Dehydration of the cyclodextrins: A model system for the interactions of biomolecules with water

Amedeo Marini, Vittorio Berbenni, Giovanna Bruni, Vincenzo Massarotti, Piercarlo Mustarelli, and Marco Villa

Citation: The Journal of Chemical Physics **103**, 7532 (1995); doi: 10.1063/1.470321
View online: http://dx.doi.org/10.1063/1.470321
View Table of Contents: http://scitation.aip.org/content/aip/journal/jcp/103/17?ver=pdfcov
Published by the AIP Publishing

Dehydration of the cyclodextrins: A model system for the interactions of biomolecules with water

Amedeo Marini, Vittorio Berbenni, Giovanna Bruni, Vincenzo Massarotti, and Piercarlo Mustarelli
Dipartimento di Chimica Fisica e C.S.T.E. C.N.R. Via Taramelli 16, 27100 Pavia, Italy

Marco Villa
Istituto di Fisica Università di Urbino, Via S. Chiara, Urbino, Italy

(Received 30 November 1994; accepted 25 July 1995)

The thermodynamics of hydration of biomolecules is experimentally studied in the β-cyclodextrin (β-CD), which contains water molecules in a range of configurations and has been proposed as a model system for complex biomolecules. The thermal measurements point to the role of a structural transition from the hydrated β-CD (phase I) to a ''dehydrated'' form (phase II). We show that dehydration in phase I is assisted by a ''compensation mechanism'' for which β-CD contributes a constant amount of energy for each H_2O mole. Despite the presence of different types of H_2O's, water losses in phase I are accurately described in terms of this energy and the isosteric molar enthalpy of dehydration. Moreover, in going from the fully hydrated to the fully dehydrated form, the contribution of β-CD to dehydration is over all equal to the enthalpy of transition from phase I to phase II. Our analysis yields the changes of an enthalpy associated with the biomolecule alone as a function of the water content. In the case of β-CD, we can sketch a qualitative phase diagram, which assists the interpretation of details of our thermal experiments. The role of kinetic factors in the attainment of the thermodynamic equilibrium is investigated with ^{2}H-NMR in samples recrystallized from heavy water. We find that, over a wide range of hydration levels, water molecules have a liquidlike diffusion, which, together with the compensation mechanism, explains the fast and nearly reversible dehydration of the β-CD.

I. INTRODUCTION

Two recent comments in Science[1,2] about the entropy cost of binding water to biomolecules remind us that classical thermodynamics is still a useful tool; we add that it may nicely complement, and even inspire, the computational modeling and statistical mechanical calculations that are in fashion today, and are applied to solvation,[3,4] absorption,[5] wettability,[6] and, of course, to hydration. The interactions—water–organic molecules, or water ''surfaces'' are at the core of the problem. Monte Carlo[7] and molecular dynamics[8] calculations are quite successful when the system is relatively simple, and appropriate potentials are known. Unfortunately, the forces of interest often result from nearly complete cancellation of large terms, which may be insufficiently known in complex systems. Furthermore, there are theoretical,[9] and, possibly, semantic difficulties in decomposing the free energy into contributions due to the ''van der Waals,'' the ''electrostatic,'' the ''hydrogen bond,'' or the ''hydrophobic'' interactions.

The need to learn from real systems how to most appropriately describe the major phenomena arising from water–biomolecule interactions is widely felt. Saenger and Steiner[10] have suggested that cyclodextrins (CDs) make an excellent model system. CDs are barrel-shaped cyclo-amyloses with well-characterized crystalline structures; there is interstitial water, water hydrogen bonded to the amylose, and water within the barrel cavity, where hydrophobic interactions are dominant. In solution, the CDs bind with a wide variety of molecules, many of them usable as drugs. An empirical rule of ''entropy–enthalpy compensation'' describes the thermodynamics of these complexes,[11,12] and still awaits an explanation.[13]

We investigate hydration of the best known member of the CD's family, the β-CD or hepta-amylose, hereafter indicated as β-CD $n H_2O$. Within its complex crystalline structure, shown in Fig. 1, several hydrogen bond patterns have been identified at room temperature (rt)[14] and below an ordering transition at ~ -50 °C;[15] the cooperative behavior of the hydrogen bonds do not seem to play an important role in hydration of β-CD, and will not be further discussed.

More relevant to our purposes is the question of the β-CD stoichiometry. Up to few years ago, the prevailing opinion[14–17] was that β-CD in equilibrium with ambient atmosphere has 11 water molecules per unit formula while a saturation water content $n_{sat}=12$ was favored in earlier studies.[18] Although changes of water content were associated with details of sample preparation, age, and storage,[16] the implication was that β-CD forms two stoichiometric hydrates. Now, it is accepted that the β-CD hydrate is nonstoichiometric,[19–21] and that n_{sat} may be larger than 12. We find a systematic difference between n_{sat} of water-recrystallized (''native'') samples and of samples rehydrated in air moisture, which is probably related to the early hypothesis of two hydrated forms. Furthermore, we show that n_{sat} is a relevant parameter, which enters the expression for the enthalpies of dehydration.

Recently, it has been found that n changes from 12.3 to 9.4[20] in a continuous and reversible way when the relative humidity at rt changes from 100% to 15%. This stage of the dehydration process involves almost exclusively some of the

 0021-9606/95/103(17)/7532/9/$6.00

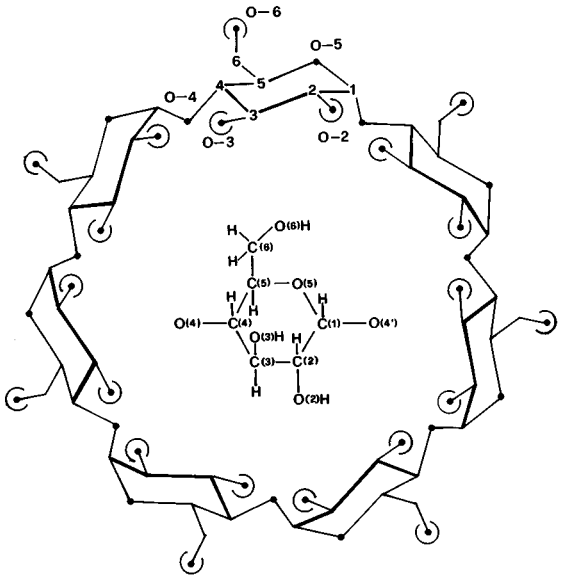

FIG. 1. Structure of β-CD, adapted from Ref. 18.

