Percolation Transition of Hydration Water: From Planar Hydrophilic Surfaces to Proteins

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The formation of a spanning hydrogen-bonded network of hydration water is found to occur via a 2D percolation transition in various systems: smooth hydrophilic surfaces, the surface of a single protein molecule, protein powder, and diluted peptide solution. The average number of water-water hydrogen bonds n_H at the percolation threshold varies from 2.0 to 2.3, depending on temperature, system size, and surface properties. Calculation of n_H allows an easy estimation of the percolation threshold of hydration water in various systems, including biomolecules.

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The existence of a spanning network of hydration water in biosystems enables their biological functions [1–6]. With an increasing hydration level, an ensemble of finite (nonspanning) clusters of hydration water transforms via a quasi-2D percolation transition into a state with a spanning water network [4,7]. The full dynamics of biomolecules is restored, when they are covered by about a "monolayer" of water. The first appearance of such a monolayer corresponds to a quasi-2D percolation transition of the hydration water at the surface of a single biomolecule [7,8]. Simulation studies of various properties of hydrated biosystems below and above the percolation threshold of the hydration water can help to clarify the role of the spanning water network in the onset of biological functions. Such studies require the knowledge of the percolation threshold of water in the system of consideration. This information can be obtained by conventional percolation analysis of water clustering [9–12], which is extremely time consuming. In this Letter we propose a simple method to locate the percolation threshold of hydration water even in complex systems, using the average number of water-water hydrogen bonds. This method is derived from extensive computer simulation studies of the percolation transition of hydration water in various systems: water adsorbed at smooth hydrophilic planes and spheres [7,13,14], at surfaces of rigid and flexible single lysozyme molecules and lysozyme powder [7,8], hydration water in protein solutions [15].

Water molecules are considered to belong to the same cluster if they are connected by an uninterrupted path of hydrogen bonds. Water clustering was studied by computer simulations at various hydration levels in low-hydrated systems [7,14] and at various temperatures and widths of the hydration shell for proteins in solution [15]. The percolation threshold of water at planar surfaces and in a model lysozyme powder (both systems are infinite due to the periodic boundary conditions) can be located by using the spanning probability, cluster size distribution, and fractal dimension of the largest cluster. In closed systems, such as a surface of a finite object, an *infinite* cluster cannot appear and the spanning probability is not defined. However, the formation of a *spanning* network of hydration water at the surface of a smooth hydrophilic sphere and of a single biomolecule, was found to occur in a way similar to infinite systems [7,8,13–15].

The probability distribution n_S of cluster sizes S obeys a universal power law $n_S \sim S^{-\tau}$ in the widest range of S at the percolation threshold, with $\tau \approx 2.05$ for 2D and $\tau \approx 2.2$ for 3D percolation [9]. In Fig. 1 we show the cluster size distributions n_S of water molecules in the hydration shell of the smallest studied system, the fully hydrated elastinlike peptide GVG(VPGVG)$_3$, for various

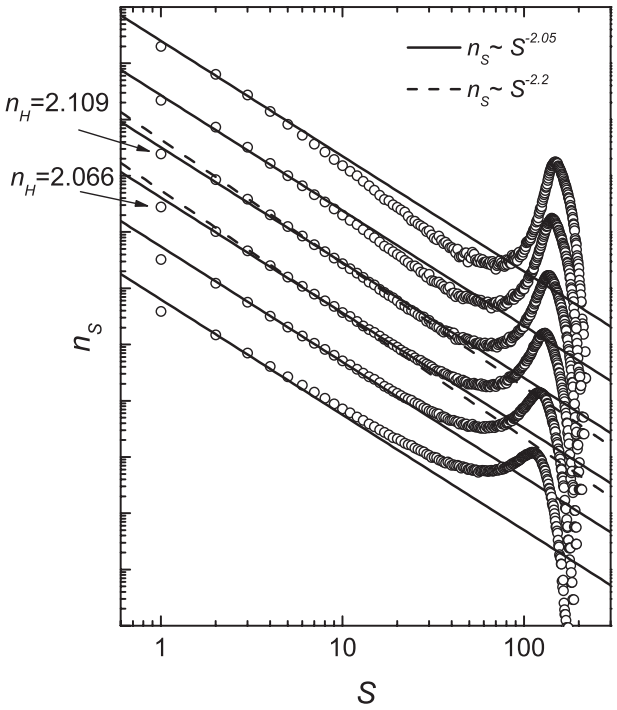

FIG. 1. Cluster size distribution n_S in the hydration shell of an elastinlike peptide at $T = 300$ K for several widths D of the hydration shell, which corresponds to the following numbers N_w of water molecules in the shell: 135, 141, 147, 153, 159, and 164 (from bottom to top). Power laws for 2D and 3D percolation thresholds are shown by solid and dashed lines, respectively.

sizes of the shell width D. A hump in n_S at large S reflects the truncation of the large clusters due to the finite size of the hydration shell. The behavior of n_S allows the location of the percolation threshold between $N_w = 147$ and $N_w = 153$, even without any assumption about the dimensionality of the transition.

