Contribution from the Department of Chemistry and the Materials and Molecular Research Division, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

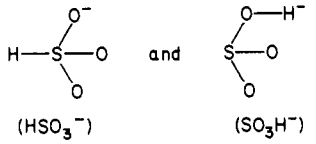
Equilibrium Quotient for the Isomerization of Bisulfite Ion from HSO_3^- to SO_3H^-

David A. Horner† and Robert E. Connick*

Received October 7, 1985

Separate peaks in the oxygen-17 NMR spectra of sodium bisulfite solutions provide direct evidence for the existence in solution of two isomers of bisulfite ion: one with the proton bonded to the sulfur atom (HSO_3^-) and the other with the proton bonded to an oxygen atom (SO_3H^-). The equilibrium quotient for the isomerization reaction was determined from measurements of peak areas and was found to be 4.9 ± 0.1 at 298 K in solutions of ionic strength 1.0 *m*. The more abundant isomer exchanges oxygen atoms with water more rapidly than does the other and, on this basis, is identified tentatively as SO_3H^-.

Two plausible structures for the bisulfite ion are



(HSO_3^-) (SO_3H^-)

The appearance of the H–S stretch in Raman spectra shows that the HSO_3^- isomer exists in both aqueous solutions and solid salts,[1-3] while positive but less conclusive evidence from Raman spectra[4,5] supports the existence of SO_3H^- in aqueous solution. Ab initio molecular orbital calculations[6,7] predict that the gaseous ions of the two isomers have comparable stability.

The species HSO_3^- is analogous to the isoelectronic HPO_3^{2-}, where the proton is held so tightly it cannot be removed in aqueous solution. This difference in acidity can be rationalized in terms of the formal charge on the central atom, in turn leading to the prediction that isoelectronic chloric acid exists only in the form ClO_3H, as is generally assumed.

We have found that under certain experimental conditions the two isomers of bisulfite ion produce separate peaks in the oxygen-17 NMR spectra of bisulfite solutions, providing direct evidence of the existence of both isomers. The equilibrium quotient for the isomerization reaction was determined through measurements of the areas of the spectral peaks.

Experimental Section

Analytical reagent grade sodium metabisulfite, sodium chloride, hydrochloric acid, and sodium hydroxide from Mallinckrodt Chemical Co. were used as supplied. Aqueous sodium bisulfite solutions were prepared in a nitrogen-atmosphere glovebag by dissolving weighed amounts of sodium metabisulfite and sodium chloride in 3.00 mL of deoxygenated water enriched to about 50 mol % ^{17}O and 40 mol % ^{18}O but having natural-abundance hydrogen and deuterium. The water was obtained from the Mound Facility of Monsanto Research Corp. and was purified by distillation. Enough sodium chloride was added to each solution to give a calculated ionic strength of 1.0 *m* at 298 K. The pH of each solution was measured with an Orion Research Model 601A digital pH meter and a Beckman Altex Model 531164 combination pH electrode, which was calibrated with standard buffers. Adjustments of the pH of each bisulfite solution were made by addition of small amounts of 6 M HCl or 6 M NaOH. The solutions were placed in 10-mm NMR sample tubes that were then sealed with pressure caps and Parafilm.

Oxygen-17 nuclear magnetic resonance spectra were acquired on a pulsed NMR spectrometer consisting in part of a Cryomagnet Systems 4.7-T superconducting magnet (corresponding to an ^{17}O resonance frequency of 27.4 MHz), a home-built receiver and transmitter, a Nicolet Instrument Corp. 293A′ programmable pulser, a Nicolet Instrument Corp. 1180 Data System, and a broad-band, multinuclear, 10-mm probe. All experiments were run without a field-frequency lock on samples spinning at roughly 20 rps. The sample temperature was controlled by passing cooled or room-temperature nitrogen gas through a heater and then past the sample. A relay device connected to the heater and a thermocouple located directly below the sample provided temperature regulation to ±0.2 K. The actual temperature of the sample was determined by measuring the chemical shift difference between the proton NMR peaks of an ethylene glycol sample[8] contained in a sealed capillary placed within the 10-mm sample tube. The published calibration curve has an estimated uncertainty of ±0.9 K.