$\sim$7 water molecules within the β-CD cavity, and is accompanied by a reduction of cell volume,[7,8] by modifications of solid-state ^{13}C NMR spectrum,[21] and by a breaking down of large crystals. When heating, all water is apparently released in a single stage,[22] and a different crystalline structure is created.[23] We find that the crystalline structure of the ''hydrated form,'' or phase I, can be maintained down to a water content as low as $n=\sim 2$; a slow transformation to the ''dehydrated form,'' or phase II, takes place over a wide range of temperatures and appears to be primarily driven by the dehydration process itself. With carefully designed experiments, we determine the *isobaric heat of dehydration*, as a function of n, and the enthalpy of transformation.

If equilibrium between the two forms and the vapor phase would be attained in reasonable times, a phase diagram would be readily constructed; this not being the case, we fit to a Langmuir expression[24] the isothermal dehydration data reported for phase I

$$n = n_{\mathrm{sat}}\left[\frac{p}{p+p_0}\right], \tag{1}$$

where p is the partial pressure of water vapor and p_0 is a temperature dependent parameter. We obtain $p_0=112$ Pa from the data of Steiner at 18 °C[19] and $p_0=457$ Pa from those of Nakai[23] at 40 °C, which allows us to estimate an *isosteric heat of dehydration* for phase I. The difference between the isosteric and isobaric heats of dehydration is the ''contribution of the biomolecule'' to the dehydration process. We find that the enthalpy intake of the phase I→phase II transformation is essentially equal to the total energy contribution of the β-CD molecules to the dehydration. In other words, we need to assign the same internal energy to the β-CD molecule in the fully hydrated and the fully dehydrated configurations.

In the last part of the paper, we introduce educated guesses which lead to a phase diagram for β-CD–H_2O. This diagram has a qualitative value but, with its help, we are able to interpret details of thermal measurements during which the sample may be in nonequilibrium states.

II. EXPERIMENT

The ''native'' β-CD has been obtained by slow evaporation at rt of an aqueous solution of commercial β-CD (Roquette, France). After a few hours in ambient atmosphere, the powder was grinded and fractionally sieved; at this point, its water content (thermogravimetrically determined, see below) was $n=10.75\pm0.15$, which will be taken as the saturation value for the *native* β-CD, $n_{\mathrm{sat}}(N)$; since no dependence upon the sieve fraction (from 38–45 to 180–250 μm) has been detected, we infer that native β-CD has no surface water, within our experimental error. With the exception of the deuterated samples, all data reported below refer to experiments performed using the 38–45 μm sieve fraction as the starting material. Rehydration of fully dehydrated β-CD is complete in less than an hour in wet atmosphere ($p\cong 3\times10^3$ Pa) and leads to samples with $n=12.6\pm0.2$, which will be taken as the saturation value for the *rehydrated* form, $n_{\mathrm{sat}}(R)$. We will represent the uncertainty of our data with the standard deviation (rather than the standard deviation of the mean), as estimated over a set of ten or more experiments. When a smaller set of data is available, the results are simply rounded off to the least significant digit.

Powder x-ray diffraction patterns have been collected under controlled atmospheres with a Philips PW 1710 powder diffractometer, equipped with a Philips PW 1050 vertical goniometer, a graphite crystal monochromator (Cu $K\alpha$ radiation), and a homemade thermal attachment.[25] Most thermogravimetric (TGA) and differential scanning calorimetric (DSC) experiments have been performed at a heating rate of 1 °C/min with a TA2000 system interfaced to DuPont cells 951 and 910, respectively. ^{2}H NMR spectra, either from free precessions or quadrupolar echoes, have been collected at 61.4 MHz with a solid-state probe and AMX-400WB (Bruker Spectrospin) spectrometer.

III. X-RAY RESULTS

Powders of β-CD were placed in the temperature-controlled x-ray chamber under a flow of dry nitrogen, or nitrogen bubbled through water at rt (wet N_2). Changes of weight were estimated with parallel thermogravimetric experiments made with samples from the same batches. Figure 2 shows the evolution of the x ray diffraction patterns of a native sample at rt as a function of the time spent in dry nitrogen. The bottom spectrum (a) was taken in air at the beginning of the experiment (nominal time $t=0$). In the next trace (b), taken after 17 h, intensities and widths have changed, but positions of the main peaks have not; after 42 h (c) the pattern has changed radically. Spectra taken in the following days do not change any more. However, the sample has swollen appreciably; as a consequence, positions of the peaks, particularly those at low angles, are somewhat displaced. The last spectrum (d) has been taken at 90 °C after nearly five days in dry nitrogen. Before this measurement, the chamber had to be briefly opened to level the sample

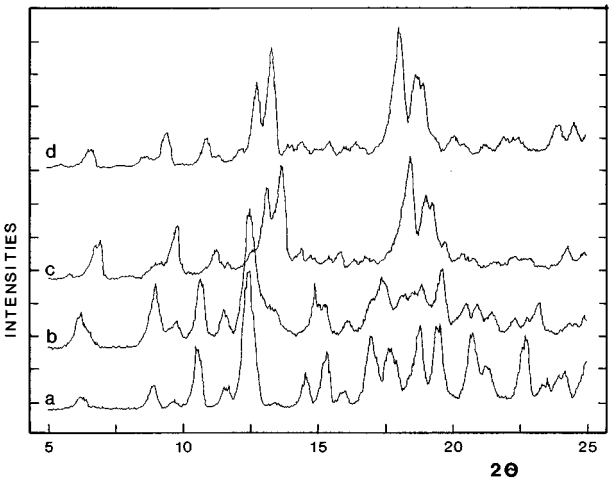

FIG. 2. X-ray diffraction patterns of native β-CD after 0 h (a), 17 h (b), and 42 h (c) in dry atmosphere and rt. The last spectrum (d) has been taken at 90 °C after nearly five days in dry nitrogen (see the text).

