Free Energy and Hydration Free Energy of N-acetyl-N'-methylalaninamide

Young Kee Kang and Mu Shik Jhon*

Department of Chemistry, Chungbuk National University, Cheongju 360-763
*Department of Chemistry, and Center for Molecular Science, Korea Advanced Institute of Science
and Technology, Seoul 130-650. Received April 9, 1991*

The changes in the free energy and hydration free energy of N-acetyl-N'-methylalaninamide in the unhydrated and hydrated states were calculated with ECEPP/2 and the hydration shell model. The configurational entropy change of each conformation in both states were computed by a harmonic method. To understand the hydration structure of each hydrated conformation, the hydration-shell coordination numbers of functional groups of the molecule were estimated from water-accessible volumes, and the contributions of water-accessible volume and polarization of each group to the hydration free energy were analyzed. The results show a reasonable agreement with those of recent theoretical studies and experiments.

Introduction

Hydration is an important factor determining the conformation and thermodynamic properties of peptides and proteins in aqeous solution.[1-4] The hydration shell model has been developed to introduce hydration into conformational energy calculations of small organic molecules, peptides, and proteins.[5-10] In a recent paper, Kang et al.[8] improved the hydration shell model by the exact computation of the water-accessible volume of each group in a molecule and by the explicit consideration of the conformational flexibility of the solutes. An alternative method with the surface area exposed to water has been used to compute the hydration free energies of peptides[11,12] and nucleotides.[13] Free energy perturbation methods were developed to evaluate the hydration properties of basic biological and organic molecules using molecular dynamics and Monte Carlo simulations.[14] Also, a method based on the extended RISM formulation of Ornstein-Zernike type integral equation theories was applied to calculate the hydration free energy of alanine dipeptide.[15] To estimate the configurational entropy contribution to the free energy of biomolecules, harmonic approximations were employed[16-19] and quasi-harmonic treatments through computer simulations were proposed.[20,21]

In this paper, we report the computation of free energy change for conformational transitions and the hydration of N-acetyl-N'-methylalaninamide (Ac-Ala-NHMe), which is often used as a model system for the polypeptide. To obtain more detailed understanding of the conformation and thermodynamics for this molecule in the unhydrated and hydrated states, the configurational entropy, as well as the conformational energy and hydration free energy, is evaluated for each low-energy conformation by a harmonic method.[16-19]

Methods

The nomenclature and conventions used follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature.[22] Backbone conformations are denoted by the letter codes of Zimmerman et al.,[23] because all the low-energy conformations of Ac-Ala-NHMe are studied herein.[24]
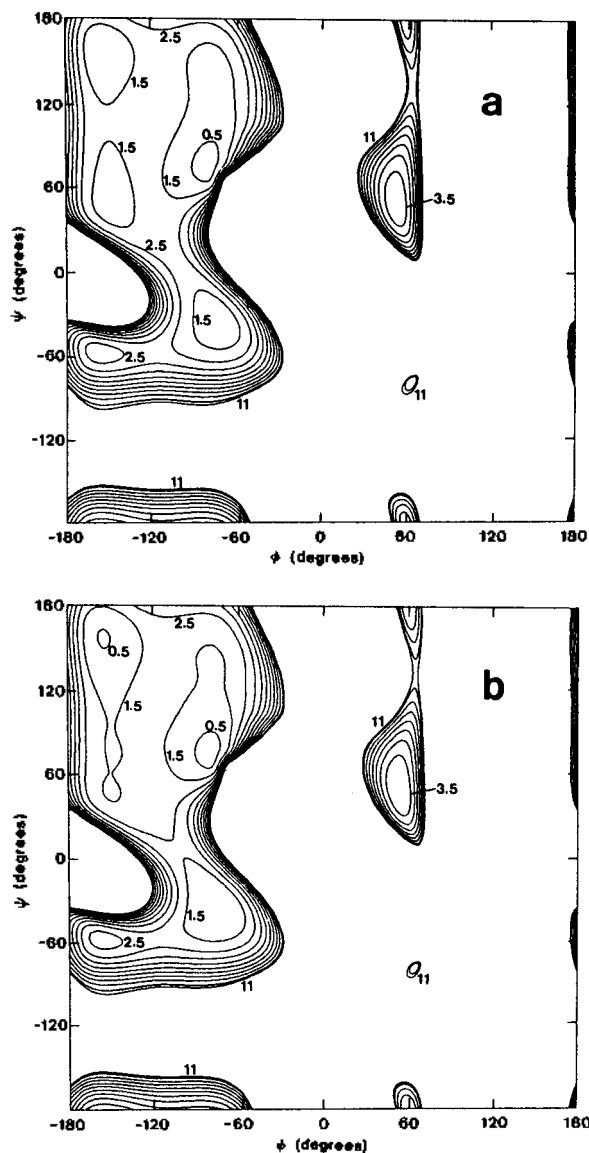
The conformational energy computations were carried out with ECEPP/2.[25] The hydration shell model improved recently[8] was used to compute the hydration free energy of each conformation of Ac-Ala-NHMe in the hydrated state, where the hydration free energy was obtained as the sum of two contributions from water-accessible volume and polarization. A quasi-Newton algorithm SUMSL (Secant-type Unconstrained Minimization problem SoLver)[26] was used for energy minimization. All the dihedral angles of the peptide backbone, side chain, and end groups were allowed to vary during minimization. For the hydrated Ac-Ala-NHMe, the total free energy (i.e., the sum of the conformational energy and hydration free energy) of each conformation was minimized. Seven low-energy conformations of Vásquez et al.[27,28] were used as starting points for energy minimization of Ac-Ala-NHMe in the unhydrated and hydrated states.[29]

