Cooperative Hydration Effect on the Binding of Organic Vapors by a Cross-Linked Polymer and Beta-Cyclodextrin

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Summary: A cooperative hydration effect being favorable for the binding of organic vapors by cross-linked poly(N-6-aminohexylacrylamide) and beta-cyclodextrin was observed in ternary systems in the absence of liquid phase. For these systems the vapor sorption isotherms were determined by the static method of headspace gas chromatographic analysis at 298 K. The obtained isotherms show an increase of binding affinity for vapor of hydrophobic sorbates above a threshold value of receptor hydration. Further hydration gives a saturation of this affinity for the studied hydrophilic polyacrylamide derivative, while the affinity of beta-cyclodextrin for the hydrophilic sorbate ethanol even decreases. A similar behavior of this polymer and beta-cyclodextrin at the change of their hydration helps to explain the observed cooperative hydration effect in terms of clathrate formation.

Keywords: clathrate formation; cooperative hydration effects; headspace GC analysis; macromolecular receptor; vapor sorption isotherms

Introduction

An important feature of an efficient and selective macromolecular receptor is a substrate binding cooperativity that mimics the cooperative properties of proteins. The molecular design of such a receptor is often confined to the synthesis of molecularly imprinted polymers with the binding sites similar to those of antibodies and enzymes.[1] Thermotropic polymers with their specific hydrophobic-hydrophilic balance mimic the cooperative stimulus-responsive behavior of proteins[2] including protein folding.[3] This property linked to the change of a receptor hydration is used in the encapsulation/release of drugs by polymers in biomedical applications.[2] The change of protein hydration gives also a number of other cooperative effects such as the

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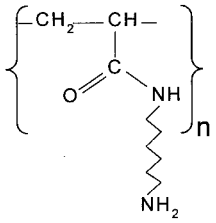
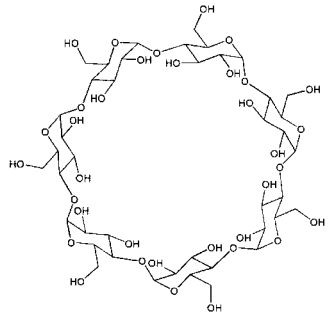
cooperative hydration influence on the rates of enzymatic reactions,[4] on the binding of hydrophobic and relatively large neutral monofunctional compounds[5] and on the protein specific heat capacity.[6] This hydration cooperativity can be seen in a hydration history effect for enzymes.[7] Besides, some proteins have more specific cooperative behavior as in the binding of oxygen by hemoglobin in water solution.[8]



cross-linked poly(N-6-aminohexylacrylamide)
(PAA)



beta-cyclodextrin
(BCD)

To reveal the structural requirements for a receptor to have the biomimetic cooperative properties the structure-property relationship was studied in this work for hydration effect on the binding properties of very different types of hydrophilic receptors: cross-linked poly(N-6-aminohexylacrylamide) (PAA) and beta-cyclodextrin (BCD). The vapor sorption isotherms obtained for these receptors in ternary systems with water-organic vapors in the absence of liquid phase were compared with the data on the cooperative behavior of human serum albumin in the same conditions[5] and hydrophobic calixarene, for which the cooperative binding of guest vapor is observed in binary host-guest systems.[9] This is the first study showing a cooperative hydration effect that helps a hydrophilic polymer and macrocyclic receptor to bind hydrophobic substrates instead of interfering with this process.

Experimental Part

The cross-linked poly(N-6-aminohexylacrylamide) (PAA) and beta-cyclodextrin (BCD) were purchased in Aldrich and ICN, respectively. The PAA studied was in the shape of beads with the average diameter of 100 μm. It was dried for 5 months over P_2O_5 at room temperature before the

sorption experiment to remove hydration memory effects. BCD was dried for 8 hours at 100°C and 1 mm Hg. To prepare the prehydrated BCD its dried 200-mg samples in open 15-ml glass vials were saturated by water vapor in hermetically closed vessel at 298 K. The residual hydration of dried PAA and BCD and the water contents in the prehydrated BCD were determined using the method of thermogravimetry as described elsewhere.[10]

The vapor sorption isotherms were determined by static method of headspace GC analysis as described earlier.[5,10] For this experiment the samples of dried PAA (100 mg), dried (200 mg) or prehydrated (234 mg) BCD were equilibrated for 72 hours in the hermetically closed 15-ml vials with organic or water-organic vapors at 298 K. The equilibration time was chosen using the estimations of the sorption kinetics and kinetics of sorbate evaporation. The liquid sorbate or water-organic mixture with 6:94 and 1:4 organic component/water volume ratios for PAA and BCD, respectively, was dosed in the open small internal vials to avoid a direct contact between a liquid sorbates and solid receptor. The absence of liquid phase after equilibration was checked visually. The error of sorbate thermodynamic activity P/P_0 determination is in the range from 10% at P/P_0 <0.10 to 5% at P/P_0 >0.5. The error of sorbate uptake A determination is 5%. Each error is mostly systematic and has low influence on the shape of obtained sorption isotherms. The total hydration value h of receptor was calculated using its initial hydration value and the amount of water added with the sorbate. The correction on the water contents in the vapor phase of the system was made. The estimated error of the receptor hydration value is ±0.002 h (= 0.002 g H_2O per g of dry receptor).