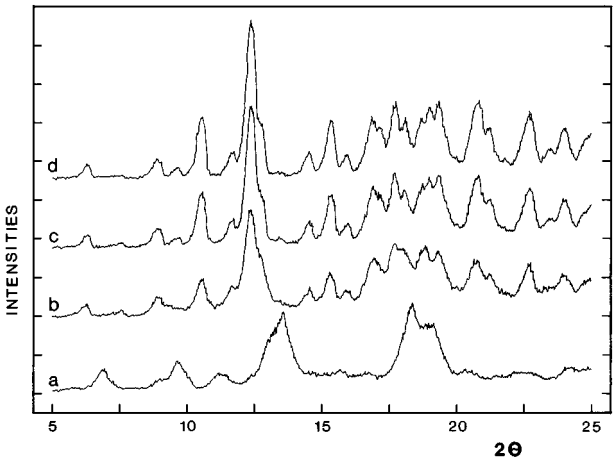

FIG. 4. Diffraction patterns of a β-CD sample at 170 °C (a) and during subsequent rehydration in wet atmosphere and at rt (see the text).

surface; the temperature was then raised to offset any possible intake from air moisture. Note that this final spectrum would almost coincide with trace (c) if displacements due to the distorted geometry are discounted. We estimate that transformation from phase I (trace a) to phase II (trace d) takes place, in this sample, after about one day in dry N_2, when n is between 2 and 3.

The traces of Fig. 3 have been recorded in a native β-CD sample kept in wet nitrogen above rt. After 46 h at 56 °C, the sample still has four water molecules and shows peak positions (but not intensities!) which are consistent with phase I (trace a of Fig. 1). After increasing the temperature to 64 °C (in about 5 min), some minor modifications are noted (trace b), which become more evident after a further hour at 64 °C (trace c). The distinctive pattern of phase II (trace d in Fig. 1) is clearly identified only after 23 h at 64 °C (trace d) but the feature near 15°, typical of phase I, is still present. In Fig. 4, we start with a sample at 170 °C in wet N_2 (trace a). All characteristic features of phase II are present, albeit much broadened relative to trace (1d), taken at a lower temperature (90 °C). The next recording (b) has been obtained at rt after ramping down the temperature at 1°C min^{-1} in wet N_2. A hydrated structure, greatly broadened relative to that of native β-CD [trace (1a)], is obtained. Further ''annealing'' at rt causes only minor changes; after one hour (trace c) the peaks between 10° and 13° have grown, and narrowed, appreciably. This ordering process is completed in the first few hours, since trace (c) is essentially equal to that taken after a week in ambient atmosphere (trace d).

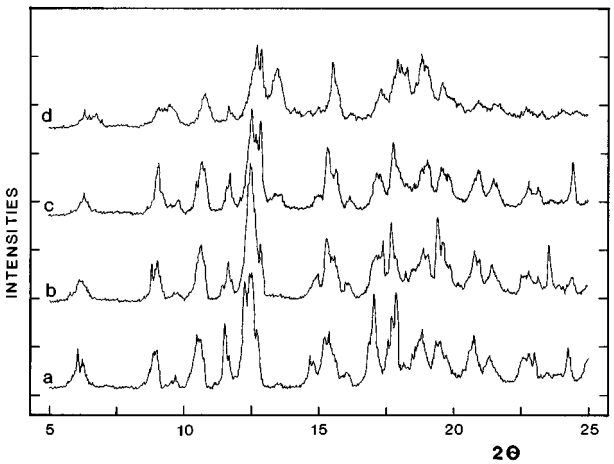

FIG. 3. Diffraction patterns of native β-CD during progressive annealing ($a \rightarrow d$) between 59 and 64 °C in wet atmosphere (see the text).

We have followed the evolution of the x-ray powder pattern for a range of samples, under different conditions and found the following.

(1) A slow transition to the crystalline phase II occurs in the partially dehydrated β-CD when the water content is near $n \sim 2$.
(2) The transition has been observed in the 20–64 °C temperature range; its rate, estimated from intensities of x-ray reflections, increases with temperature but it does not follow an Arrhenian trend.
(3) The transition is accompanied, or followed, by a substantial expansion (up to 10%) of our powder, which requires us to open the x-ray experiment chamber. This operation, and the long transition times, make the evaluation of the critical water content somehow uncertain.
(4) In wet air and near rt, phase II reverts very quickly to phase I. An hours long process of ''reordering'' can be followed in the freshly rehydrated samples, which remain substantially more disordered than the native ones.
(5) The rate of the transition, and/or of the water release, appears to be influenced by several factors, such as age of the sample, average particle size, and use of deuterated water.

IV. THE THERMAL ANALYSIS

Upon heating at 1 °C min^{-1} in air, native β-CD loses about 10.7 H_2O molecules through an apparently unique process, taking place between ~30 and 100 °C [see the TGA

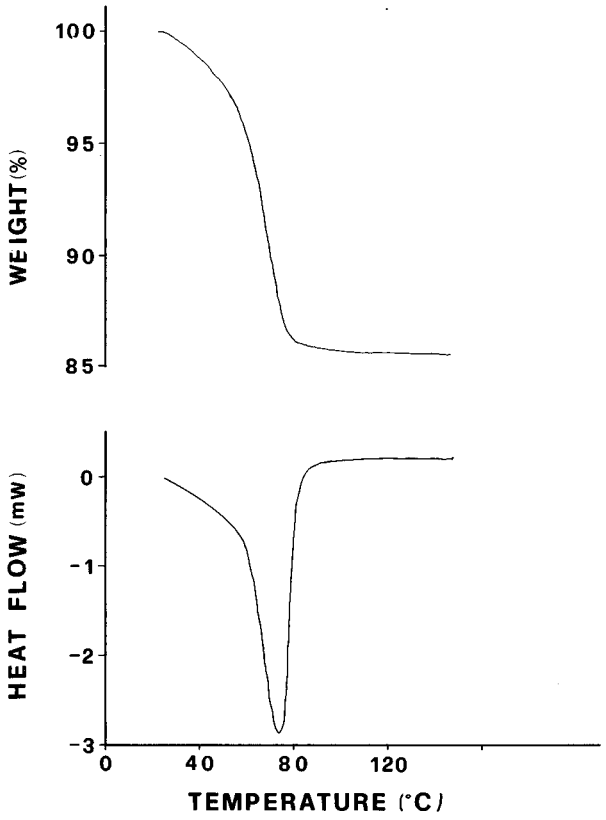

FIG. 5. TGA and DSC curves of native β-CD in air.

