two reaction products, ΔF at 37° was, respectively, 25,200, 22,800, 25,300 and 27,400 cal. per mole. These figures are of the same order as that obtained for the formation of insulins IU and IIU in the present studies. For these gonadotropins the respective entropy changes of activation varied from $+133$ to -17 cal. per degree per mole. The energy changes in the urea insulin reaction are therefore akin to those found for the other protein hormones studied. In the case of the mare serum gonadotropin, the reaction product was devoid of biologic activity, while in the case of the chorionic gonadotropin a product of decreased biologic activity was obtained. The qualitative biologic response of the latter compound was unchanged. If the urea reaction is an unfolding of the protein molecular chain, biologic activity must be dependent upon the spacing of active groups in relation to each other.

Waugh, *et al.*,[13] studied the ability of insulin to form fibrils or crystals after solution in concentrated urea solution (8 *M*) with subsequent recovery. Two of their experiments cover the approximate temperature range, *p*H and concentrations used in our experiments. Waugh, *et al.*, found that 24 hours at 0°, *p*H 7.0, abolished neither the ability to form fibrils nor form crystals. We found that 141 hours at 3° produced no measurable amount of insulin IIU, and that insulin OU remained intact. Waugh, *et al.*, found that 24 hours at 37° abolished completely the ability to crystallize but not to form fibrils. We found that under these conditions insulin OU has disappeared and 30% of insulin IIU has appeared. The failure to crystallize must therefore appear as a property of insulins IU and IIU, and the ability to crystallize is retained by insulin OU. The ability of insulin IIU to form fibrils and of insulin OU to crystallize has been observed in this Laboratory.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY]

Infrared Investigation of Bound Water in Hydrates

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The infrared absorption spectrum of water bound in crystalline salt hydrates has been studied for a variety of systems in the region 4000 to 800 cm.$^{-1}$, by the KBr pellet technique. The positions of the fundamental absorption bands of water were found to vary slightly with the nature of the hydrate. The variation appears to be different for each of the three bands studied. The effects of the salt on the 3425 cm.$^{-1}$ band are in most cases reversed with respect to the 1616 cm.$^{-1}$ bands whereas the position of the absorption in the 2915 cm.$^{-1}$ region is not significantly altered.

Introduction

Several studies have been reported on the Raman[1] and infrared[2-5] spectra of hydrates, both in solution and in the solid phase. The positions of the absorption bands of water in such studies have been used to gain insight into the nature and strength of binding of the water in the hydrate.[6-8] With the development of the KBr pellet technique by Stimson[9] and Schiedt,[10] it has become possible to measure infrared spectra of solid samples in pressed KBr pellets with elimination of scattering losses and background interference, and with a considerably improved separation and sharpness of bands. In the present study, this technique was employed to measure the infrared absorption spectrum of various salt hydrates and to determine whether any systematic shifts in the water bands could be observed with changing nature of the salt.

Experimental

Materials and Apparatus.—All salts and salt hydrates were of AR grade. Pressed KBr pellets of these materials, at the weight concentrations listed in Table I, were prepared with the Perkin-Elmer evacuable dye and a Loomis 20-ton hydraulic press. Spectra were recorded on the Perkin-Elmer Model 21 Recording Spectrophotometer, with the pellets mounted on the pellet holder and microcell adapter supplied by the manufacturer. All spectra were measured with CaF_2 optics and wave length calibrations were made using the known bands of water vapor and CO_2.

Sample Preparation.—Since some of the materials are extremely hygroscopic, it was necessary to work under conditions as dry as could be obtained. The pellets were pressed under vacuum for two minutes under 23,000 pounds total load. All materials were sifted through a 300-mesh screen. All sampling and mixing operations were carried out in a moisture free dry box in an atmosphere of dry nitrogen. Thus, the pellets were actually exposed to atmospheric water vapor only during the time required to scan the spectrum. Repeating the scanning showed that during this time the intensity or position of the water bands was not altered significantly.