Results and Discussion

The vapor sorption isotherms obtained for ternary systems with PAA and BCD hydrated *in situ*, together with the saturation by the vapor of organic component, are given on Figure 1 and 2, respectively, showing two different 2D cross sections of the three-dimensional isotherms. On the Figures 1A and 2A 'pure liquid sorbate – receptor' partition coefficient $A/(P/P_0)$ is plotted as a function of receptor hydration h. On Figures 1B and 2B the sorbate uptake A is plotted as function of sorbate activity for the same isotherms. Besides, on Figures 1 and 2 the vapor sorption isotherms for ternary systems of human serum albumin[5] prepared in the same way are shown for comparison. The contents of the water/organic mixtures added to the studied receptors were

chosen to reduce the contribution of PAA plasticization by organic component and to create optimal conditions for the clathrate formation with BCD. A simultaneous saturation of dried hydrophilic macromolecular receptors by water-organic vapors provides more reproducible sorption data because it is easier to remove the hydration memory effects from this initial state of sorption process.

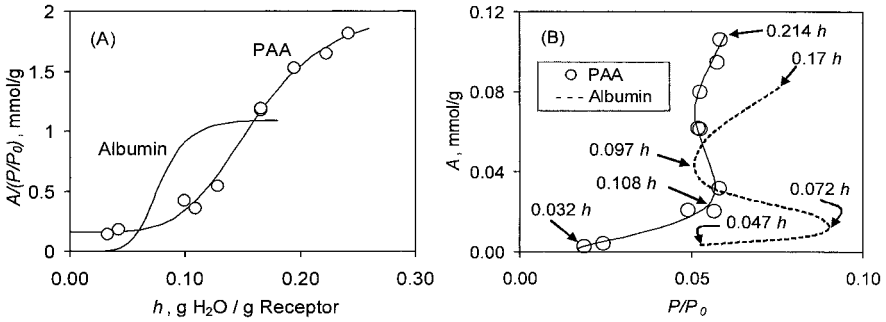


Figure 1. The hydration effect on the vapor sorption of ethyl acetate by cross-linked poly(N-6-aminohexylacrylamide) (PAA) with initial hydration 0.004 *h* at 298 K in two different presentations of the same sorption isotherm: (A) as a function of 'pure liquid sorbate – polymer' partition coefficient [*A/(P/P0) vs.* polymer hydration *h*] and (B) sorbate uptake *A vs.* sorbate activity P/P_0. The contents of organic component in liquid water-organic mixture added to the initially dried polymer is 6 vol. %. The vapor sorption isotherm of ethyl acetate by human serum albumin (initial hydration 0.01 *h*) determined in the same conditions is from Ref.5. The lines are drawn to guide the eye.

The sigmoidal shape of the vapor sorption isotherm of ethyl acetate by the studied cross-linked polyacrylamide derivative (Figure 1A, B) reveals rather high cooperativity of the hydration effect on the binding properties of this polymer. The hydration up to 0.11 *h* does not increase much the free volume of PAA accessible for organic component. Above hydration level of 0.11 *h* the sorption affinity of PAA for ethyl acetate cooperatively increases (Figure 1A) indicating a small decrease of sorbate activity P/P_0 at the increase of sorbate uptake *A* (Figure 1B). This cooperativity is similar to that of albumin[5] in the same conditions (Figure 1). The observed inflections of sorption isotherm for PAA on Figure 1B are less deep than for albumin. A separate liquid phase is not observed after equilibration in all studied range of PAA hydration.