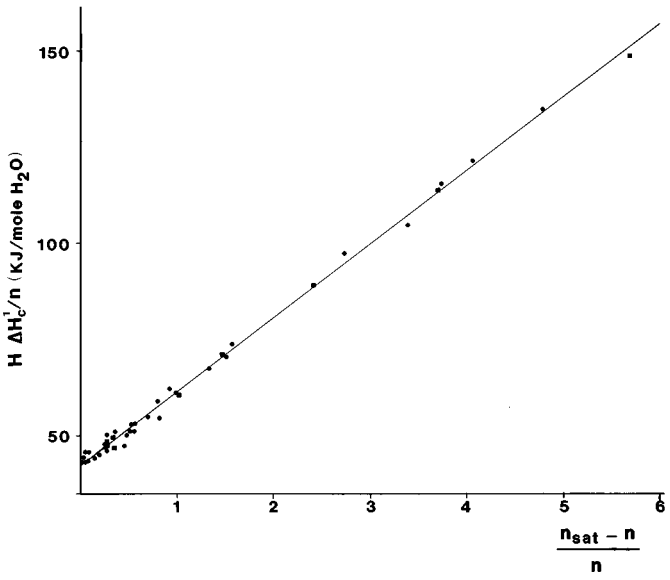

FIG. 6. Dehydration enthalpy per mole of water $\Delta H_c'/n$ vs $(n_{\mathrm{sat}}-n)/n$ [see Eq. (4)] for native (■) and rehydrated (●) samples.

Fig. 5(a)]. The parallel DSC run [Fig. 5(b)] displays a structured endothermic peak with an area of $\sim$460 kJ/mol$_{\beta\text{-CD}}$ or $\sim$43 kJ/mol$_{\mathrm{H_2O}}$ per mole of released water. We have repeated this TGA+DSC experiment with about $\sim 10^2$ fully and partially hydrated samples, according to the following protocols for native (N) and rehydrated (R) samples.

N. Native samples have been kept for a time t_a at temperature T_a in a controlled atmosphere, before starting parallel TGA and DSC runs to 120 °C.

R. Completely dehydrated samples (in dry nitrogen at 120 °C) have been fully or partially rehydrated at rt in atmosphere with controlled $p_{\mathrm{H_2O}}$, before performing TGA and DSC scans to 120 °C.

If we consider only samples with an initial water content $n>2$, we find that the area of the endothermic DSC peak normalized to a mole of β-CD, $\Delta H_c'$, is a linear function of n:

$$\Delta H_c' = A' + Bn. \tag{2}$$

Here and in the following, the prime will mark thermodynamic quantities referred to a mole of β-CD, whereas quantities referred to a mole of water will be designed with unprimed capital letters. Data of N and R samples follow lines with the same slope, $B \cong 23.5$ kJ, different intercepts, and correlation coefficients (r) of 0.977 and 0.983, respectively. Within the experimental uncertainty, the best fit values of the intercepts, $A'(N)$ and $A'(R)$, are proportional to the mean values $\langle n_{\mathrm{sat}}(N)\rangle$ and $\langle n_{\mathrm{sat}}(R)\rangle$ (see Sec. II)

$$\begin{aligned} A'(N) &= 204 \text{ kJ} \cong A\langle n_{\mathrm{sat}}(N)\rangle, \\ A'(R) &= 239 \text{ kJ} \cong A\langle n_{\mathrm{sat}}(R)\rangle \end{aligned} \tag{3}$$

with $A \cong 19$ kJ/mol$_{\mathrm{H_2O}}$. Equation (2) can now be written in a form that applies to all samples:

$$\Delta H_c' = A(n_{\mathrm{sat}} - n) + (A+B)n. \tag{4}$$

Figure 6 plots the enthalpy referred to a mole of water, $\Delta H_c'/n$ vs the hydration parameter $(n_{\mathrm{sat}}-n)/n$; n_{sat} was individually determined for each of the N samples, and set equal to 12.6 for all R samples. The correlation coefficient with the best fit line of Fig. 6 is $r=0.998$, much improved relative to the two fits obtained with Eq. (1). While almost equivalent, Eqs. (2) and (4) correspond to different descriptions of the dehydration process. According to Eq. (2), the dehydration enthalpy has a constant value

$$\frac{d\Delta H_c'}{dn} = B \cong 23.5 \frac{\text{kJ}}{\text{mol}_{\mathrm{H_2O}}} \tag{5}$$

and the enthalpy of transition to the phase II (A') is of the order of $\sim$200 kJ/mol$_{\beta\text{-CD}}$.

On the other hand, to attain full dehydration, we must supply an energy $A+B$ for each mole of water that is present, and an energy A for each mole of water that is missing from the fully hydrated β-CD [see Eq. (4)]. This last contribution should be associated with a β-CD substrate which becomes more stable, i.e., more relaxed, the more water is taken out; this ''relaxation process'' of the substrate supplies, on average, a portion (A) of the overall energy ($A+B$) needed to take out a mole of water.

A direct estimate of the transition enthalpy can be obtained by comparing the DSC scans of native samples which have been kept at 3.3 h (Fig. 6 lower trace) and 24 h (Fig. 7 top trace) in wet nitrogen at 59 °C. The water lost during the scan is $n=1.6$ and $n=1.2$ for the bottom and the top traces

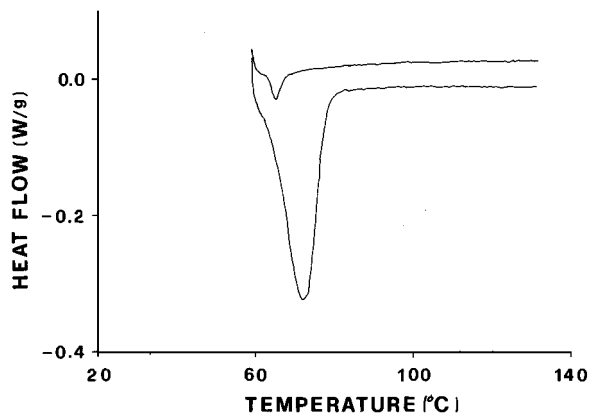

FIG. 7. DSC scans of samples annealed 3.3 h (bottom trace) and 24 h (top trace) in wet nitrogen at 59 °C.