Each spectrum was obtained by Fourier-transforming the sum of between 5000 and 10000 free decays produced with the standard one-pulse method or with a two-pulse sequence (θ_x–τ–θ_{-x}–acquire; $\theta \leq 45°$), which suppressed the strong solvent signal.[9] In the two-pulse sequence the frequency of the pulses is set equal to the Larmor frequency of the nuclei in the solvent site, and the time τ is set equal to half the inverse of the difference in frequency between the solute and solvent peaks, so that at the end of time τ the solute and solvent magnetizations are 180° out of phase with each other. The second pulse returns the solvent magnetization to the z axis of the rotating frame of reference but tilts the solute magnetization further toward the xy plane.

To eliminate rolling base lines caused by pulse breakthrough, a delay of approxmately 200 μs was inserted between the end of the pulse and the beginning of data acquisition. In the one-pulse experiments the exact duration of this delay was set equal to the inverse of the frequency difference between the water and one of the bisulfite peaks[10] to minimize or eliminate the need for first-order phase correction.

All chemical shifts were measured relative to the water peak in the same spectrum; downfield shifts were assigned positive values.

Calculations of the concentrations of the various S(IV) species in the solutions were carried out by using values of the dissociation equilibrium quotient of water and the two ionization quotients of sulfurous acid: pQ_w = 13.79, pQ_{a1} = 1.37, and pQ_{a2} = 6.34,[11] which are appropriate for conditions of 1.0 M, 1.0 *m*, and 1.0 *m* ionic strength, respectively, and 298 K. The value of the equilibrium quotient for the dimerization of bisulfite ion to form $S_2O_5^{2-}$ was estimated to be 0.082 from the data of Connick, Tam, and von Deuster[5] by first obtaining the equilibrium quotient at $[Na^+]$ = 5.0 M and a particular concentration of S(IV) by interpolation of the data in their Table IV and by then extrapolating the result to an ionic strength of 1.0 M by assuming that the slope of their plot of log Q_d vs. $\mu^{1/2}$ is independent of the S(IV) concentration and that the ionic strengths of their $[Na^+]$ = 5.0 M solutions were 5.0 M. The same value was used for all calculations since it changed only trivially over the range of S(IV) concentrations used, and the very small temperature dependence[5] was ignored. The ionization equilibrium quotients listed above are given in units of molarity; they were assumed to have the same values in units of molality. Hydrogen ion concentrations were calculated from the pH meter reading, assumed to equal $-\log_{10}(a_{H^+})$, and an activity coefficient of 0.754, which is the mean activity coefficient of 0.01 M HCl in 1 m NaCl.[12] This activity coefficient was considered to be a reasonable substitute for the unknown activity coefficient in the bisulfite solutions. (The pH meter reading will be referred to by the term "pH".) Concentrations at temperatures other than 298 K were calculated

†Current address: Department of Chemistry, North Central College, Naperville, IL 60566.

(1) Simon, A. *Angew. Chem.* **1947**, *59*, 247.
(2) Simon, A.; Waldmann, K. *Z. Anorg. Allg. Chem.* **1955**, *281*, 113.
(3) Simon, A.; Schmidt, W. *Z. Elektrochem.* **1960**, *64*, 737.
(4) Simon, A.; Kriegsmann, H. *Chem. Ber.* **1956**, *89*, 2442; and earlier papers by Simon and co-workers cited therein.
(5) Connick, R. E.; Tam, T. M.; von Deuster, E. *Inorg. Chem.* **1982**, *21*, 103.
(6) Baird, N. C.; Taylor, K. F. *J. Comput. Chem.* **1981**, *2*, 225.
(7) Strömberg, A.; Gropen, O.; Wahlgren, U.; Lindqvist, O. *Inorg. Chem.* **1983**, *22*, 1129.
(8) Kaplan, M. L.; Bovey, F. A.; Cheng, H. N. *Anal. Chem.* **1975**, *47*, 1703.
(9) Horner, D. A. Ph.D. Dissertation, University of California, Berkeley, CA, 1984.
(10) Canet, D.; Goulon-Ginet, C.; Marchal, J. P. *J. Magn. Reson.* **1976**, *22*, 537.
(11) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1976; Vol. 4.
(12) Harned, H. S.; Owen, B. B.; *The Physical Chemistry of Electrolyte Solutions*; Reinhold: New York, 1943; p 575.