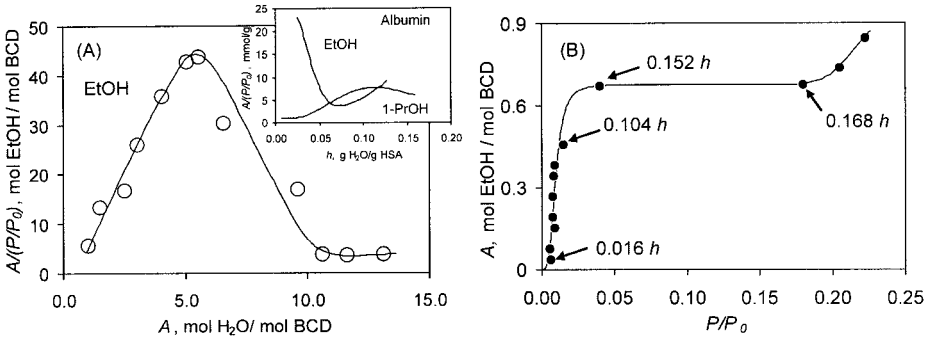


Figure 2. The hydration effect on the vapor sorption of ethanol by beta-cyclodextrin (BCD) with the initial hydration of 0.008 *h* at 298 K in two different presentations of the same sorption isotherm: (A) as a function of 'pure liquid sorbate – polymer' partition coefficient $A/(P/P_0)$ *vs.* BCD hydration and (B) sorbate uptake A *vs.* sorbate activity P/P_0. The contents of organic component in liquid water-organic mixture added to the initially dried polymer is 20 vol. %. The inset shows the vapor sorption isotherms of ethanol and 1-propanol by human serum albumin (initial hydration 0.01 *h*, contents of the organic component in the water-organic mixture added is 6 vol. %, $T = 298$K) from Ref.5. The lines are drawn to guide the eye.

The sigmoidal shape of sorption isotherms both of PAA and albumin can be explained supposing formation of clathrate 'water + ethyl acetate + receptor'. The formation of similar clathrates for γ-chymotrypsin was confirmed by X-ray data.[11] The same may be supposed to make a significant contribution to the sorption of ethyl acetate by PAA.

To make more definite conclusion on the nature of the molecular interactions causing the sorption of organic component in this system, we studied the binding properties of another hydrophilic receptor, BCD, which binds organic compounds in solid phase only through clathrate formation.[12] For BCD the isotherm of ethanol vapor sorption in the presence of the simultaneously added water show a definite saturation, Figure 2. In the '$A/(P/P_0)$ *vs.* *h*' presentation this ethanol sorption isotherm resembles the isotherm of 1-propanol vapor sorption by albumin in comparable conditions[5] (Figure 2A, inset), which also has a maximum in these coordinates, but less expressed. The isotherm of ethanol vapor sorption by albumin has a very different shape,[5] Figure 2A, inset. For albumin no saturation by ethanol at high hydration is observed, but this isotherm has a minimum at 0.07 h, which reflect a Langmuir shape of ethanol sorption isotherm (A *vs.* P/P_0) at low ethanol activity, P/P_0, for dried albumin[13] (Figure 3, inset).

The experimental point on the saturation part of the sorption isotherm on Figure 2B at the BCD hydration 0.152 *h* corresponds to the molar ratio of solid phase components 1 : 0.7 : 9 (BCD : ethanol : water), which is not much far from the composition of BCD-ethanol-water clathrate 1 : 1 : 8 studied by neutron diffraction and X-ray methods.[14] The isotherm inflection above BCD hydration 0.168 *h* is caused by the existence of separate liquid phase in the system after its equilibration. In binary BCD-water system a separate liquid phase forms above hydration 0.175 *h* at water activity P/P_0>0.93.[15] So, according to the slope of sorption isotherms at high receptor hydration, the ability of three hydrophilic macromolecular receptors to perform a saturation limit by organic component at the simultaneous hydration increase decreases in the order: BCD>albumin>PAA. This may be caused by the decreasing contribution of clathrate formation for the sorbate binding by these receptors in the same order. The influence of the receptor homogeneity on the shape of hydration effect on the ethanol binding by BCD and its cooperativity can be seen in the comparison of the binding properties of dried and hydrated BCD. The obtained vapor sorption isotherms of ethanol by dried BCD with residual hydration 0.008 *h* and of benzene by BCD dried and prehydrated to 0.172 *h* are shown on Figures 3 and 4, respectively. For comparison the ethanol sorption isotherms for albumin[13] and clathrate forming host *tert*-butylcalix[4]arene (TBC4)[16] earlier obtained in the same conditions are shown on Figure 3. Besides, the isotherm of benzene vapor sorption by TBC4[16] is given on Figure 4. No sorption of propanols, ethyl acetate and benzene from vapor phase exceeding 0.2 mol/mol BCD was observed for dried BCD (0.008 *h*) at sorbate activity P/P_0 < 0.8. The observed sigmoidal shape of the ethanol sorption isotherm for dried BCD is the same as