respectively; the large difference between the $\Delta H_c'$ ($\sim$200 kJ/mol$_{\beta\text{-CD}}$) is due to the transition phenomena, and nicely agrees with $A'(N)$. From the top trace of Fig. 7, the dehydration enthalpy of phase II would be

$$\frac{d\Delta H_c^*}{dn} = B^* \cong 35 \frac{\text{kJ}}{\text{mol}_{\text{H}_2\text{O}}}. \tag{6}$$

Here, and in the following the symbols with asterisks will refer to phase II. An estimation of the energy needed to extract a mole of water in the absence of a modification of the substrate, ΔH_i, can be derived from dehydration isotherms taken at different temperatures [see Eq. (1)]. Within the usual ideal gas approximation, the Clausius–Clapeyron equation is

$$\left(\frac{\partial(\ln p)}{\partial T}\right)_n = \frac{\Delta H_i}{RT^2}, \tag{7}$$

where the partial derivative is performed under isosteric (n =const.) conditions. If n is a function of p/p_0, as is the case of the Langmuir law, we have

$$\frac{\partial(\ln p)}{\partial T} = \frac{\partial(\ln p_0)}{\partial T}. \tag{8}$$

By assuming a temperature-independent enthalpy we obtain

$$p_0(T) = p_\infty \exp\left(-\frac{\Delta H_i}{RT}\right). \tag{9}$$

From the dehydration isotherms we obtain $\Delta H_i \cong 50$ kJ/mol$_{\text{H}_2\text{O}}$, and $p_\infty \cong 9.5\times10^{10}$ Pa. ΔH_i is close enough to $A+B$, and uncertain enough, to allow us to conclude that DSC and isothermal dehydration data are in agreement.

V. THE NMR RESULTS

We have studied the ^{2}H NMR spectra of β-CD samples as a function of time spent at rt under dry N_2. The samples had been recrystallized from heavy water, and stored 10 months in a sealed vial in a dessicator. The initial water content was, on average, $n \cong 10$. Since parallel DSC scans did not yield a consistent rate of water loss (presumably because of heterogeneity of grain size), the water content was estimated directly from the NMR signal, collected under nonsaturating conditions. In Fig. 8 we display only the central portion of the ^{2}H spectrum, which has been assigned to the water deuterons.[26] The upper trace refers to a sample immediately after placement in the probe ($n \cong 10$) while the middle and the lower ones refer to the same sample after 11 h ($n \cong 6$) and 45 h ($n \sim 3$), respectively, in dry atmosphere.

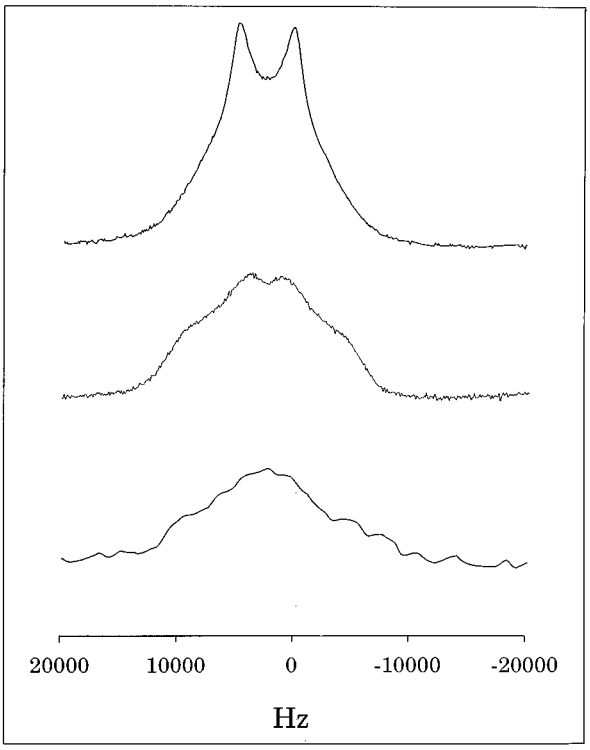

FIG. 8. Room temperature ^{2}H NMR spectra of water in β-CD nD$_2$O as a function of time spent in dry atmosphere: 0 h (top), 11 h (center), 45 h (bottom).

The structure seen in the upper trace is similar to that reported in the literature[26] for β-CD 11 D$_2$O at rt; it is due to fast dynamic averaging among cavity waters and interstitial waters within a single crystal, which leads to a residual coupling $\langle q_{cc}\rangle \cong 10$ kHz (or $\sim$5% of the expected rigid lattice value) with an average asymmetry parameter $\langle\eta\rangle$ of about 0.3.[26] With decreasing water content, we observe: (i) the growth of a central feature, (ii) the disappearance of the characteristic ''Pake doublet'' structure, (iii) a progressive and slight narrowing of the line. The last fact implies that the ^{2}H exchange rate among neighboring positions is always much larger than 10^6 s^{-1}.

If one could rule out any effect associated with long range diffusion of water among different crystals, it would be concluded that dehydration drastically changes the distribution of water within a crystal, leading to a spectrum roughly consistent with $\langle\eta\rangle = 1$ (bottom trace) and, generically, to a more disordered water arrangement (decreasing $\langle q_{cc}\rangle$). While it is expected[20] that occupancies of water positions and the associated residual coupling constant $\langle q_{cc}\rangle$ and $\langle\eta\rangle$ change with hydration, we believe that diffusion-assisted averaging among neighboring crystals dominate the evolution of the ^{2}H

spectrum shown in Fig. 8. Accordingly, the central spectral feature in the middle spectrum is due to a minority fraction of deuterons that move between neighboring crystals in a time $\tau \leqslant 10^{-5}$ s. This spectrum also implies a wide distribution of exchange times between crystals, with the majority of deuterons living in the original crystal more than 10^{-4} s. The bottom spectrum would result from a Pake doublet under ''intermediate'' exchange conditions, where most water deuterons migrate to another crystal in about $\sim 2\times 10^{-5}$ s.

