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Effect of high pressure on the infrared spectra of the thiamine enzyme 'active aldehyde' intermediate 2-(α-hydroxybenzyl)thiamine chloride (HBT) and the mercury(II) complex, $Hg(HBT)Cl_3$

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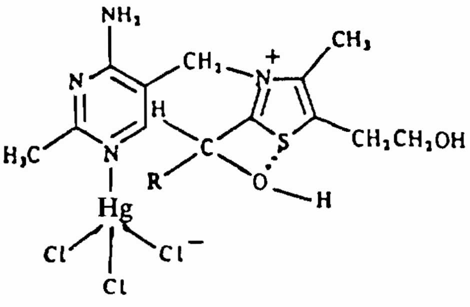
Abstract

The effects of high external pressures (~30 kbar) on the IR spectra of the protonated form of 2-(α-hydroxybenzyl)thiamine chloride (HBT·HCl) and the zwitterionic mercury(II) complex $Hg(HBT)Cl_3$ have been investigated at room temperature. With the exception of the ν(OH) peak of the HBT ligand, all the observed peaks shift towards higher wavenumbers with increasing pressure. The most pressure-sensitive peaks ($d\nu/dP > 0.6$ cm^{-1}/kbar) are chiefly associated with vibrations of the pyrimidine ring. The *S* conformation of the pyrimidine and thiazolium rings is apparently retained under high pressure in both HBT and $Hg(HBT)Cl_3$, as is also the case in solution.

Introduction

Vibrational spectroscopy has long been the method of choice for investigating the effect of complexation of a ligand to a metal. The shifts observed for specific vibrational modes can often provide useful information about the nature of the bonding involved. These studies are particularly important for biological molecules where several potential ligating sites exist. Lowering the temperature improves the quality of the observed vibrational spectra, and sometimes phase changes can be detected. Another approach is to examine the effect of high pressures on the spectra of the solid materials [1]. These measurements can readily be accomplished for both IR and Raman spectra by using commercially-available diamond-anvil cells, which are simple to operate at pressures approaching 100 kbar (~100 000 atm). There has been considerable interest lately in examining the effects of high pressures on the vibrational spectra of solid coordination compounds [1], including organometallic complexes [2], but similar studies of biochemical systems are quite rare. Some high-pressure Raman data have been reported for α-glycine [3], the copper protein azurin [4] and uridine [5]. There has also been some related IR work on organic solids of biological interest, e.g. urea, thiourea and vanillin [6], palmitic acid and L-α-dipalmitoylphosphatidylcholine [7], and salicylic acid [6, 8].

Substitution at the C(2) position of the thiazole moiety of thiamine leads to a series of 'active aldehyde' derivatives which are intermediates in the action of thiamine enzymes, e.g. decarboxylation of α-keto acids or formation of α-ketols [9]. These intermediates adopt the so-called *S* conformation for the relative orientation of the pyrimidine and thiazolium rings [10], which is also retained in complexes of 2-(α-hydroxybenzyl)thiamine (HBT) with Zn^{2+}, Cd^{2+} and Hg^{2+} [10, 11] and with Co^{2+} and Ni^{2+} [12]. The N(1') position of the pyrimidine moiety of HBT is the coordination site in every case (see structure). The various conformations (*S*, *V* or *F*) that can possibly be adopted by the ligand, the 'active aldehyde' derivative of thiamine, in its metal complexes are clearly important in un-