At each energy minimum in both states, the configurational entropy was evaluated by a harmonic method.[16,19] The elements of a hessian matrix of second derivatives at each minimum were numerically computed.[19] The seven- and five-point formulas were employed for the calculation of off-diagonal and diagonal elemental, respectively, with the step size of each variable equal to 1°. All the thermodynamic quantities have been calculated for $T = 25°C$.

Results and Discussion

Conformational energy contour maps for Ac-Ala-NHMe in the unhydrated and hydrated states are drawn in parts a and b of Figure 1, respectively. The revised version of ECEPP gave us some different features in conformational energy surface of the unhydrated Ac-Ala-NHMe from the earlier results.[27] Hodes et al.[7] employed the earlier versions of ECEPP and the hydration shell model. Thus, the results presented here is not compared to those of Hodes et al.

The steep boundaries of the low-energy regions coincide on the maps of both states. Inside the low-energy regions, slight shifts of individual contour lines occur through the hydration. The noticeable alterations occur between regions E and D and between regions F and C. The relative energies of these regions are lowered due to the hydration, and this

496 *Bull. Korean Chem. Soc., Vol. 12, No. 5, 1991*

Young Kee Kang and Mu Shik Jhon

Figure 1. Conformational energy (or free energy) contour maps of N-acetyl-N'-methylalaninamide in the unhydrated (a) and hydrated (b) states. Energies were calculated at 10° intervals of ϕ and ψ, for fixed values of $\chi^1=60°$ and other dihedral angles equal to 180°. Contour lines are drawn at 1 kcal/mol intervals and are labeled with energy in kcal/mol above the minimized energy of conformation C in each state.

Table 1. Calculated Thermodynamic Quantities for Ac-Ala-NHMe[a,b]

	C	E	A	D	F	G	A*
			Unhydrated				
ΔE	0.00	0.71	0.81	1.11	1.12	1.74	2.37
$-T\Delta S$	0.00	−0.60	−0.55	−0.75	−1.27	−0.05	−0.03
ΔA_{tot}	0.00	0.11	0.26	0.36	−0.15	1.69	2.34
			Hydrated				
ΔE	0.00	0.71	0.81	1.11	1.13	1.73	2.37
$-T\Delta S$	0.00	−1.09	−1.04	−0.73	−1.41	−0.54	−0.51
ΔA_{hyd}	0.00	−0.34	0.63	0.28	0.20	0.06	0.74
$\Delta A_{tot}{}^{c}$	0.00	−0.72	0.40	0.66	−0.08	1.25	2.60
	0.0	−0.4	1.3		−0.5		0.6
	0.0		· 0.3± 0.9		0.2± 0.6		

[a] Energies are in kcal/mol. [b] Entropic and free energetic contributions are calculated at $T=25°C$. [c] The values of ΔA_{tot} in the second and third rows are taken from refs. 15 and 21, respectively.

