Translational Diffusion Constants of the Amino Acids: Measurement by NMR and Their Use in Modeling the Transport of Peptides

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Received: November 30, 2006; In Final Form: December 22, 2006

In this work, the translational self-diffusion constants, D_T's, of 12 amino acids (Ala, Arg, Asn, Asp, Cys, Glu, His, Ile, Lys, Met, Phe, and Ser) are measured by field gradient NMR and extrapolated to infinite dilution. The experiments were carried out in D_2O at 298 K at pD ≅ 3.5 in 50 mM sodium phosphate buffer. Of these 12 amino acids, 6 are being reported for the first time (Asp, Cys, Glu, His, Lys, and Met) and the remaining 6 (Ala, Arg, Asn, Ile, Phe, and Ser) are compared with D_T's from the literature. When corrected for differences in solvent viscosity and temperature, the discrepancy between D_T's measured in the present work and those reported previously is always <8%, which is reasonable given the range of values reported previously by different groups. With the present work, D_T's for all of the amino acids are now available. These diffusion constants are then used in modeling studies of the diffusion and free solution electrophoretic mobility, μ, of several model peptides. For this set of peptides, it is shown that modeling using revised input parameters results in improved agreement between model and experimental mobilities.

Introduction

The translational self-diffusion constant, D_T, of molecules in solution plays a fundamental role in a wide range of processes in biology and chemistry. Self-diffusion constants are sensitive to particle size and conformation. This sensitivity, coupled with a wide range of available techniques that make D_T a comparatively simple quantity to measure, are responsible for its importance. For macromolecules with a molecular weight in excess of 20 kD, dynamic light scattering has long been the method of choice.[1] For small molecules that do not scatter much light, methods related to boundary spreading due to a concentration gradient have long been used.[2−5] In addition, NMR has become a useful method[6−8] and has the advantage of being applicable at very low concentrations. In recent years, measurement of diffusion by NMR has been used to characterize peptide conformation/aggregation in both free solution and micelle environments.[9,10]

The focus of the present study concerns the self-diffusion constants of the amino acids in aqueous solution in the limit of infinite dilution. Such D_T's are of considerable interest in modeling the diffusional[11−13] and electrophoretic[14] transport of peptides and proteins. Despite their fundamental importance, we are unaware of literature values for the D_T's of six amino acids: Asp, Cys, Glu, His, Lys, and Met. Of the studies reported in the literature,[2,3,5,15] the early work by Polson[2] and a recent study by Ma and co-workers[5] are the most extensive. All of these involve "boundary spreading"[2,3,5] or "pore diffusion"[15] methodologies. The principal objective of this work is to report diffusion constants of the "unknown" amino acids. Diffusion constants of a number of "known" amino acids are also reported and compared with literature values. The method employed is field gradient NMR and is described in detail elsewhere.[6,7]

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Materials and Methods

Amino Acids Samples. Amino acid samples (Sigma) were prepared in D_2O in a buffer consisting of 50 mM sodium phosphate. The amino acid concentration of most samples was 40 mM. For four amino acids (Ile, Lys, Phe, and Ser), however, the concentration was varied from 10 to 160 mM (for Ile and Phe) and from 10 to 40 mM (for Lys and Ser). The pH* (uncorrected meter reading) was adjusted to 3.01−3.08 with DCl. This corresponds to a pD of approximately 3.5.[16]

NMR Spectroscopy. NMR spectra were acquired on a Bruker Avance 600 MHz spectrometer equipped with a 10 A gradient amplifier using a 5 mm QXI probe head $^1H\{^{31}P, ^{13}C, ^{15}N\}$ with a shielded Z-gradient coil. The gradient strength was calibrated as 5.48 (Gauss/(cm A)) using a 5 mm Shigemi NMR tube (part number Z529451, Aldrich) with a 11 mm sample window.