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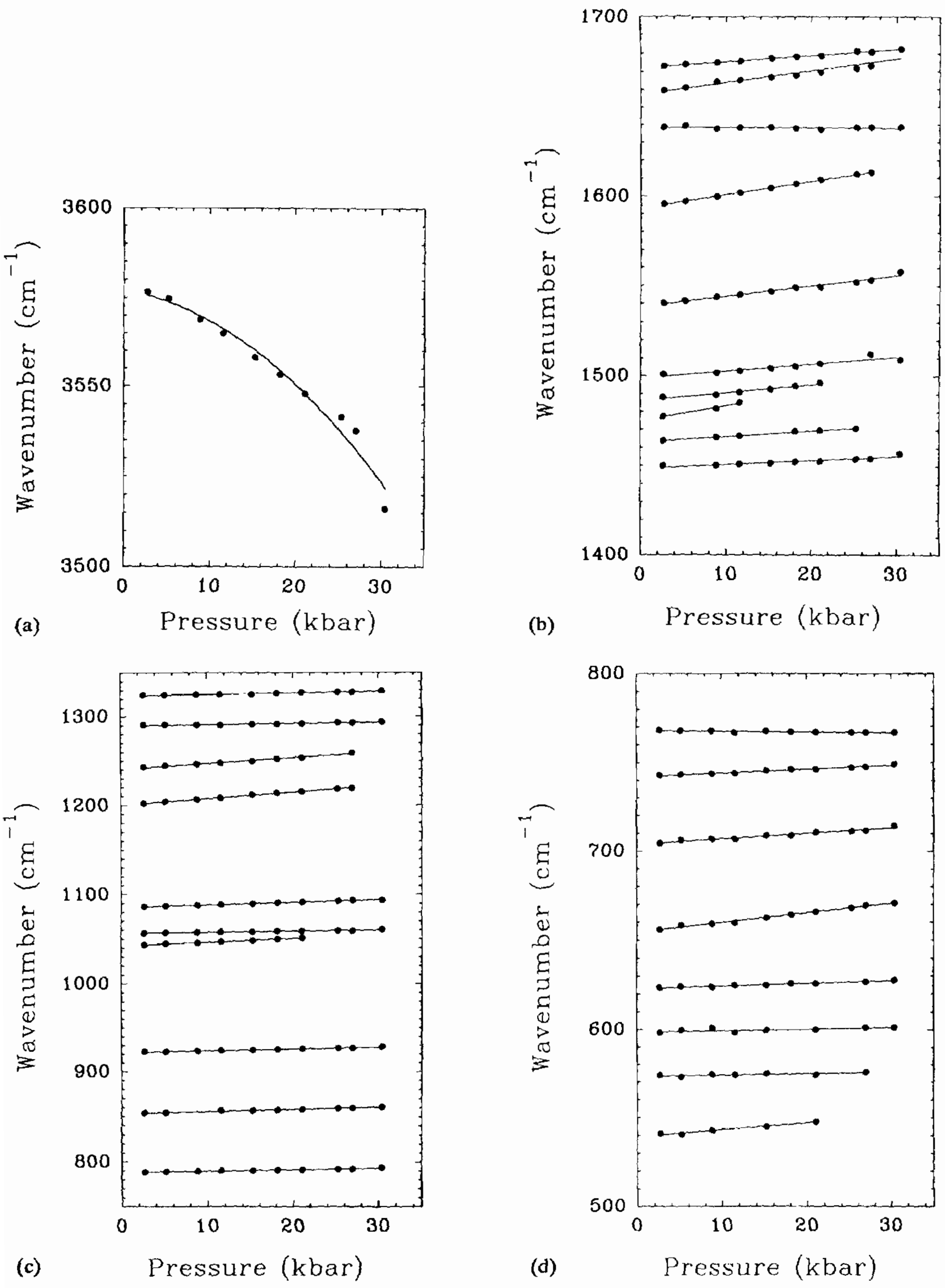

Fig. 1. Pressure dependences of some of the observed IR bands for the solid HBT ligand.

derstanding the enzymatic action of thiamin. Consequently, we recently decided to undertake a wide range of structural studies of metal–HBT complexes (some of which have already been mentioned [10–12]) in an effort to throw more light on the catalytic function of thiamine enzymes.