NHMe in the unhydrated and hydrated states generally are similar to those obtained by Vasquez *et al.*[27] Although all the dihedral angles were allowed to vary during minimization here, there are only small changes in all the torsion angles within about ± 0.5° in both states. Therefore, the minimum-energy geometries are not listed here. It is particularly notable that the values of (ϕ, ψ) for conformations F and A* are changed slightly through the hydration from $(-76°, 139°)$ to $(-78°, 137°)$ and from $(54°, 46°)$ to $(55°, 45°)$, respectively (see Figure 1).

The calculated thermodynamic quantities for the selected conformations of Ac-Ala-NHMe, relative to conformation C, are shown in Table 1. In our results, the calculated conformational energies in both states show almost the same values as those of Vasquez *et al.*[27] because of very small changes in the conformations, as noted previously.

In the unhydrated state, the entropic contribution (i.e., $-T\Delta S$) lowers in general the relative free energies of low-energy confromations, but the similar trends of stability are still conserved whether the entropic contribution is included or not. However, conformation F has a somewhat larger entropic contribution than other conformations and this is caused from the flat potential energy surface in region F, as described in ref. 27.

The hydrated Ac-Ala-NHMe has a similar trend in the entropic contribution to free energy changes for conformational transitions. The relative magnitude of entropic contribution for each conformation is increased about twice than that of the unhydrated state except for conformations D and F, and this tells us that the potential energy surface becomes generally flatter through the hydration. The flatness of region F still gives $-T\Delta S$ to be the most negative value. The free energy of hydration for Ac-Ala-NHMe is not largely changed from conformational transitions and thus the hydration does not alter the conformations considerably. The interaction of water molecules with the CO and NH groups of peptide backbone results in that conformation E is most favorable than other conformations, especially than conformation C.

Our results where possible with those obtained by the

may come from the fact that the extended conformations favor the hydration more than the folded ones and that the hydration free energy changes computed by the hydration shell model are not so large for conformational transitions. Also the hydration makes the region D narrower (around $\phi=-150°$ and $\psi=60°$) and lowers the relative energy of the "bridge region" (near $\psi=0°$). The overall free energy contour maps of the hydrated Ac-Ala-NHMe is similar to that of Ooi *et al.*[12] (see Figure 1 of ref. 12), in which they used ECEPP/2 to compute the conformational energy, but a different approach was employed to evaluate the hydration free energy. The major difference in contour maps of two works is in regions E and D, and in inter-region of them.

The computed minimum-energy conformations of Ac-Ala-

Free Energy of Ac-Ala-NHMe

Bull. Korean Chem. Soc., Vol. 12, No. 5, 1991 497

Table 2. Configurational Entropy Differences for Ac-Ala-NHMe[a,b]

System	ΔS		
	This work	Ref. 20b	Ref. 21
E(unh) to C(unh)	−2.02	−1.78	−1.0± 1.3
A(unh) to C(unh)	−1.84		−1.1+ 1.6
F(unh) to C(unh)	−4.26		−1.6± 1.2
C(unh) to C(hyd)	−1.67	−1.41	
A(unh) to A(hyd)	−0.02		
F(unh) to F(hyd)	−1.19		
F(hyd) to A(hyd)	−1.25	−1.51	
F(hyd) to C(hyd)	−4.74	−1.60	

[a] Entropies are in cal/(mol K). [b] *Unh* and *hyd* refer to the unhydrated and hydrated states, respectively.

extended RISM[15] and Monte Carlo[21] methods on the hydrated Ac-Ala-NHMe are compared in Table 1. Since the hydration shell model can not give the entropic and energetic contributions to the hydration free energy separately, only the total free energy changes for conformational transitions from conformation C are compared each other. The present study yields the free energy changes from conformation C to conformations A and F to be 0.4 and −0.1 kcal/mol, respectively, whereas the extended RISM method yielded 1.3 and −0.5 kcal/mol and Monte Carlo yielded 0.3± 0.9 and 0.2± 0.6 kcal/mol, respectively. Also, our study yields −0.7 and 2.6 kcal/mol for conformational transitions from conformation C to conformations E and A*, whereas the extended RISM method[15] yielded −0.4 and 0.6 kcal/mol, respectively. Although different potential functions and entropy expressions are used in each study, the computed results are in good agreement with each other. The largest discrepancy is found for the transition from conformation C to conformation A* and this can be easily understood if we compare the free energy contour maps of both studies (see Figure 1 of this work and Figure 4 of ref. 15).