All measurements were recorded at 298 K; typically, 8−32 scans were collected for each experiment, using a relaxation delay of 8 s, 6000 Hz spectral width and 1.5 Hz line broadening. A 1D stimulated echo pseudo pulse sequence (step1s1d) was used to optimize gradient length and diffusion time (typical parameters are 1.5−1.7 ms sine shaped gradients and 200 ms diffusion time) to record the decay function fully. Subsequently, a pseudo 2D pulse sequence (stegp1s) was used with a gradient ramp from 1.1 to 52.1 G/cm in 16 increments. The data were processed and analyzed using the T1/T2 package of XwinNMR 3.5. Diffusion experiments were carried out in duplicates; multiple decay data were obtained for individual protons for each amino acid and averaged.

Results

It is well-known that the translational diffusion constant, D_T, is concentration-dependent.[2,5] Shown in Figure 1 is the variation in D_T versus concentration, as measured by NMR, for the amino acids Ile (open squares) and Phe (filled). In the limit of zero concentration, D_T extrapolates to 6.18 and 5.96 × 10^{-10} m^2/s

10.1021/jp068217o CCC: $37.00
Published on Web 02/02/2007

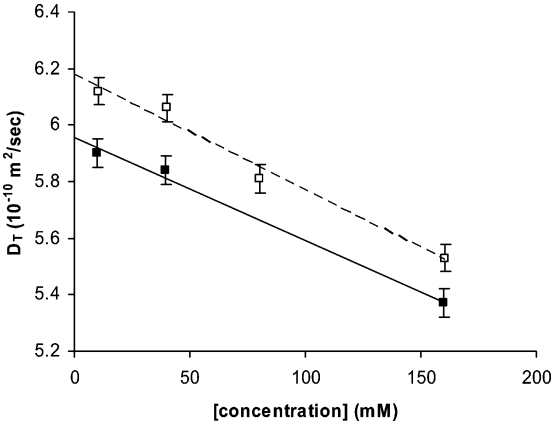

Figure 1. D_T versus amino acid concentration for Ile and Phe. The open and filled squares correspond to experimental D_T's for Ile and Phe, respectively. Measurements were carried out in D_2O at 298 K in 50 mM phosphate buffer at pD ≅ 3.5. The dashed and solid lines correspond to linear fits of the data.

TABLE 1: Amino Acid Self-diffusion Constants[a]/ Hydrodynamic Radii[b]

a.a.	D_T(raw)[c]	s.d.	$D^0_{20,w}$ [d,e]	$D^0_{20,w}$ [d,f]	R_h[b]
Ala[g]	7.39	0.03	8.26,[2] 7.95,[3] 8.02[5]	8.07	0.266
Arg[g]	5.45	0.01	5.76,[2] 6.40[5]	5.96	0.360
Asn[g]	6.57	0.01	7.25[3]	7.18	0.298
Asp	6.49	0.01		7.09	0.302
Cys	6.87	0.06		7.50	0.286
Glu	6.25	0.05		6.82	0.314
His	5.62	0.02		6.14	0.349
Ile[g]	6.18	0.06	6.74[5]	6.62	0.324
Lys	5.31	0.01		5.80	0.369
Met	6.38	0.05		6.96	0.308
Phe[g]	5.96	0.05	6.18[15]	6.39	0.335
Ser[g]	7.10	0.05	8.06[5]	7.75	0.276

[a] Diffusion constants are in 10^{-10} m^2/s. [b] Hydrodynamic radii are in nm. [c] "raw" data are in D_2O at 25 °C; determined by NMRin the present study. [d] $D^0_{20,w}$ refers to a water solvent at 20 °C. [e] Literature values (references given as a superscript number). [f] From present NMR experiments. [g] D_T's of these amino acids have been reported previously in the literature.

for Ile and Phe, respectively. Also, $D_T(0\ mM)/D_T(40\ mM) = 1.019$ and 1.020 for Ile and Phe, respectively. Similar concentration studies were also carried out for Lys and Ser and we found $D_T(0\ mM)/D_T(40\ mM) = 1.024$ and 1.003 for Lys and Ser. Other investigators have observed a similar concentration dependence,[2,5] which appears, with the possible exception of serine, to be relatively uniform among the different amino acids. (Ma and co-workers also observed a weak concentration dependence of D_T for Ser.[5]) For the remaining 8 amino acids measured, one concentration, 40 mM, was studied and the infinite dilution limit was estimated by simply multiplying the measured D_T by 1.02. Since D_T values can be readily measured by NMR at the 10 mM level, the concentration correction to infinite dilution is approximately 0.5% which is comparable to the error level seen in these measurements. The principle results of the present work are summarized in Table 1. Translational diffusion constants, D_T, of 12 amino acids were measured by field gradient NMR in D_2O at 298 K in 50 mM phosphate buffer at pD ≅ 3.5. These "raw" diffusion constants are listed in the second column of the table. A hydrodynamic radius, R_h, is related to D_T by the standard expression[17]