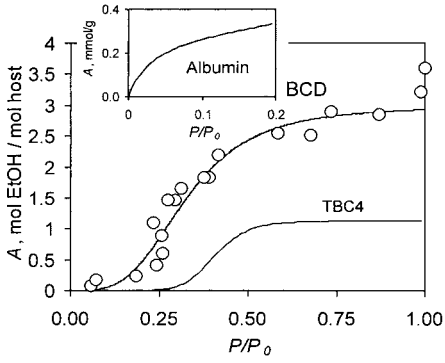


Figure 3. The vapor sorption isotherm of ethanol by the dried beta-cyclodextrin (BCD) with the residual hydration of 0.008 *h* at 298 K. The inset shows the isotherm of ethanol vapor sorption by dried human serum albumin (hydration 0.01 *h*) determined in the same conditions from Ref.13. The vapor sorption isotherm of ethanol by solid *tert*-butylcalix[4]arene (TBC4) at 298 K is from Ref.16. The sigmoidal lines were calculated using Hill equation (1).

for TBC4 (Figure 3) and is typical for clathrate formation at the binding of guest vapor by homogeneous solid hosts.[9,17] Any inhomogeneity caused, for example, by the presence of second guest in the solid host may reduce the threshold value of guest activity P/P_0 almost to zero removing the apparent cooperativity.[16] This may be a cause of Langmuir-like isotherm shape of ethanol vapor sorption by dried albumin at low ethanol activity P/P_0 (Figure 3, inset). Dry albumin has practically no surface of protein-air interface that can give a significant Langmuir contribution to the sorption.[13]

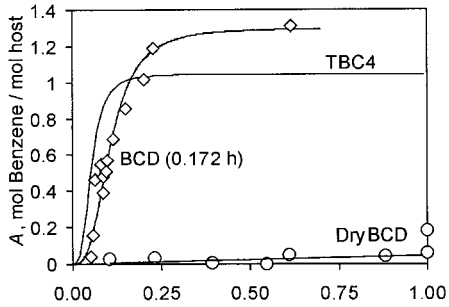


Figure 4. The vapor sorption isotherms of benzene by the dry and prehydrated beta-cyclodextrin (BCD) with the constant hydration of 0.008 *h* and 0.172 *h*, respectively, at 298 K. The vapor sorption isotherm of benzene by *tert*-butylcalix[4]arene (TBC4) at 298 K is from Ref. 16 . The sigmoidal lines were calculated using Hill equation (1).

The isotherms obtained for BCD were fitted by Hill equation[9]:

$$A = SC(P/P_0)^N / (1 + C(P/P_0)^N) \qquad (1)$$

where inclusion stoichiometry S, a co-operativity parameter N and a sorption constant C are the fitting parameters. Their values for ethanol isotherm are S = 3.0 (mol EtOH/mol BCD), N=3.5 and C = 49.3. The ethanol threshold activity at the 50% saturation is equal to $a_{0.5S} = \exp(-(\ln C)/N) = 0.33$. The lower is the value of $a_{0.5S}$, the higher is host-guest affinity.[9]

The comparison of isotherms given on Figures 2-4 shows how much the hydration changes the binding properties of BCD. Hydrated BCD has much lower threshold value of ethanol activity, $a_{0.5S}$ <0.007, and lower inclusion stoichiometry, S = 0.7, than the dried receptor. These changes cannot be non-cooperative, but a measure of this cooperativity is not easily seen. The more profound hydration effect is observed for benzene sorption by BCD. Dry BCD with residual hydration 0.008 *h* does not bind benzene vapors (Figure 4). The same was observed at least up to BCD hydration of 0.06 *h* and benzene activity P/P_0 = 0.8. But BCD with high hydration of 0.172 *h* binds benzene with almost the same efficiency (S=1.3, N=3.0, C=800, $a_{0.5S}$ = 0.11) as TBC4 (Figure 4), which does not need water for it. So, as well as albumin and PAA in systems with ethyl acetate, BCD has a hydration threshold

for the binding of benzene. This threshold is a cause of S-like shape of sorption isotherms on Figure 1A.

Conclusions

Obtained results are the first data describing a favorable cooperative hydration effect on the binding of organic vapors by a hydrophilic cross-linked polymer and beta-cyclodextrin. Despite these two hydrophilic receptors and earlier studied protein (albumin) have very different structure, they have important common features. Without hydration all of them have rather tight packing preventing from the binding of relatively large or/and hydrophobic compounds like ethyl acetate and benzene. Still the structure of the studied receptors is relatively flexible, which can be seen from the cooperative increase of their binding ability above a threshold hydration value. The shape of this hydration effect is similar for these three types of receptors. Albumin occupies an intermediate position between the studied cross-linked polyacrylamide derivative and beta-cyclodextrin by its ability to perform saturation by organic compound at the simultaneous hydration increase. This ability, decreasing in the order BCD>albumin>PAA, may be linked to the capacity of hydrated receptor to form clathrates with organic component.

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