In this paper, we report the results of an investigation of the effects of high external pressures on the IR spectra of the protonated form of the ligand, HBT·HCl, and the zwitterionic mercury(II) complex, Hg(HBT)Cl_3. The IR and Raman spectra of both compounds at ambient pressure have been published [11], and their

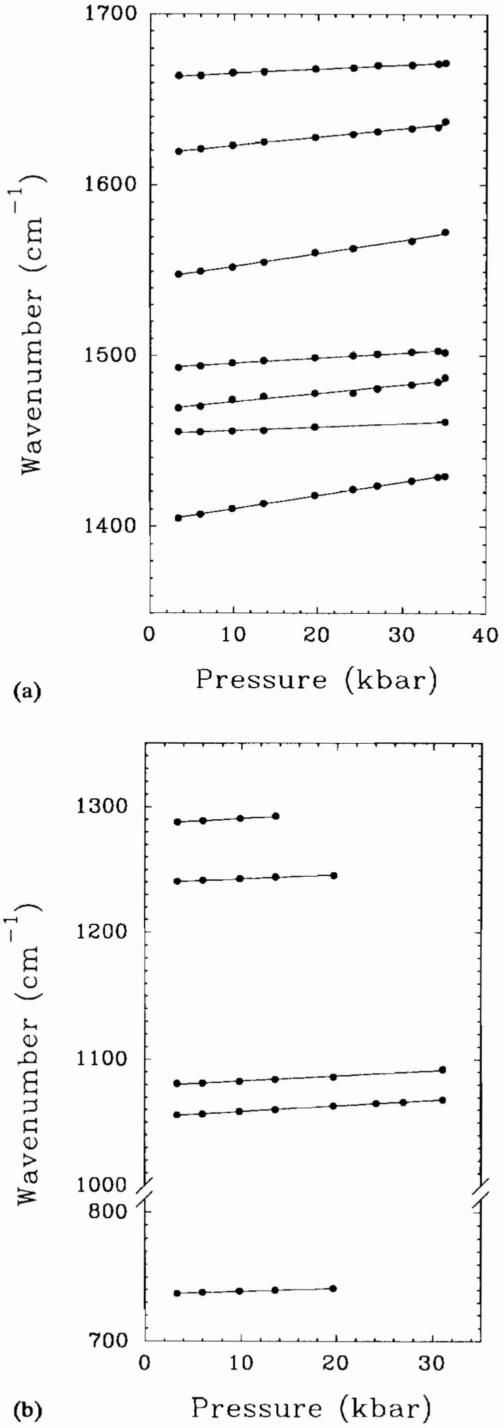

Fig. 2. Pressure dependences of some of the observed IR bands for the solid Hg(HBT)Cl_3 complex.

molecular structures have been established by X-ray diffraction [10, 13]. We have also recently shown from comparisons of the solid-state and solution ^{13}C NMR spectra that the *S* conformation of the HBT ligand is retained in Hg(HBT)Cl_3 [14].

Experimental

Materials

The HBT·HCl ligand [10] and the Hg(HBT)Cl_3 [15] complex were prepared by the literature methods indicated.

High-pressure IR measurements

The IR spectra were recorded at room temperature on a Nicolet model 6000 FT spectrometer equipped with a liquid nitrogen-cooled MCT-B detector. The DAC used for the high pressure measurements was purchased from High Pressure Diamonds Optics, Inc. The sample and a thin layer of pressure calibrant (0.14% wt. $NaNO_3$ in NaBr, prepared by the method of Klug and Whalley [16]) were placed in the 300-μm hole of a 200-μm thick, stainless-steel gasket located between the parallel faces of the two diamonds. The DAC was mounted onto a Spectrabench (Spectra Tech, Inc.) fitted with a f4 beam condensor. The position of the DAC on the Spectrabench was then adjusted to maximize the IR throughput. The pressure in the DAC was determined from the observed wavenumber shift of the antisymmetric N–O stretch of the NO_3^- ion [16].

Results and discussion

Some representative IR wavenumber versus pressure plots for HBT·HCl and Hg(HBT)Cl_3 are shown in Figs. 1 and 2, respectively, while the pressure dependences ($d\nu/dP$) of the principal peaks observed are listed in Tables 1 and 2, respectively. There is no evidence for a phase transition in either case.