Table 2 contains the calculated entropy changes associated with the conformational transitions in the unhydrated and hydrated states, and between both states. The large negative value for the conformational transition of conformation F to conformation C in both states is expected to come from the flat potential energy surface of region F. The small entropy change of conformation A through the hydration tells us that the hydration does not have an effect on the potential surface of region A. Our results show the same trends as those obtained by molecular dynamics[20b] and Monte Carlo[21]

simulations, although dihedral angles are considered only as variables in this work and the correlations of the dihedral angles with other internal coordinates seem to be important as noted by Karplus *et al.*[17]

To explore the hydration structure of the hydrated Ac-Ala-NHMe in detail, the calculated hydration-shell coordination numbers of the CH₃, CO, and NH groups for seven conformations are listed in Table 3. Water-accessible volume of the α-CH group of the peptide backbone of each conformation keeps constant and thus their values are not included in Tables 3 and 4. Each coordination number was calculated from ΔV_{wa} divided by 30.0 Å³, where ΔV_{wa} is the relative water-accessible volume of each group to that of conformation C and the effective volume of a water molecule is assigned to be 30.0 Å³ from the density of liquid water at 25°C.[30] For conformations A and A* (*i.e.*, α-helical conformations) relative to conformation C, there is a decrease of about 1.5 water molecules, whereas other conformations have 1.2 to 3.0 water molecules more than conformation C. There is a large discrepancy between our results for conformations E, A, and F, and those of Monte Carlo study by Mezei *et al.*[31a] This may be caused from the difference in the way in which conformations and coordination numbers[31b] are defined in two studies.[32] The present results show that the major contributions to the total coordination numbers come from those of the CO and NH groups for conformation E and of the NH group only for conformation F, whereas the contributions from the CH₃ and NH groups for conformation A are significantly important. The large values of coordination numbers of the CO and NH groups for conformation E tells us that the interaction of water molecules with these groups may be most favorable for the extended confromation E.

To aid in further analysis of the contributions to the hydration free energy, the decomposed free energies due to the water-accessible volume and polarization are listed in Table 4. The polarization free energies of conformations except conformation E relative to conformation C show positively large values and this may be caused by the destabilization of water molecules between polar groups as noted in ref. 8b. The total hydration free energies purely coming fron the water-accessible volumes are compensated by the corresponding polarization free energies and thus all the conformations studies herein except conformation E are destabilized through the hydration. However, conformation E has the most negative hydration free energy because of the largest water-accessible volume contributions and the smallest polarization contributions. The most important part for conformation E is the hydration of the NH group. Polarization free energies of the CO and NH groups largely contribute the

Table 3. Calculated Hydration-Shell Coordination Numbers for Ac-Ala-NHMe[a,b]

	C	E	A	D	F	G	A*
CH₃	0.0(0.0)	0.1(−2.3)	−0.5(−2.0)	0.1	0.4(−1.0)	−0.1	−0.6
CO	0.0(0.0)	1.2(0.7)	−0.2(0.6)	1.4	−0.3(1.2)	1.6	−1.0
NH	0.0(0.0)	1.7(0.1)	−0.7(0.0)	−0.2	1.1(0.1)	0.0	−0.1
Total	0.0(0.0)	3.0(−1.5)	−1.4(−1.4)	1.3	1.2(0.3)	1.5	−1.7

[a] Coordination numbers are calculated from $\Delta V_{wa}/30.0$, where ΔV_{wa} ia the relative water-accessible volume of each group to that of conformation C and the effective volume of a water molecule is taken as 30.0 Å³ from the density of liquid water at 25°C (ref. 30). [b] The values in parentheses are taken from ref. 31a.