0020-1669/86/1325-2414$01.50/0

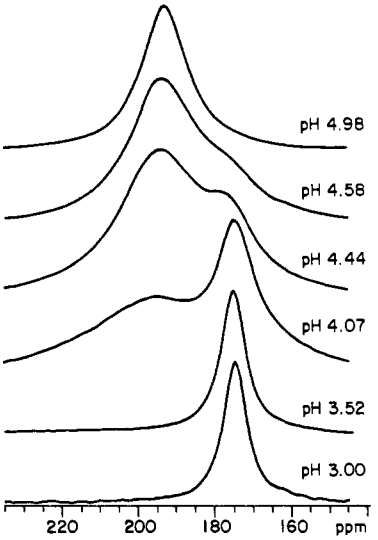


Figure 1. Traces of portions of the ^{17}O NMR spectra of sodium bisulfite solutions of various acidities, showing how the number of peaks in the spectra as well as the widths and chemical shifts of the peaks are dependent upon the pH of the solution. Every solution had a S(IV) concentration of 0.20 *m* and an ionic strength of 1.0 *m*. The temperature of each solution was between 277 and 283 K. The room-temperature pH meter readings are indicated. The ^{17}O spectra were recorded at a radio frequency of 27.377 MHz by using the two-pulse sequence described in the Experimental Section. The chemical shift scale is one on which the water peak in the one-pulse spectrum of each solution has a chemical shift of zero. Downfield shifts are positive. The vertical expansion is not the same for different traces.

after first correcting the values of Q_w, Q_{a1}, and Q_{a2} for changes in temperature by using the enthalpies of reaction 13.55, −4, and −3 kcal/mol,[11] respectively. (These reaction enthalpies are for solutions of ionic strength 0.5, 0, and 0, respectively; the values at unit ionic strength were not available.)

The traditional definition of molality was thought to be unsatisfactory for this work because it yields different concentrations of S(IV) for two solutions prepared in exactly the same manner save for the use of water having different mole fractions of the oxygen isotopes. It was decided to employ a unit of concentration that would yield equal S(IV) concentrations for solutions prepared by using an equal number of moles of S(IV) and equal volumes of water and would correspond to the traditional definition of molality if the water used were of normal isotopic composition. The unit chosen, moles of S(IV)/55.5 mol of H_2O, is called "molality" and is indicated by the symbol *m* throughout this paper. Equilibrium quotients based on this definition will correspond exactly to those for normal water except for the small chemical isotope effect of replacing ^{16}O by ^{17}O and ^{18}O.

Results and Discussion

In the pH range 3–5 the ^{17}O NMR spectra of 0.2 *m* sodium bisulfite solutions contain two or three peaks: a large water peak (greatly diminished in the two-pulse spectra) and one or two small peaks, presumably due to oxygen in S(IV) solute species, located between 170 and 200 ppm downfield from the water. Variations in acidity (as well as temperature and S(IV) concentration) affected the widths and chemical shifts of the peaks as well as the number of resonances observed (Figure 1). Below pH 3.6 only one peak, located at about 175 ppm, appeared in addition to the water peak. As the pH was increased above 3.6, a second solute peak appeared at about 195 ppm, but at pH 5 only a single solute resonance, located at 193 ppm, was observed.