VI. DISCUSSION

The thermodynamics of the irreversible water absorption in biopolymers has been studied, by Bryan, among others.[27–29] He concludes that the difference between the isosteric enthalpy, ΔH_i, and the calorimetric enthalpy per mole of water absorbed, dH'/dn, is due to a positive entropy term (ΔS) arising from the irreversible nature of the absorption process

$$\Delta H_i = -\frac{dH'}{dn} - T\Delta S = \frac{d\Delta H_c'}{dn} - T\Delta S. \tag{10}$$

Since $d\Delta H_c'/dn = B$ is a positive quantity, the isosteric enthalpy should be smaller than the heat released when absorbing a mole of water. If Eq. (10) also described the desorption process, we would conclude that the isosteric energy equals the difference of the heat of desorption and of a negative entropy term. Since ΔH_i has been estimated in the range 43/50 kJ/mol$_{H_2O}$, it would be, at rt, $-\Delta S > 64$ J/mol$_{H_2O}$K.

Such a large value cannot come from ordering of the hydrogen bond network,[15] which, in addition, appears to be incompatible with our NMR findings. In the absence of major changes in the vibrational spectra of partially dehydrated crystals (unreported observations), it is also difficult to imagine a modification of the β-CD substrate that is described in purely entropic terms. It seems more likely that some strain energy of the hydrated β-CD structure is released, which may be associated with reduction of the unit cell volume,[20] and rearrangement of the carbon environment, particularly of C4.[21] We may generically say that ''bonds'' among β-CD units are formed in place of bonds between β-CD and interstitial waters. Down to $n \cong 2$, and despite the existence of different types of water, dehydration seems to be a smooth process with constant $d\Delta H_c'/dn$ [see Eq. (4)]. The enthalpy of transition to phase II is equal to the overall contributions of these compensation mechanisms.

The existence of a structural phase transition with, apparently, a critical hydration level, but no critical temperature, is now discussed with the help of a model where we neglect higher order terms in an expansion of the thermodynamic potentials in n. In other words, we assume that enthalpy and entropy of β-CD in the phase I are linear and temperature-independent functions of the water content (n):

$$H'(n) = H_0' + n\frac{dH'}{dn}, \quad S'(n) = S_0' + n\frac{dS'}{dn} \tag{11}$$

and that similar equations hold for phase II, from $n=0$ up to a saturation limit n_{sat}^*:

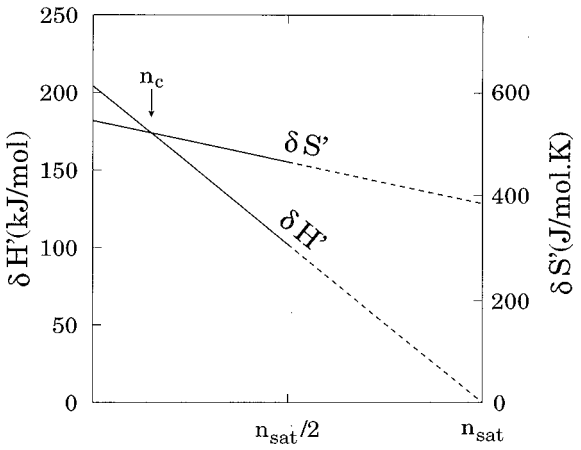

FIG. 9. Behavior of enthalpy (left-hand scale) and entropy (right-hand scale) differences between phase II and I as a function of n.

$$H'^*(n) = H_0'^* + n\frac{dH'^*}{dn}, \quad S'^*(n) = S_0'^* + n\frac{dS'^*}{dn}, \quad n \leqslant n_{sat}^*. \tag{12}$$

We use the experimental parameters A and B as follows:

$$-\frac{dH'}{dn} = B, \quad -\frac{dH'^*}{dn} = A + B. \tag{13}$$

The second equation, which is in agreement with Eq. (6), translates the expectation that no compensation is at work in phase II. The terms dS'/dn and dS'^*/dn are the entropies of water in phase I and in phase II, respectively. At most, they may range from $\sim$70 J/mol$_{H_2O}$K (liquid water) to $\sim$40 J/mol$_{H_2O}$K for tightly bonded water.[1] We will assume an intermediate value of 55 J/mol$_{H_2O}$K for the partially hydrophobic phase I, and the lower value (40 J/mol$_{H_2O}$K) for phase II. The limited range of choices make the assumptions about the thermodynamic parameters of water almost irrelevant in our case. The difference $H_0'^* - H_0'$ is experimentally determined. The only parameter left is n_{sat}^*, which may be expected to be of the order of the interstitial molecules ($n \cong 5.5$); therefore, we will set $n_{sat}^* = n_{sat}/2$.

A transition takes place at a critical hydration $n_c \leqslant n_{sat}^*$ where the free energies of the two phases become the same

$$\delta G = H'^*(n_c) - H'(n_c) - T[S'^*(n_c) - S'(n_c)] = 0. \tag{14}$$

A plot of $\delta H' = [H'^*(n) - H'(n)]$ and $\delta S' = (S'^* - S')$ versus n is shown in Fig. 9. It has been drawn by taking into account that a transition occurs at 59 °C with $\Delta H'(n_c = 1.6) \cong 200$ kJ; the extrapolation above $n > n_{sat}^*$ has been indicated with dashed lines: the entropy values can be read on the enthalpy scale by dividing by $T = (273.15 + 59)$ K. Some features of the model are illustrated in the (n,T) diagram of Fig. 10, where the coexistence curve given by Eqs. (12)–(14) is represented by a dotted line. Since in ordinary experiments the independent variables are p and T, we should use the Langmuir and Clausius–Clapeyron equations for the hydrated β-CD [Eqs. (1), (8)], and implicitly assume

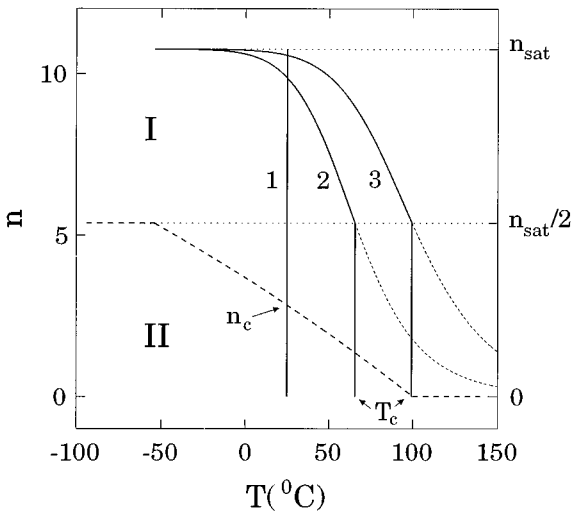

FIG. 10. Phase diagram of the β-CD–H_2O system: coexistence curve (dots), dehydration isotherm (1), isobaric transformations at $p=2000$ Pa (2), and $p=10^4$ Pa (3).

that the corresponding (and unknown) relationships for the dehydrated phase are compatible with the coexistence curve. We may distinguish the following regions.