$$D_T = \frac{k_B T}{6\pi\eta R_h} \tag{1}$$

where k_B is Boltzmann's constant, T is absolute temperature, and η is the solvent viscosity. For a low ionic strength D_2O buffer solution at 298 K, $\eta(D_2O, 298) \cong 1.091$ cp.[18] Physically, R_h represents the radius of a sphere that would have the same diffusion constant as the amino acid in question. These are summarized in the last column in Table 1 and are computed using the D_T's obtained in the present study by NMR. It is also convenient to report D_T's under "standard" conditions of 20 °C in H_2O ($\eta(H_2O, 293.15) = 1.002$ cp, $D^0_{20,w}$. Since R_h should be invariant to solvent viscosity and temperature,

$$D^0_{20,w} = D_T(\text{raw})\left(\frac{293.15\eta(D_2O, 298)}{298\eta(H_2O, 293.15)}\right) \tag{2}$$

These standard D_T's are also included in Table 1 and compared with literature values in the case of 6 of the amino acids denoted by asterisks. Most of these come from "boundary spreading" experiments and are extrapolated to zero concentration. The D_T's for the remaining 6 amino acids, to the best of our knowledge, are being reported for the first time.

For arginine, $D^0_{20,w}$ determined by NMR value falls between the other two, but is closer to the lower value reported by Polson.[2] The relative *random* error in the NMR D_T's is approximately 0.5% and that for Arg at the lowest concentration (14 mM) studied by the "boundary spreading method" of Polson[2] is approximately 1.7%. In this example, it is worth pointing out that the two "boundary spreading" values differ from each other by 10%. This, in turn, indicates that *systematic* errors between different groups using similar boundary spreading methodologies substantially exceed random error. Alanine is perhaps the most thoroughly studied amino acid and the current NMR value lies between −2.3 and +1.5% of the other (boundary spreading) values. Given the inherent random and systematic error discussed in connection with arginine, these discrepancies are entirely reasonably. For the 4 remaining amino acids that are compared with earlier studies, the discrepancy between the D_T's measured by NMR and other methods is 1.0, 1.8, 3.3, and 3.8% for Asn, Ile, Phe, and Ser, respectively. It should be emphasized that these discrepancies fall well within the 10% discrepancy seen for Arg as discussed earlier.

Finally, we examined the pD dependence of D_T for Lys over the limited range $3.0 < pD < 4.1$. At a concentration of 10 mM for Lys and pD = 3.02, 3.46, and 4.08, $D_T = 5.50$, 5.60, and 5.58×10^{-10} m^2/s, respectively. Thus, little if any pH dependence is seen in this case. We shall return to this point again at the end of the next section.

Application to the Transport of Peptides

To illustrate the usefulness of the D_T's or equivalently the R_h's of the amino acids in modeling studies, we shall consider the transport (translational diffusion, D_T, and electrophoretic mobility, μ) of peptides. The methodology is described in detail elsewhere[14] and only a brief outline is given here.

When free amino acids condense to form peptides, a single water molecule is lost for each amino acid that is added to the growing chain, and a van der Waals volume, $\delta v = 0.0186$ nm^3, is lost.[19] For small molecules with R_h in the range 0.2−0.6 nm^3, $R_h \cong (3v_w/4\pi)^{1/3}$ where v_w is the van der Waals volume of the molecule. For the amino acids, R_h falls in this range. If a small molecule with initial hydrodynamic radius R_h loses volume δv, the resultant hydrodynamic radius, R_s, is then given by