The areas of the S(IV) peaks were measured. Rough measurements of the relative areas of the 175 and 195 ppm peaks were made by comparing simulated spectra of two overlapping Lorentzian lines to those two-pulse spectra in which two S(IV) peaks appeared. Although exact fits were not obtained due to the presence of chemical exchange and phase differences between the two peaks, it was possible to determine that the ratio of the area of the 195 ppm peak to that of the 175 ppm peak was between 5 and 10. Precise measurements of the areas of the peaks at 193 and 175 ppm, at high and low pH, respectively, were obtained from the one-pulse spectra containing only one S(IV) resonance, by using the water peak in the spectrum as an internal standard. The area of each peak was taken to be the product of its height and half-width. A Lorentzian curve-fitting computer program was used to obtain both the height and half-width of the water peak but did not give precise results for the line width and height of the small solute peak because the shape of that portion of the spectrum consisting of the superimposition of the solute peak and a wing of the water peak was very sensitive to slight phase errors. Therefore, the width of the S(IV) peak was obtained by fitting a Lorentzian curve to the corresponding peak in the two-pulse spectrum of the same solution at the same temperature. The height of the S(IV) peak in the one-pulse spectrum was then determined in the following manner: the wing of the water peak was approximated by a nonhorizontal straight line in the region of the solute peak. From T_2 of the two-pulse spectrum was determined the segment of this line whose end points were at the frequencies $\omega_0 + 1/T_2$ and $\omega_0 - 1/T_2$. (ω_0 is the location of the peak maximum, and $1/T_2$ is the half-width at half-height.) This line segment was then positioned so that its end points coincided with the spectral trace. The height of the peak was taken to be twice the vertical distance from the midpoint of the line segment to the trace of the peak.

Table I. Variation of S(IV) Peak Area with S(IV) Concentration and Acidity in ^{17}O NMR Spectra of Sodium Bisulfite Solutions in Which Only One S(IV) Peak Appears[a]

[S(IV)],[b] *m*	pH meter reading[c]	S(IV) peak chem shift, ppm	{(S(IV) peak area)/(H_2O peak area)} × 55.5/[S(IV)][d]
0.201	4.98	193	3.24 ± 0.07
0.449	4.97	191–193	3.05 ± 0.04
0.101	3.01	175–178	0.50 ± 0.08
0.199	3.00	175–178	0.42 ± 0.06
0.456	3.02	173–177	0.42 ± 0.06

[a] The ionic strength of each solution was 1.0 *m*. [b] S(IV) was added as $Na_2S_2O_5$. [c] Measured at room temperature. [d] Averages of values obtained from spectra recorded between 273 K and 303 K, including a correction for the signal decay that occurred during the preacquisition delay time (see Experimental Section).

The peak areas were corrected for the effect of signal decay that occurred during the finite delay time between the end of the pulse and the beginning of data acquisition by multiplying the area of each peak by $\exp(\tau/T_2)$, where τ is the delay time.

Table I shows the results of the determination of peak areas. The area of the peak at 193 ppm is so large that it can be accounted for only by assigning it to the bisulfite ion, which was by far the major solute species in the solutions studied. According to the calculations of the concentrations of the various S(IV) species, over 90% of the S(IV) was present in the form of bisulfite ion, while SO_2, $S_2O_5^{2-}$, and SO_3^{2-} accounted for no more than 3%, 7%, and 3%, respectively. Given this information and the fact that the chemical shifts of SO_2 and SO_3^{2-} are 520 and 210 ppm, respectively,[13,14] the peak at 175 ppm cannot be assigned to either SO_2 or SO_3^{2-}. Because its area is proportioanl to [S(IV)] and not $[S(IV)]^2$, the 175 ppm peak also cannot be assigned to $S_2O_5^{2-}$.