It was found that, provided 300-mesh material was used throughout, the position of the bands could be reproduced within the limits given in the next section. Within these limits, the positions could be reproduced for different periods of grinding and mixing. However, this reproducibility could only be obtained when the samples were prepared under anhydrous conditions.

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Results and Discussion

Table I summarizes the results of this research. In all cases, 3 absorption peaks were investigated. For pure KBr, pellets made under anhydrous conditions gave weak water bands at 3425, 2915 and 1616 wave numbers. These bands are named I, II and III, respectively. The reproducibility of the measurements in Table I was found to be ±7, ±7 and ±3 cm.$^{-1}$ for bands I, II and III. Spectra were taken of anhydrous SrF_2, $Sr(NO_3)_2$ and Na_2SO_4, and these salts gave weak residual water bands that agreed with those of the KBr sample within the reproducibility of duplicate measurements. The intensities obtained with the salt hydrates in KBr were much greater than the weak intensities of the anhydrous salts, and, in most cases, the bands were shifted. The table summarizes these observed shifts relative to the positions of the residual water bands; *i.e.*, if (I) is the observed position of the water absorption band for a given hydrate in the 3425 cm.$^{-1}$ region, then (I) = 3425 − I. Similarly, (II) = 2915 − II and (III) = 1616 − III, where (II) and (III) refer to the observed bands in the 2915 and 1616 cm.$^{-1}$ regions, respectively. Hence, a negative sign in the table signifies a shift of the band toward higher wave numbers, and a positive sign a shift toward lower wave numbers.

TABLE I

OBSERVED SHIFTS IN THE POSITIONS OF THE WATER BANDS FOR SALT HYDRATES IN KBr PELLETS

Hydrate	*a*	*b*	(I)	(II)	(III)
$LiI \cdot 3(H_2O)$	1.67	335	+ 8	0	−10
$SrCl_2 \cdot 6(H_2O)$	1.67	342	−12	0	−13
$SrBr_2 \cdot 6(H_2O)$	1.67	323	−16	0	+13
$Na_3PO_4 \cdot 12(H_2O)$	1.67	350	−14	−5	+10
$AlCl_3 \cdot 6(H_2O)$	1.67	337	+15	0	− 9
$Al(NO_3)_3 \cdot 9(H_2O)$	1.67	329	+18	0	− 7
$SnCl_2 \cdot 2(H_2O)$	1.67	321	−25	−9	− 8
$SnCl_4 \cdot 5(H_2O)$	1.67	311	−40	−9	+10

[a] Concentration of hydrate in KBr, parts per thousand by weight. [b] Total weight of the pellet in mg.

Data on the applicability of Beer's law are not tabulated. For $SrCl_2 \cdot 6(H_2O)$, in the concentration region up to 5 p.p.t. by weight of hydrate in KBr the absorbance at 1616 cm.$^{-1}$ increased linearly with concentration.

Correlations between the absorption spectra of bound water in hydrates and the nature and strength of binding of the water are, at best, extremely difficult. From the analysis of the water vapor bands, it may be assumed that the regions studied correspond to the fundamental vibrations of the water molecule.[11] Therefore, if the reported shifts are significant, they reflect changes in the modes of vibration of the water molecules as influenced by such factors as the nature and valence of the salt cation and anion, the coördination number, the structure of the hydrate, etc. No simple correlation was obtained in this work. Considering the effects on a given water band caused by changes in the nature of the hydrate, there is no gradual shift with variations in coördination number or cation charge. Indeed, the differences observed (III) for the Sr halides indicate that the nature of the anion influences this particular mode of vibration. On the other hand, with the possible exception of the tin hydrates, the 2915 cm.$^{-1}$ band appears to remain unaltered throughout the series. Across the table, for a given hydrate the influences on one absorption band do not give corresponding effects on another. In most cases, a given shift in the 3425 cm.$^{-1}$ band is accompanied by little or no shift of the 2915 cm.$^{-1}$ band, whereas the 1616 cm.$^{-1}$ absorption is shifted in the opposite direction.

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