Table 4. Calculated Hydration Free Energies for Ac-Ala-NHMe[a,b]

	C	E	A	D	F	G	A*
			Water-accessible Volume				
CH₃	0.00	0.00	−0.02	0.01	0.02	−0.01 ·	−0.03
CO	0.00	−0.12	0.02	−0.17	0.06	−0.19	0.13
NH	0.00	−0.24	0.09	0.06	−0.20	0.03	−0.02
Total	0.00	−0.36	0.09	−0.10	−0.12	−0.17	0.08
			Polarization				
CH₃	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CO	0.00	0.07	0.52	0.58	0.08	0.50	0.56
NH	0.00	−0.05	0.02	−0.20	0.24	−0.27	0.10
Total	0.00	0.02	0.54	0.38	0.32	0.23	0.66
			Total				
CH₃	0.00	0.00	−0.02	0.01	0.02	−0.01	−0.03
CO	0.00	−0.05	0.54	0.41	0.14	0.31	0.69
NH	0.00	−0.29	0.11	−0.14	0.04	−0.24	0.08
Total[c]	0.00	−0.34	0.63	0.28	0.20	0.06	0.74
	0.0	−4.1	−9.9		−11.4		−10.4
	0.0		−3.6		−3.2		

[a] Free energies are in kcal/mol. [b] Total hydration free energy of each group is calculated as the sum of water-accessible volume and polarization contributions, as noted in the text. [c] The value in the second and third rows are taken from refs. 15 and 21.

destabilization of conformations A and F, respectively. We can compare our results with those from the extended RISM method of Pettitt et al.[15] and from Monte Carlo simulation of Ravishanker et al.[21] The extended RISM method gave the negatively large values of hydration free energies within −4.1 to −11.4 kcal/mol, and Monte Carlo simulation yielded −3.6 and −3.2 kcal/mol for conformations A and F from conformation C, respectively. Although the changes of hydration free energy for the conformational transitions in the latter two simulations are large in magnitude, the total free energy changes associated with the transitions are relatively small in magnitude since the conformational energy changes are also large (see Table 1 of refs. 15 and 21). As a result, their calculated total free energy changes for the transitions are generally similar to ours as shown in Table 1.

It was found from the CD and NMR experiments[33] that the probable conformations of Ac-Ala-NHMe are conformations C, E, A, and F in the nonpolar and polar solvents, and that some conformation C in the nonpolar solvents is replaced by conformations A and F in aqueous solution. Our results indicate that the free energies of the four conformations are very similar and thus these conformations are reasonably expected to be thermally populated at ambient temperatures, although the distinctive transitions of conformation C to conformations A and F were not predicted due to the hydration.

The approach used in this work may have the advantage to estimate the free energy for large biomolecules of interest in aqeous solution over other methods based on simulations. The sufficient data on the structure and thermodynamics of the systems will provide as further validity of the present approach.

References

1. W. Kauzmann, Adv. Protein Chem., 14, 1 (1959).
2. H. A. Scheraga, Acc. Chem. Res., 12, 7 (1979).
3. J. T. Edsall and H. A. McKenzie, Adv. Biophys., 16, 53 (1983).
4. L. Packer, ed., Methods Enzymol., 217 (several relevant articles) (1986).
5. K. D. Gibson and H. A. Scheraga, Proc. Natl. Acad. Sci. U.S.A., 58, 420 (1967).
6. A. J. Hopfinger, Macromolecules, 4, 731 (1971).
7. Z. I. Hodes, G. Nemethy, and H. A. Scheraga, Biopolymers, 18, 1565 (1979).
8. (a) Y. K. Kang, G. Nemethy, and H. A. Scheraga, J. Phys. Chem., 91, 4105 (1987); Erratum, ibid., 92, 1382 (1988); (b) ibid., 91, 4109 (1987); (c) ibid., 91, 4118 (1987); (d) Y. K. Kang, K. D. Gibson, G. Nemethy, and H. A. Scheraga, ibid., 92, 4739 (1988).
9. K. Takahashi, Y. K. Kang, G. Nemethy, H. A. Scheraga, and V. S. Ananthanarayanan, Biopolymers, 26, 1781 (1987).
10. B. Jayaram, G. Ravishanker, and D. L. Beveridge, J. Phys. Chem., 92, 1032 (1988).
11. D. Eisenberg and A. D. McLachlan, Nature (London), 319, 199 (1986).
12. T. Ooi, M. Oobatake, G. Nemethy, and H. A. Scheraga, Proc. Natl. Acad. Sci. U.S.A., 84, 3086 (1987).
13. P. S. Ho, G. J. Quigley, R. F. Tilton, Jr., and A. Rich, J. Phys. Chem., 92, 939 (1988).
14. (a) D. L. Beveridge and W. L. Jorgensen, eds., Ann. N. Y. Acad. Sci., 482 (several relevant articles) (1986); (b) P. A. Bash, U. C. Singh, R. Langride, and P. A. Kollman, Science, 236, 564 (1987); (c) W. L. Jorgensen and J. K. Buckner, J. Phys. Chem., 91, 6083 (1987).
15. B. M. Pettitt and M. Karplus, J. Phys. Chem., 92, 3994 (1988).