$$R_s = R_h\left(1 - \frac{3\delta v}{4\pi R_h^3}\right)^{1/3} \tag{3}$$

TABLE 2: Comparison of Various Radii (in nm) for the Amino Acids

a.a	ref[a]	R_h^{old} [b]	R_h^{NMR}	R_s(int)[c]	R_s(end)[c]	a_s(int)	a_s(end)
Ala (A)	5	0.267	0.266	0.243	0.255	0.174	0.192
Arg (R)	5	0.335	0.360	0.348	0.354	0.312	0.319
Asn (N)	3	0.296	0.298	0.280	0.289	0.228	0.239
Asp (D)		(0.296)	0.302	0.285	0.294	0.234	0.246
Cys (C)		(0.285)	0.286	0.267	0.277	0.210	0.224
Gln (Q)	3	0.323		0.308	0.316	0.264	0.273
Glu (E)		(0.323)	0.314	0.298	0.306	0.251	0.261
Gly (G)	5	0.232		0.200	0.217	0.0818	0.127
His (H)		(0.310)	0.349	0.336	0.343	0.298	0.306
Ile (I)	5	0.318	0.324	0.309	0.317	0.265	0.275
Leu (L)	2	0.339		0.326	0.332	0.285	0.294
Lys (K)		(0.343)	0.369	0.358	0.363	0.323	0.329
Met (M)		(0.308)	0.308	0.292	0.300	0.243	0.254
Phe (F)	15	0.347	0.335	0.321	0.328	0.280	0.288
Pro (P)	2	0.268		0.246	0.257	0.178	0.196
Ser (S)	5	0.266	0.276	0.255	0.265	0.192	0.207
Thr (T)	5	0.304		0.287	0.296	0.237	0.248
Trp (W)	3	0.350		0.337	0.344	0.299	0.306
Tyr (Y)	3	0.357		0.345	0.351	0.308	0.316
Val (V)	5	0.332		0.318	0.325	0.276	0.284

[a] When multiple values are given in the literature, the most recent one is taken. [b] Where no literature value exists, R_h^{old} is estimated by the "volume increment method."[14,19] These are indicated by parentheses. [c] Computed using eq 3 and R_h^{NMR}, when available, and R_h^{old} otherwise.

For an interior amino acid, $\delta v = 0.0186$ nm^3 (loss of a single water molecule), and for an end amino acid, δv is taken to be half of this. These, along with R_h, are summarized in Table 2 for all 20 amino acids. For the 12 amino acids reported in Table 1, R_h values are estimated from D_T measured by NMR in the present work. For the remaining 8 amino acids, the R_h values are estimated from other literature values of D_T. For the 6 amino acids for which D_T has not been reported until the present study, R_h has been previously estimated using space-filling models.[14,19] These estimates appear in parentheses under the R_h^{old} column in Table 2. For the most part, these are in good agreement with the values determined in the present study, (R_h^{NMR}). The one exception is histidine, where R_h^{NMR} exceeds R_h^{old} by about 12%.

A peptide made up of X amino acids is modeled as $N = 2X$ beads with each amino acid represented by two touching beads. The radius of the "backbone bead" is taken to be 0.19 nm and the distance between neighboring backbone beads is 0.38 nm. This reproduces the C_α to C_α distance in peptides.[20] Modeling each amino acid as a dimer of two touching beads, it is straightforward to determine the radius of the side bead, a_s, that reproduces the hydrodynamic radius of the amino acid minus one or one-half of a water molecule, R_s.[21] The side bead radius for each amino acid for "end" and "interior" amino acids are also listed in Table 2. More information regarding the generation of peptide conformations as well as assigning charges to the amino acids is described in ref 14. In addition, more detailed accounting of the finite size of the model beads as well as the effect of "ion relaxation" on the free solution electrophoretic mobility, μ, of model peptides has been made.[22]