On the basis of the measured areas of the S(IV) peaks, we conclude that the peak at 175 ppm must be assigned to one of the isomers of bisulfite ion and the peak at 195 ppm to the other. Oxygen exchange occurs between the two isomers at a rate that increases with increasing pH, resulting in the coalescence of the two bisulfite resonances at pH 5 to give a single resonance located at 193 ppm. This interpretation of the spectrum is consistent with the results of a study of the kinetics of the system,[15] which also shows that the 195 ppm isomer exchanges oxygen with water much more rapidly than does the 175 ppm isomer. The rate of oxygen

(13) Jackson, J. A.; Taube, H. *J. Phys. Chem.* **1965**, *69*, 1844.
(14) We observed an ^{17}O chemical shift of 210 ppm for SO_3^{2-} in 0.3 *m* Na_2SO_3.
(15) Connick, R. E.; Horner, D. A., to be submitted for publication.

Table II. Values of Parameters Used in the Calculation of Q_2, the Equilibrium Quotient for the Reaction $HSO_3^- = SO_3H^-$ (Ionic Strength 1.0 *m*)

[S(IV)], *m*	pH meter reading[a]	S(IV) peak chem shift, ppm	[SHO_3^- oxygens][b]/ [total S(IV) oxygens]	*T*, °C	[SHO_3^-],[b] *m*	{(S(IV) peak area)/ (H_2O peak area)} × 55.5[c]	Q_2[d]
0.201	4.98	193	0.945	2–29	0.189	0.65 ± 0.01[e]	
0.199	3.00	175–178		3.7	0.188	0.0719	7.52
0.199	3.00	175–178		10.6	0.188	0.0830	6.37
0.199	3.00	175–178		15.2	0.188	0.0946	5.46
0.449	4.97	191–193	0.919	1–29	0.408	1.37 ± 0.02[e]	
0.456	3.02	173–177		1.1	0.416	0.155	7.28
0.456	3.02	173–177		7.7	0.416	0.163	6.86
0.456	3.02	173–177		13.9	0.416	0.191	5.69
0.456	3.02	173–177		21.4	0.415	0.211	5.06
0.456	3.02	173–177		21.9	0.415	0.211	5.06
0.456	3.02	173–177		29.3	0.415	0.217	4.89

[a] Measured at room temperature. [b] Calculated by using literature values of equilibrium quotients, as explained in the text. [c] Includes a correction for the effect of signal decay that occurred during the preacquisition delay time (see Experimental Section). [d] See text for method used to calculate Q_2. [e] Averages of values obtained from spectra recorded in the listed temperature range. The area of the 193 ppm peak should not have a significant temperature dependence.

exchange between water and the 195 ppm isomer is relatively slow at pH 5 and increases with increasing acidity, so that below pH 3.5 the 195 ppm resonance is too broad to observe.

The assignment of each of the two bisulfite peaks to a particular isomer was made by comparing the rates of oxygen exchange between water and each of the two bisulfite species. Exchange rates were obtained by studying the broadening of the 175 ppm resonance and the water peak;[15] it was found that the pseudo-first-order rate constant for oxygen exchange from the 195 ppm site to water is at least 500 times as large as the pseudo-first-order rate constant for the exchange from the 175 ppm site to water. The rate law for the exchange from the 195 ppm site to water was found to be first order in both hydrogen ion and bisulfite ion. The SO_3H^- isomer offers the simplest mechanism for this process:

$$SO_3H^- + H^+ = SO_2 + H_2O \qquad (1)$$

in which the incoming hydrogen ion need only attack the oxygen on which the bisulfite hydrogen is located. The corresponding mechanism involving HSO_3^- is more complicated because it requires in addition the migration of a hydrogen from the sulfur atom to an oxygen atom and, therefore, is expected to occur more slowly.[16] On this basis the 195 ppm resonance is assigned to SO_3H^-.