The dimensionality of the percolation transition can be determined from the effective fractal dimension d_f of the largest water cluster, which can be obtained from a fit of its mass distribution: $m(r) \sim r^{d_f}$. The range of r, used in the fits of $m(r)$, is determined by the system size. We limited this range by the half of the simulation box L for planar surfaces and lysozyme powder, by the diameter of the spherical surfaces, and by the shortest value of the effective axis of a single lysozyme molecule, which is about 24 Å. At the percolation threshold, the fractal dimension of the largest cluster is $d_f^{2D} \approx 1.9$ for a 2D system and $d_f^{3D} \approx 2.53$ for a 3D system [9]. We have found that in low-hydrated systems the value of d_f is close to the value d_f^{2D} [7,14]. This indicates the quasi-2D character of the percolation of adsorbed water. In particular, the threshold hydration level and the dimensionality of the water percolation transition in model lysozyme powder remarkably agrees with experimental results [4]. Note that the fractal dimension of the largest water cluster in the hydration shell of the elastinlike peptide in solution cannot be determined due to the small system size (the average radius of gyration of this peptide does not exceed about 8 Å).

The percolation thresholds of hydration water in all systems studied are given in Table I in terms of N_1, the number of water molecules in the first hydration shell per unit surface area. For lysozyme powder, N_1 is simply the total number of water molecules divided by the number of protein molecules and the surface area of a single protein. The values obtained for the threshold hydration level in terms of N_1 noticeably vary from one studied system to another and also depend on temperature. These variations of N_1 at the threshold should be expected, as this surface coverage is similar to the occupancy variable in lattice models, which is essentially nonuniversal. For random percolation in 2D lattices, the threshold value of site and bond occupancy depends on the lattice type [9]. The honeycomb lattice with 3 neighbors and the square lattice with 4 neighbors seem to be the most relevant for water adsorbed at hydrophilic surfaces [13,16]. For these lattices, the threshold values of the occupancy variable are ~0.70 and ~0.59 for site percolation and ~0.65 and 0.50 for bond percolation, respectively. These threshold values are more universal in terms of the average numbers of bonds, which are ~2.09 and ~2.37 for site percolation and ~1.96 and 2.00 for bond percolation, on the honeycomb and square lattices, respectively. As the water percolation process can be considered as a correlated site-bond percolation [17], one may expect a rather universal threshold value in various hydrated systems in terms of n_H, the average number of water-water H bonds, formed by each water molecule. Such expectation is supported by the observed similarity between percolation in bulk liquid water and random bond percolation in 3D lattice. In bulk liquid water, n_H at the percolation threshold is about 1.53 [18], i.e., rather close to the value 1.55 for bond percolation in a 3D diamond lattice with 4 neighbors [9].

The dependence of the fractal dimension d_f on n_H for some of the systems studied is shown in Figs. 2 and 3. Below the percolation threshold, d_f is essentially an *effective* fractal dimension, because most of the largest water

TABLE I. Number of water molecules in the first (N_1) and second (N_2) hydration shells per unit surface area and the average number of water-water hydrogen bonds n_H at the quasi-2D percolation thresholds of various model systems and proteins.

System	T/K	$N_1/100$ Å^2 (± 0.05)	N_2/N_1 (± 0.005)	n_H (± 0.01)
Plane 80×80 Å^2	425	7.5	0.043	2.20
Plane 100×100 Å^2	425	7.4	0.043	2.22
Plane 150×150 Å^2	425	7.3	0.044	2.22
Sphere $R = 15$ Å	425	8.6	0.12	2.11
Sphere $R = 30$ Å	425	8.5	0.11	2.14
Sphere $R = 50$ Å	425	8.3	0.10	2.15
Sphere $R = 15$ Å	475	9.1	0.36	1.95
Lysozyme powder	300	2.1[a]	···	2.32
Rigid lysozyme	300	4.9	0.38	2.30
Flexible lysozyme	300	5.0	0.44	2.30
Lysozyme powder	400	2.5[a]	···	2.02
Rigid lysozyme	400	5.1	0.98	2.05
Elastin	320	10.8	···	2.08
Elastin	300	10.3	···	2.10
Elastin	280	9.8	···	2.10
Elastin	260	9.2	···	2.08

[a]Total number of water molecules per surface area of all lysozyme molecules.

