a) As defined in Figure 2.9

	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
CoHCA I	7.14	7.21	8.45	8.38
CoHCA II	5.95	5.62	6.62	6.95
CoBCA II	6.12	6.28	7.75	7.59

Recall that the activity and spectroscopic profiles follow one another (see Figure 2.2 and Section IV.B). Furthermore, similar microconstant values had been obtained on ZnHCA II by analyzing the pH dependence of the maximum velocity of the hydration reaction, V_{max} , assuming that the two hydroxo-containing species had the same activity.⁴⁹ The present analysis implies that species (2) and (3) of Figure 2.9 are distinguishable, although their interconversion may be fast.

Metal coordination lowers the pK_a of coordinated water. Factors affecting the acidity of the coordinated water are many, and their effects are probably overlapping, making the analysis quite complex (see also Section III.A). Nonetheless, the following factors probably contribute to the lowering of the pK_a :

1. the charge of the chromophore, which in this case is 2+, although it may be somewhat lowered by the H-bonding between a coordinated histidine and a negative glutamate residue;
2. the coordination number (which is four), since a higher value leads to a larger electron density on the metal ion ligands;
3. the presence of other acidic groups with which the coordinated water interacts;
4. the presence of positively charged residues inside the metal binding cavity that favors the removal of a proton from the cavity.

This last factor is presumably operating for CA III, which contains several arginine residues in the cavity; the same factor may also induce changes in the microscopic properties of the solvent inside the active cavity. These considerations account for the observation that most model complexes have a significantly higher pK_a value than the protein itself.

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