With the exception of the broad peak associated with the OH groups of the uncomplexed HBT ligand, all the peaks exhibit the expected blue shifts towards higher wavenumbers with increasing pressure. The ν(OH) peak steadily decreases in wavenumber due to the effect of increased O–H···O hydrogen bonding upon application of pressure which leads to a decrease in O–H bond strength (Fig. 1). Such a pressure effect has been noted previously for the hydrogen bonds in organic solids [17] and in the present case may also reflect an increased HO···S interaction in the HBT ligand. The most pressure sensitive peaks ($d\nu/dP > 0.60$ cm^{-1}/kbar) are at 1659.7 (pyrimidine ring (8a) + δNH_2), 1595.5 (pyrimidine ring (8b)), 1477.3 (δCH (cyclohexane + methyl)), 1242.6 (phenyl) and 1201.6 (δCH (phenyl) (9a)) cm^{-1}.

In the case of the Hg(HBT)Cl_3 complex, the IR spectra under pressure closely resemble that of the complex in the absence of pressure. The most pressure sensitive peaks are those at 1548.2 (pyrimidine ring (8b)) and 1404.5 (phenyl ring ($\nu_{16} + \nu_5$)) cm^{-1}. The comparatively slight spectral changes brought about by

TABLE 1. Pressure dependences of the observed IR bands of HBT·HCl

Observed band (cm^{-1})[a]	dν/dP (cm^{-1}/kbar)	Vibration assignment[b]
3577	−0.39	ν(OH)
1673.0	0.33	pyrimidine ring (8a) + δNH_2
1659.7	0.66	
1638.5	−0.01	
1595.5	0.74	pyrimidine ring (8b)
1540.2	0.58	
1500.7	0.40	
1488.0	0.45	δCH (cyclohexane + methyl)
1477.3	0.85	
1463.8	0.31	
1450.1	0.22	
1324.2	0.17	
1290.4	0.12	δCH_2 aliphatic
1242.6	0.65	phenyl ring
1201.6	0.73	δCH (phenyl) (9a)
1086.0	0.29	
1056.0	0.13	δC–OH
1042.9	0.43	
922.6	0.21	thiazole breathing
853.6	0.25	
788.1	0.17	
767.9	−0.04	
742.6	0.22	CH_2 rocking
704.3	0.31	
655.6	0.55	
623.3	0.15	
598.1	0.09	
573.5	0.09	
541.0	0.39	

[a] At 2.7 kbar, initial pressure in the DAC. [b] From ref. 11.

TABLE 2. Pressure dependences of the observed IR bands of Hg(HBT)Cl_3

Observed band (cm^{-1})[a]	dν/dP (cm^{-1}/kbar)	Vibration assignment[b]
1664.2	0.23	pyrimidine ring (8a) + δNH_2
1619.1	0.50	
1548.2	0.76	pyrimidine ring (8b)
1493.0	0.30	δCH (cyclohexane + methyl)
1469.3	0.49	
1455.4	0.20	
1404.5	0.78	phenyl ring ($\nu_{16}+\nu_5$)
1287.8	0.47	δCH_2 aliphatic
1240.4	0.32	phenyl ring
1080.7	0.41	
1055.7	0.46	δC–OH
737.0	0.24	CH_2 rocking

[a] At 3.38 kbar initial pressure in the DAC. [b] From ref. 11.

pressure indicate that the *S* conformation is retained, as is also the case in solution [14]. This conformation is clearly quite robust and this may be the reason why it plays such an important role during the enzymatic action of thiamine.

In conclusion, the pressure dependences of both HBT·HCl and Hg(HBT)Cl_3 are quite similar to those typically observed for organic molecules, e.g. uridine [5], being less than 1.0 cm^{-1}/kbar. In the case of uridine, anomalous values greater than 1.0 cm^{-1}/kbar were found only for the νCH and CH_2 rocking modes. The νCH modes in the present work were too broad to provide any useful information.

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