The equilibrium quotient for the isomerization reaction (2) was determined by using the areas of the 175 ppm peak in the spectra of the pH 3 solutions and the coalesced peak at 193 ppm in the spectra of the pH 5 solutions. If the only S(IV) species present

$$HSO_3^- = SO_3H^- \qquad Q_2 = [SO_3H^-]/[HSO_3^-] \qquad (2)$$

in the solutions were HSO_3^- and SO_3H^-, then the quantity $\{[HSO_3^-] + [SO_3H^-]\}/[HSO_3^-]$ would be equal to the ratio of the area of the 193 ppm peak at pH 5 to the area of the 175 ppm peak at pH 3. However, between 3% and 10% of the S(IV) in each of the solutions was present in the form of SO_3^{2-}, $S_2O_5^{2-}$, and SO_2, the ^{17}O resonances of which could perhaps be coalesced with the resonance of one of the isomers of bisulfite ion, creating the possibility that the areas of the 175 and 193 ppm peaks were not proportional to the HSO_3^- and total bisulfite ion concentrations, respectively. It was therefore necessary to make some assumption concerning which S(IV) species contribute to the areas of the two peaks. It was assumed that the area of the peak at 175 ppm was due only to HSO_3^-, while the area of the peak at 193 ppm arises from all the S(IV) species, i.e. HSO_3^-, SO_3H^-, SO_3^{2-}, SO_2, and $S_2O_5^{2-}$. The SO_2 resonance (at 520 ppm) and the SO_3H^- resonances were coalesced over the entire pH range from 3 to 5 due to the rapid oxygen exchange that occurs through reaction 1. An investigation of the ^{17}O NMR spectra of two very concentrated bisulfite solutions ([S(IV)] > 4 *m*, pH 3.2 and 4.5), in which the $S_2O_5^{2-}$ concentration is large enough to make a significant contribution to peak areas, revealed that at high concentrations the $S_2O_5^{2-}$ and bisulfite ion resonances are coalesced. It was assumed that the $S_2O_5^{2-}$ and the SO_3H^- resonances were also coalesced at lower S(IV) concentrations.

The assumption that SO_3^{2-} contributes to the area of the 193 ppm peak at pH 5 is justified by the coalescence of the 175 and 195 ppm resonances at that acidity. The increase with pH of the oxygen-exchange rate between HSO_3^- and SO_3H^-, which produces the coalescence of the 175 and 195 ppm resonances at pH 5, is a consequence of the increase with pH of the rate of the following step in the exchange mechanism:

$$(HSO_3^-)^* + SO_3^{2-} \rightleftarrows (SO_3^{2-})^* + SHO_3^- \qquad (3)$$

where SHO_3^- refers to both isomeric forms of bisulfite ion.[15] $(SO_3^{2-})^*$ can then be converted to $(SO_3H^-)^*$ by addition of a hydrogen ion from SO_3H^-, HSO_3^-, or H^+. The necessary condition for coalescence of two resonances is that the sum of the pseudo-first-order rate constants for oxygen exchange between the two sites be much larger than the difference between the precessional frequencies in the two sites. From the resonance frequency differences and the relative rate constants obtained from the concentration ratios it is readily shown that the SO_3^{2-} resonance will be coalesced with the two bisulfite resonances if the latter are coalesced at pH 5 due to rapid oxygen exchange occurring via reaction 3. Thus SO_3^{2-} will contribute to the 193 ppm resonance. The assignment of the SO_3^{2-} oxygen population to either the 175 ppm or the 195 ppm site is unimportant at low pH because the concentration of SO_3^{2-} is very small at high acidity.