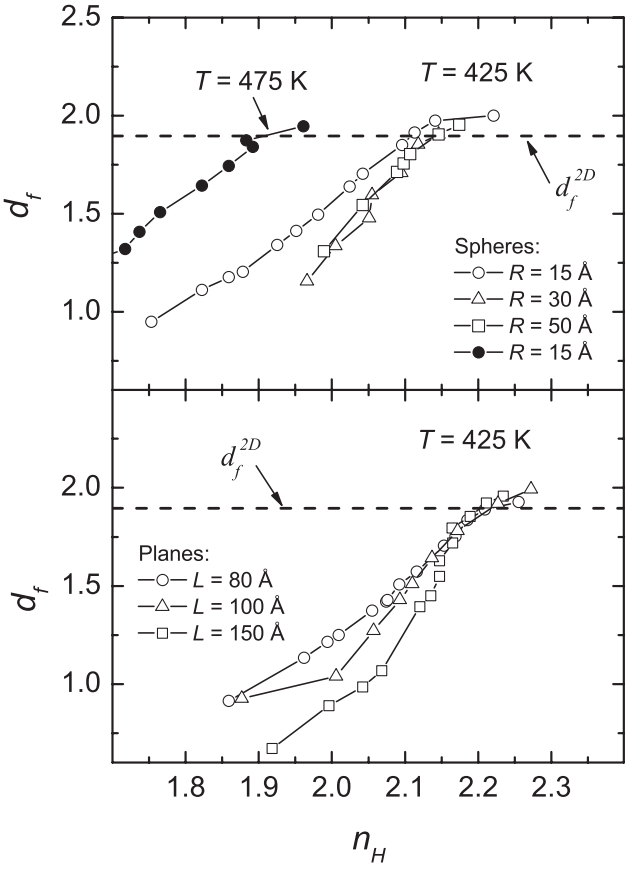

FIG. 2. Fractal dimension of the largest cluster d_f as a function of the average number n_{H} of H bonds between water molecules near smooth hydrophilic planar (lower panel) and spherical (upper panel) surfaces.

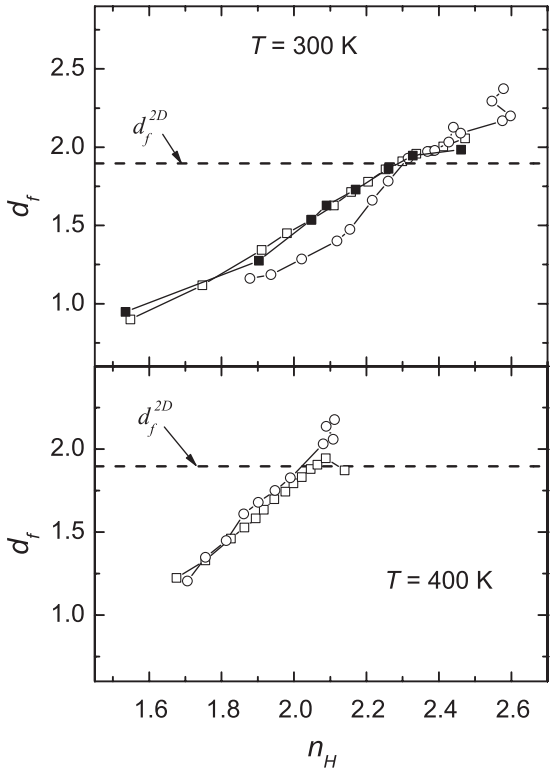

FIG. 3. Fractal dimension of the largest cluster d_f as a function of the average number n_{H} of H bonds between water molecules at the surface of rigid (open squares) and flexible (solid squares) lysozyme and in the hydrated lysozyme powder (open circles).

clusters are not fractal objects. That is why the values of d_f noticeably depend on the system size and geometry at low hydration levels. At the percolation threshold, the structure of the largest water cluster is close to a fractal and d_f approaches the threshold fractal dimension $d_f^{2\mathrm{D}}$ for 2D percolation. For three studied planar surfaces of different sizes, the percolation thresholds of hydration water practically coincide: at $T = 425$ K they are located at $n_{\mathrm{H}} = 2.21 \pm 0.01$ and $N_1 = 0.074 \pm 0.001$ Å^{-2}. At the spherical surfaces, the percolation threshold corresponds to $N_1 = 0.085 \pm 0.002$ Å^{-2}, that is about 15% higher than the threshold value of N_1 for planar surfaces. However, the value of n_{H} at the threshold is 2.13 ± 0.02 for spherical surfaces, that is, just 4% lower than for the planar surface. The higher threshold hydration levels N_1 for the spheres are accompanied by higher concentrations of water molecules in the second hydration shell N_2. The ratio N_2/N_1 is almost 12% for spheres, whereas it is only 4% for the planar surfaces of the same hydrophilicity (see Table I). The presence of water molecules in the second shell effectively reduces the number of water-water H bonds in the first shell, thus resulting in a higher threshold value of N_1 and a lower threshold value of n_{H}. Such a shift of n_{H} to lower values can be considered as a trend toward three dimensionality, where n_{H} is about 1.53 at the percolation threshold of bulk liquid water [18].