(i) Low temperatures. Below -54 °C the critical water content becomes a constant because the coexistence curve reaches the n^*_{sat} limit. Since -54 °C is very close to the freezing temperature of the hydrogen network, this region would be extremely difficult to explore because of the slow kinetics. The critical pressure at -54 °C would be ~ 0.1 Pa.

(ii) Intermediate temperatures and pressures. Beginning with fully hydrated β-CD, we discuss an isothermal experiment at rt (curve 1) and an isobaric experiment at ambient humidity ($p=2000$ Pa) (curve 2). In both cases, phase I may become unstable before n_c through a mechanism described by the sequence of events

$$\beta\text{-CD(I)}n\text{H}_2\text{O} \Leftrightarrow \beta\text{-CD(II)}n\text{H}_2\text{O} \Rightarrow \beta\text{-CD(II)}n'\text{H}_2\text{O}+(n-n')\text{H}_2\text{O}\uparrow. \quad (15)$$

The first reaction may begin near $n=n^*_{sat}$ where the free energies of the two phases are rather similar; creation of phase II may be assisted by defects, surface effects, fluctuations of n or T, and any other cause that broadens a transition. The second reaction leads to an irreversible release of water, and a thermodynamically stable phase II with a lower ($n'<n$) water content. If the second reaction is so slow as to be neglected in the time needed to bring phase I from n^*_{sat} to n_c, the transition is observed near $n\cong n_c$. This is always the case of our isothermal dehydration experiments performed in dry atmosphere, since TGA experiments show that phase I desorbs faster than phase II. The rt isotherm (curve 1) is just a vertical line that crosses the coexistence curve at $n_c=2.8$. In the isobaric experiment at ambient humidity ($p=2000$ Pa, curve 2), the instability point is predicted at ~ 66°C, where $p_0(T)=p$ and $n=n_{sat}/2$. If desorption from phase II is fast enough (or the temperature scan is slow enough), n goes rapidly from n^*_{sat} to n_c, where the transition completes, and then to the near-zero equilibrium value of phase II.

(iii) High temperatures and pressures. Above $T=100$ °C, $n_c=0$, and only phase II exists below the instability point ($n<n^*_{sat}$). At 100 °C, phase I can exist only with $p>p_0(100\ ^\circ\text{C})=10^4$ Pa. Curve 3 traces the isobaric desorption at this pressure.

We are now in position to discuss the evidence supporting the assumptions of our model.

(a) Our isothermal dehydration experiments allow determination of n_c, as discussed before. However, while our estimates of n_c are consistent with the theoretical values, their experimental uncertainty is such that only the trend of the coexistence curve has been demonstrated;

(b) our choice $n^*_{sat}=n_{sat}/2$ is a simple way to translate the expectation that phase II may exist only after most of the hydration water has been released. From this assumption and the Langmuir isotherms, it follows that the transition temperature, under isobaric conditions, is a function of pressure; it should occur at 66 °C in air ($p=2000$ Pa, Fig. 10, trace 2) and at a partial pressure five times higher at 100 °C. In the DSC runs in ambient and wet atmospheres, we have consistently observed a sharp inflection point near 65 °C (see Fig. 5) when the instability point is reached. In fact, a faster heat intake is needed, initially, to sustain the faster desorption associated with the scheme of Eq. (15), and then, the full onset of the transition. Our interpretation is supported also by the observation[23,30] of a sharp increased rate of water release near 66 °C for experiments in air moisture.

Our diffraction data, and those of the literature,[14,19,20] suggest that dehydration causes a native crystal to break down, progressively and almost irreversibly, to a size (l) where Bragg's peaks broaden noticeably ($l\sim 10$ nm). Qualitatively, the exchange rate τ^{-1} of deuterons among crystals with average size l should be proportional to their long-range self-diffusion coefficient D:

$$\tau \sim \frac{l^2}{D}. \quad (16)$$

If we put $l=10$ nm, from our ^{2}H NMR spectrum, at rt and $n=6$, (middle trace of Fig. 7) we have that a substantial fraction of the deuterons has $D>10^{-11}$ m^2 s^{-1}. Since x-ray data imply that l cannot go below several lattice spaces ($a\cong 2.1$ nm), and τ is found to increase only modestly when dehydrating to $n=3$ (see the comments to Fig. 8, bottom trace), deuterons in phase I have a self-diffusion coefficient only 1–3 orders of magnitude smaller than in bulk water. We expect H^+ diffusion within a continuous hydrogen bond network to be percolative in nature, and strongly dependent upon hydration; on the other hand, the jumping of water molecules among neighboring sites may occur at a rate rather independent of n, and should be the mechanism controlling

the intergrain diffusion. For these reasons, we suggest that the changes observed in the ^{2}H NMR spectra in phase I are related with the long range diffusion of water molecules, rather than with motion of deuterons within a local hydrogen network. Computer simulations[31] and the fact that a hydration equilibrium sets up within minutes[19] (when $n>9$) confirm that water molecules have a liquidlike mobility in phase I.

VII. CONCLUSIONS

Dehydration of β-CD involves a structural phase transition to a distinct crystalline form (phase II). By assigning a simple mathematical form to the thermodynamic potentials and using experimental data, we derive an approximated phase diagram of the β-CD–water system. While qualitative, this diagram helps understanding the mechanism of phase transition and the role of nonequilibrium conditions. We have to distinguish between an ''instability point'' (n^*_{sat}) and the true thermodynamic transition, which takes place at the coexistence point (n_c). The inflections observed in both DSC and DTA runs during isobaric dehydration experiments in wet atmospheres are quantitatively interpreted as a landmark of the instability point. On the other hand, the isothermal dehydration experiments give information about the coexistence point. Our thermodynamic model is a simple way to account for the complex phenomenology of dehydration of β-CD.