Under the aforementioned assumptions the equilibrium quotient for reaction 2 could be evaluated by converting the area of the 175 ppm peak to a HSO_3^- concentration and comparing this concentration to the calculated sum of the concentrations of the two isomers for the same solution. It was thought best, however, to use the area of the 193 ppm peak as a measure of the total concentration of bisulfite ion, in the hope that any systematic error inherent in the measurement of peak areas would be at least partially canceled by the use of area measurements to obtain both the HSO_3^- concentration and the total bisulfite ion concentration. The equilibrium quotient for reaction 2 was evaluated by using the formula

$$Q_2 = (\text{peak area corresponding to total } [SHO_3^-] \text{ at pH 3})/(\text{area of 175 ppm peak at pH 3}) - 1 \qquad (4)$$

The area corresponding to the total bisulfite ion concentration at pH 3 was obtained from the area of the 193 ppm peak at pH 5 in the following manner. The concentrations of SHO_3^-, SO_2,

(16) It is interesting to note that Eigen, Kustin, and Maass (Eigen, M.; Kustin, K.; Maass, G. *Z. Phys. Chem. (Munich)* **1961**, *30*, 130) used exactly the opposite reasoning in an attempt to account for the great difference in rates of reaction of hydrogen ion with bisulfite ion and bicarbonate ion. A more likely explanation would be the much greater geometrical change that must occur when linear CO_2 is formed.

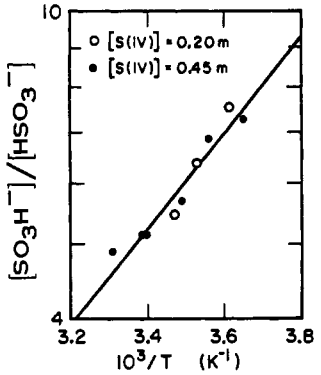


Figure 2. Temperature dependence of the equilibrium quotient Q_2 for the reaction $HSO_3^- = SO_3H^-$. The equilibrium quotient was determined at an ionic strength of 1.0 *m* for solutions having S(IV) concentrations of 0.20 and 0.45 *m*. The straight line represents the nonweighted linear-least-squares fit of the data and has the equation $\ln Q_2 = (-3.232 \pm 0.5274) + (1438 \pm 151.2)/T$, with a covariance of -79.71 between the slope and *y* intercept.

SO_3^{2-}, and $S_2O_5^{2-}$ were calculated for the pH 5 solutions, and the fraction of the 193 ppm peak area attributable to bisulfite ion was determined. The area corresponding to the total bisulfite ion concentration in the pH 3 solution was obtained by multiplying the area corresponding to the total bisulfite ion concentration in the pH 5 solution by the ratio of the calculated bisulfite ion concentration at pH 3 to that calculated at pH 5. To minimize error arising from the estimated value of the equilibrium quotient for the dimerization of bisulfite ion (which was used in calculating the bisulfite ion concentrations), a pH 5 solution having nearly the same bisulfite concentration as the pH 3 solution was always used.

The values of the parameters used in the calculation of Q_2 are listed in Table II together with the resulting values of the equilibrium quotient. Figure 2 shows a plot of log Q_2 vs. $1/T$. A nonweighted linear-least-squares treatment of the data yields

$$\ln Q_2 = (-3.232 \pm 0.5274) + (1438 \pm 151.2)/T \quad (5)$$

as the equation of the best straight line through the points, with a covariance of -79.71 between the slope and *y* intercept. From the values of the slope and intercept one obtains $\Delta H_2 = -2.9 \pm 0.3$ kcal/mol and $\Delta S_2 = -6 \pm 1$ cal K^{-1} mol^{-1}.

The oxygen-17 NMR spectra of bisulfite solutions provide the most convincing evidence to date for the existence of the two isomers of bisulfite ion, HSO_3^- and SO_3H^-, and allow the first measurement of their equilibrium concentration ratio.

Acknowledgment. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Science Division of the U.S. Department of Energy, under Contract No. DE-AC03-76SF00098.

Registry No. HSO_3^-, 15181-46-1; O_2, 7782-44-7.