A similar trend toward larger threshold values of N_1 and smaller values of n_{H} is observed with increasing temperature. When the temperature rises from $T = 425$ to 475 K, the threshold value of N_1 for a sphere with radius $R = 15$ Å increases from 0.086 to 0.091 Å^{-2}, whereas the fraction of molecules in the second hydration shell increases and N_2/N_1 changes from 0.12 to 0.36. This is accompanied by a decrease of n_{H} from 2.11 to 1.95. Hence, an increase of temperature by 100° causes a decrease of the n_{H} value at the threshold by about 15%. This trend corresponds to the growing importance of the ''bond'' percolation relative to the ''site'' percolation with increasing temperature in site-bond percolation of water.

At the surfaces of the rigid lysozyme molecule, the flexible lysozyme molecule, and in the model lysozyme powder, the percolation transition of hydration water at ambient conditions ($T = 300$ K) occurs when the average number of water-water H bonds n_{H} is 2.31 ± 0.01 (see Table I and Fig. 3). Intriguingly, the value of n_{H} at the percolation threshold remains highly universal even in such complex systems as hydrated proteins. An increase of the temperature to $T = 400$ K reduces the threshold

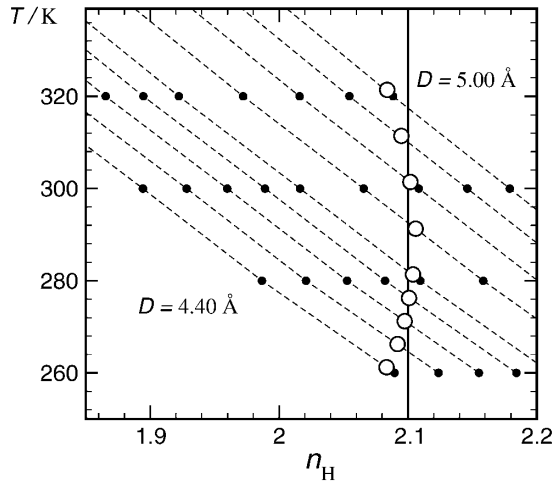

FIG. 4. The average value n_H of water-water H bonds in the hydration shell of an elastinlike peptide as a function of temperature (closed circles and dashed lines). The widths of the hydration shell D are 4.40, 4.45, 4.50, 4.55, 4.60, 4.70, 4.80, 4.90, and 5.00 Å. Percolation thresholds are shown by open circles.

value of n_H to 2.03 ± 0.02, i.e., by about 15%. Hence, the effect of temperature on the threshold value of n_H is remarkably similar at protein surfaces and at smooth surfaces of essentially hydrophilic spheres (compare the values N_2/N_1 for these systems in Table I).

The surface of a biomolecule in dilute aqueous solution is completely covered by hydration water, which forms a spanning network at low temperature. Upon heating, this spanning water network breaks up via a percolation transition, and the hydration water shell becomes an ensemble of finite (nonspanning) water clusters [15]. The location of the percolation threshold at the surface of fully hydrated elastinlike peptide, using cluster size distribution n_S, indicates that, at $T = 300$ K, n_H is about 2.09 ± 0.02 at the threshold (see Fig. 1). More accurately, the percolation threshold can be located by additional analysis of the probability distribution of the largest cluster size [16]. The estimated temperature of the percolation transitions of hydration water in the shells of various width D are shown by open circles in Fig. 4 as a function of the average value of water-water H bonds n_H. Evidently, the value of n_H at the percolation threshold is about 2.1 for any reasonable choice of the hydration shell width D. The value n_H for hydration water in protein solutions decreases almost linearly with temperature (see Fig. 4 and also Ref. [19]). This gives us the possibility of estimating the value of n_H in a wide temperature range by simulations at two temperatures and, subsequently, of estimating the temperature of the thermal breaking of the spanning network of hydration water.

Summarizing our studies of the quasi-2D percolation transition of hydration water in various model systems, we propose a relatively simple method to indicate the existence of a spanning network of hydration water by the analysis of the average number n_H of hydrogen bonds between the water molecules in the hydration shell. This value can be obtained by conventional computer simulations or estimated from experimental data [20]. At ambient temperature, the threshold value of n_H is about 2.1 for fully hydrated systems and about 2.3 for low-hydrated systems. A value n_H above the threshold indicates the presence of a spanning network of hydration water. The threshold value of n_H decreases slightly with increasing temperature.

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