We have found that phase I, at rt and above, is thermodynamically stable down to hydration levels of few water molecules, i.e., over a range of n much broader than that explored by recent x rays[19,20] and NMR studies.[21] Furthermore, dehydration is not strictly reversible in native samples, because fully rehydrated β-CDs have a systematically higher water content (n_{sat}). Both TGA and DSC data clearly show that this extra water is not absorbed on the surface because it has the same thermodynamic properties of water in native β-CD. While it was expected that the crystal breakdown which accompanies dehydration yields a more disordered structure, with a higher concentration of water defects, it is apparently difficult to understand how samples with different amounts of water within the cavity, outside the cavity, and in defect positions, may have essentially the same dehydration behavior.

An explanation of the last point is possibly related with the most important finding of this work, i.e., a ''relaxation,'' or energy release, of the β-CD substrate which assists the dehydration process. Qualitatively, the more bonded the water is, the larger the relaxation contribution of the substrate is, and a constant dehydration enthalpy per mole of water (B) may result. This is consistent with our observations because the dehydration isotherms and the $\Delta H'$ vs n curves [Eqs. (2)–(4)] give only average values of the substrate contribution (A). Thus, the compensation mechanism may be described by a single energy which embodies a property of the macromolecular substratum, rather than the microscopic details of its interactions with water. This is the core of our message which, we hope, may assist an understanding of the thermodynamics of other hydrated biomolecules. Our guess is that the compensation mechanism is at work in many biological systems and plays a role in their stability. We observed a reversible hydration which is certainly related with the fact that we may assign the same enthalpy to the fully hydrated and fully dehydrated configurations of the β-CD substrate. Dehydration is expected to be irreversible if the biomolecule reaches a dehydrated configuration which is substantially more stable than the hydrated one. We acknowledge that experimental determination of this enthalpy difference may be difficult, particularly in glasses and inherently metastable systems where a thermodynamic state may be ill known. However, if Dunitz[1] is right in arguing that the thermodynamic parameters for water in most biological systems are very similar, different hydration behaviors are related with the ''energy landscape'' of the accessible configurations of the biomolecules.

A complex hydrated system may be studied, and simulated, as a whole or we may try to understand its behavior with the help of information available about its constituents. Since there is not a unique way of breaking down the interactions among these constituents, it may be difficult to bridge between alternative descriptions and microscopic models. Our experimental determination of enthalpy changes ascribed to the biomolecular component alone may help in finding a sensible description for hydration of biomolecules.

ACKNOWLEDGMENTS

The NMR measurements have been performed at Laboratorio Risonanze Magnetiche of Centro Grandi Strumenti of the University of Pavia (Italy). M.V. is associated with the Istituto di Fisica della Materia, Unitá di Pavia.

[1] J. D. Dunitz, Science **264**, 670 (1994).
[2] W. P. Bryan, Science **266**, 1726 (1994).
[3] L. E. S. de Souza and D. Ben-Amotz, J. Chem. Phys. **101**, 9858 (1994).
[4] B. Mennucci, M. Cossi, and J. Tomasi, J. Chem. Phys. **102**, 6837 (1995).
[5] J. A. Given, J. Chem. Phys. **102**, 2934 (1995).
[6] D. J. Olbris, A. Ulman, and Y. Shnidman, J. Chem. Phys. **102**, 6865 (1995).
[7] P.-O. Åstrand, A. Vallqvist, and G. Karlström, J. Chem. Phys. **100**, 1262 (1994).
[8] G. H. Peters, S. Toxvaerd, A. Svendsen, and O. H. Olsen, J. Chem. Phys. **100**, 5996 (1994).
[9] P. E. Smith and W. F. Gunsteren, J. Chem. Phys. **98**, 13735 (1994).
[10] W. Saenger and T. Steiner, in *Water-Biomolecule Interactions*, SIF Conference Proceeding, Vol. 43, edited by M. U. Palma, M. B. Palma-Vittorelli, and F. Parak (SIF, Bologna, 1993), p. 55.
[11] B. Zhang and R. Breslow, J. Am. Chem. Soc. **115**, 9353 (1993).
[12] Y. Inoue, Y. Liu, L. Tong, B. Shen, and D. Jin, J. Am. Chem. Soc. **115**, 10637 (1993).
[13] A. Marini, V. Berbenni, G. Bruni, P. Mustarelli, F. Giordano, and M. Villa, J. Inclus. Phenom (in press).
[14] C. Betzel, W. Saenger, B. E. Hingerty, and G. M. Brown, J. Am. Chem. Soc. **106**, 7545 (1984).
[15] H. Hanabata, T. Matsuo, and H. Suga, J. Inclus. Phenom. **5**, 325 (1987).
[16] V. Zabel, W. Saenger, and S. A. Mason, J. Am. Chem. Soc. **108**, 3664 (1986).
[17] K. Pathmanathan, G. P. Johari, and J. A. Ripmeester, J. Phys. Chem. **93**, 7491 (1989).
[18] K. Lindner and W. Saenger, Carbohydr. Res. **99**, 103 (1982).
[19] T. Steiner, G. Koellner, S. Ali, D. Zakim, and W. Saenger, Biochem. Biophys. Res. Commun. **188**, 1060 (1992).
[20] T. Steiner and G. Koellner, J. Am. Chem. Soc. **116**, 5122 (1994).
[21] J. A. Ripmeester, Supramol. Chem. **2**, 89 (1993).
[22] A. Szafranek, J. Thermal Anal. **34**, 917 (1988).
[23] Y. Nakai, K. Yamamoto, K. Terada, A. Kajiyama, and I. Sasaki, Chem. Pharm. Bull. **34**, 2178 (1986).

[24] R. E. Powell, *Physical Chemistry, in Fundamental Formulas of Physics*, editor D. H. Menzel (Dover, New York, 1960), Vol. 2, p. 655.
[25] G. Spinolo, V. Massarotti, and G. Campari, J. Phys. E **12**, 1059 (1979).
[26] M. G. Usha and R. J. Witebort, J. Am. Chem. Soc. **114**, 1541 (1992).
[27] W. P. Bryan, Biopolymers **25**, 1967 (1986).
[28] W. P. Bryan, Biopolymers **26**, 387 (1987).
[29] W. P. Bryan, Biopolymers **26**, 1705 (1987).
[30] S. Kohata, K. Jyodoi, and A. Ohoyoshi, Thermochim. Acta **217**, 187 (1993).
[31] J. E. H. Koehler, W. Saenger, and W. F. Gunsteren, Eur. Biophys. J. **15**, 211 (1987).