Contribution from the Department of Chemistry and the Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Divalent Metal Ion Binding Sites on Yeast Inorganic Pyrophosphatase As Studied by CW-EPR and Electron Spin Echo Measurements on the Copper(II) Enzyme

Amit Banerjee,† Russell LoBrutto,‡ and Barry S. Cooperman*†

Received November 4, 1985

Cu(II) EPR is used to probe the divalent metal ion binding sites of yeast inorganic pyrophosphatase (PPase). Measurements of *g* and *A* values for the highest affinity site provide clear evidence for exclusive oxygen coordination to Cu(II) in a distorted octahedral environment. The absence of electron spin echo envelope modulation provides evidence against histidine side-chain binding to Cu(II). Examination of the CW-EPR spectrum along with measurement of spin-lattice relaxation times (T_1) of enzyme-bound Cu(II), as functions of Cu(II):PPase stoichiometry in both the absence and the presence of either inorganic phosphate or hydroxymethylenediphosphonate, allows formulation of a three-site model for Cu(II) binding to PPase. In this model the strongest interaction is between the two Cu(II) ions bound per subunit in the absence of a phosphoryl ligand, and there is some interaction between one of these sites and the third Cu(II) site that accompanies the binding of a phosphoryl ligand. Evidence that the Cu(II) sites observed are relevant for the active site of PPase is provided by the observation that Cu(II) serves as a divalent metal ion cofactor for enzymatic activity (although it confers only 0.1% of the activity found with Mg^{2+}), by the sensitivity of both CW-EPR and T_1 measurements to added phosphoryl ligands, and by the strong competition by Mg^{2+} for at least one of the three Cu(II) sites.

Introduction

Yeast inorganic pyrophosphatase (PPase) (E.C. 3.6.1.1) is a metal ion activated enzyme, catalyzing both hydrolysis of pyrophosphate (PPi) and oxygen exchange between water and orthophosphate (Pi).[1] PPase consists of two identical subunits of molecular weight 32 000.[2,3] Rapoport et al.[4] have shown that native PPase binds two divalent metal ions (Mg(II), Co(II), Mn(II), or Zn(II)) per enzyme subunit. Recent studies by ourselves and others have shown that in the presence of either PPi or Pi the enzyme binds a third and even a fourth divalent metal ion per subunit and, in addition, that three divalent metal ions per subunit are required for activity.[5-8] Two important questions are raised by these results: First, what are the structures of the metal ion binding sites on the enzyme? Second, what is the nature of metal ion-metal ion interaction on the enzyme surface?

In previous studies we have employed ^{113}Cd NMR[9] and Mn(II) EPR[10] to obtain at least partial answers to these questions. In the present work we continue our investigation through use of

* To whom correspondence should be addressed.
† Department of Chemistry.
‡ Department of Biochemistry and Biophysics.

(1) Cooperman, B. S. *Methods Enzymol.* **1982**, *87*, 526.
(2) Heinrikson, R. L.; Sterner, R.; Noyes, C.; Cooperman, B. S.; Bruckmann, R. H. *J. Biol. Chem.* **1973**, *248*, 2521.
(3) Cohen, S. A.; Sterner, R.; Keim, P. S.; Heinrikson, R. L. *J. Biol. Chem.* **1978**, *253*, 889.
(4) Rapoport, T. A.; Hohne, W. E.; Heitmann, P.; Rapoport, S. *Eur. J. Biochem.* **1973**, *33*, 341.
(5) Cooperman, B. S.; Panackal, A.; Springs, B.; Hamm, D. J. *Biochemistry* **1981**, *20*, 6051.
(6) Springs, B.; Welsh, K. M.; Cooperman, B. S. *Biochemistry* **1981**, *20*, 6384.
(7) Welsh, K. M.; Armitage, I. M.; Cooperman, B. S. *Biochemistry* **1983**, *22*, 1046.
(8) Knight, W. B.; Dunaway-Mariano, D.; Ransom, S. C.; Villafranca, J. J. *J. Biol. Chem.* **1984**, *259*, 2886.
(9) Welsh, K. M.; Cooperman, B. S. *Biochemistry* **1984**, *23*, 4947.
(10) Banerjee, A.; Cooperman, B. S. *Inorg. Chim. Acta* **1983**, *79* (B7) 146.

0020-1669/86/1325-2417$01.50/0