As a brief illustration of the effects of the revised bead model parameters (the a_s's) in model studies of the transport of peptides, average D_T's and μ's of seven peptides were examined. These were chosen on the basis of past study[22] and the availability of experimental free solution electrophoretic mobilities[23,24] and contain amino acids with revised hydrodynamic radii on the basis of the present work. Experimental free solution mobilities, μ_{exp}, were carried out in 50 mM phosphate buffer at 22 °C at pH = 2.5.[23,24] Under these conditions, the ionic strength is 35.3 mM (35.3 mM Na^+ and $H_2PO_4^-$, respectively). At a pH of 2.5, the peptides are expected to be largely unfolded. In the model studies, 100 different peptide conformations are randomly generated[14] and average D_T's and μ's computed.[22] The results are summarized in Table 3 for both "old" (using a_s's available before the NMR measurements of the present work) and "new" bead model parameters. The first two peptides chosen, DD and MM, have relatively minor changes in the model parameters. The last five (KKKK through ACHGRDRRT) involve at least several amino acids with substantial changes in the bead model parameters. It is evident from the results in Table 3 that the revised parameters produce a small, but significant change in D_T and μ for all peptides considered. The relative discrepancy between model, μ, and experimental, μ_{exp}, mobilities, $\langle(\mu/\mu_{exp} - 1)^2\rangle^{1/2}$, averaged over all seven peptides equals 9.3% and 7.5% for "old" and "new" model parameters, respectively. For this small set of peptides, the new parameters yield mobilities in better agreement with experiment, although this may not be the case in specific cases (MM, for example). It should be emphasized that there are other sources of error in the modeling studies. Perhaps the most important is uncertainty in the charge of the model peptides,[14,22] which will have considerable effect on μ but little effect on D_T. In general, charge does influence D_T and the physical basis of this is dielectric[25] or electrolyte[26−28] friction. However, the effect on D_T is expected to be small unless the molecule is highly charged. For the amino acids under the pH and buffer conditions of interest in this work, dielectric or electrolyte friction is expected to be negligible.

TABLE 3: Average Diffusion,[a] D_T, and Free Solution Electrophoretic Mobility,[b] μ, of Specific Peptides (35.3 mM $Na^+H_2PO_4^-$, pH = 2.5, T = 22 °C)

sequence	D_T^{old}	D_T^{new}	μ^{old}	μ^{new}	μ_{exp}
DD	5.66	5.50	0.120	0.117	0.103[c]
MM	5.57	5.39	0.138	0.133	0.139[c]
KKKK	3.55	3.29	0.341	0.320	0.330[d]
KKKKK	3.23	3.01	0.346	0.329	0.330[d]
RPPGF	3.76	3.75	0.193	0.192	0.184[c]
AAGIGILTV	2.99	2.94	0.075	0.073	0.065[c]
ACHGRDRRT	2.87	2.76	0.280	0.274	0.265[d]

[a] D_T's are in 10^{-10} m^2/s. [b] μ's are in cm^2/kV·s. [c] From ref 23. [d] From ref 24.

Summary

In this work, the translational self-diffusion constants, D_T's, of 12 amino acids are measured by field gradient NMR under conditions typically used in free solution capillary electrophoresis. Of these 12 amino acids, 6 are being reported for the first time (Asp, Cys, Glu, His, Lys, and Met) and the remaining 6 (Ala, Arg, Asn, Ile, Phe, and Ser) are compared with D_T's from the literature. The discrepancy between D_T's measured in the present work and those reported previously, when corrected for differences in temperature and solvent viscosity, is always less than 8%, which is also the range of values reported by different groups employing similar "boundary spreading" methodologies. With the present work, D_T's for all of the amino acids are now available. These diffusion constants are then used in modeling studies of the transport (diffusion and free solution electrophoretic mobility) of seven model peptides. For this set of peptides, it is shown that modeling using revised input parameters results in improved agreement between model and experimental mobilities.

Even though there is a considerable amount of experimental data available regarding the free solution electrophoretic mobili-

ties of peptides,[23,24] there is very little corresponding data available regarding their translational diffusion constants. Progress in this field is being made, however, and NMR is playing an important role.[9,10] Experimental D_T's for peptides would be of considerable use in understanding their average solution conformation.[9−13,21] In addition, the combination of experimental D_T's and μ's would enable investigators to estimate the solution charge of peptides under specific solvent/buffer conditions.[14] NMR measurement of the diffusion constants of peptides is a subject of current interest in our laboratories.

Acknowledgment. Support from NIH and the Georgia Cancer Coalition is acknowledged.

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