EPR Study of YBa₂Cu₃O₇₋ᵧ Doped with Pd or Zn

Bull. Korean Chem. Soc., Vol. 12, No. 5, 1991 499

16. (a) N. Gō and H. A. Scheraga, *J. Chem. Phys.*, **51**, 4751 (1969); (b) *Macromolecules*, **9**, 535 (1976).
17. M. Karplus and J. N. Kushick, *Macromolecules*, **14**, 325 (1981).
18. Y. K. Kang and M. S. Jhon, *Macromolecules*, **17**, 138 (1984).
19. (a) Y. K. Kang and D. W. Kim, *Bull. Korean Chem. Soc.*, **11**, 144 (1990); (b) Y. A. Shin and Y. K. Kang, *ibid.*, **12**, 61 (1991); (c) Y. K. Kang, *Int. J. Pept. Protein Res.*, **38**, 79 (1991).
20. (a) R. M. Levy, O. Rojas, and R. A. Freisner, *J. Phys. Chem.*, **88**, 4233 (1984); (b) J. Brady and M. Karplus, *J. Am. Chem. Soc.*, **107**, 6103 (1985).
21. G. Ravishanker, M. Mezei, and D. L. Beveridge, *J. Comput. Chem.*, **7**, 345 (1986).
22. (a) IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970); (b) IUPAC-IUB Joint Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **260**, 14 (1985).
23. S. S. Zimmerman, M. S. Pottle, G. Némethy, and H. A. Scheraga, *Macromolecules*, **10**, 1 (1977).
24. The letter codes of C, E, A, F, and A* denote the C_7^{eq}, the extended (*e.g.* C_5), the right-handed α-helical (α_R), the proline type II (p_{II}), and the left-handed α-helical (α_L) conformations; other letter codes of D and G represent the remaining conformations to indicate contiguity (see ref. 23 for detaills).
25. (a) G. Némethy, M. S. Pottle, and H. A. Scheraga, *J. Phys. Chem.*, **87**, 1883 (1983); (b) M. J. Sippl, G. Némethy, and

26. D. M. Gay, *ACM Trans. Math. Software*, **9**, 503 (1983).
27. M. Vásquez, G. Némethy, and H. A. Scheraga, *Macromolecules*, **16**, 1043 (1983).
28. In ref. 27, the energy was minimized with respect to backbone and side chain dihedral angles only, where the dihedral angles of peptide bonds and end groups were held fixed at 180°.
29. In this work, seven low-energy conformations with relative conformational energy $\Delta E < 3.0$ kcal/mol in ref. 27 were used as starting conformations because two remaining conformations show relatively high energy states, *i.e.*, $\Delta E > 5.0$ kcal/mol.
30. R. C. Weast, ed., *CRC Handbook of Chemistry and Physics*, 66th ed., CRC, Boca Raton, 1985.
31. (a) M. Mezei, P. K. Mehrotra, and D. L. Beveridge, *J. Am. Chem. Soc.*, **107**, 2239 (1985); (b) P. K. Mehrotra and D. L. Beveridge, *ibid.*, **102**, 4287 (1980).
32. In ref. 8b, we optimized the hydration shell radius of ether oxygen to be bigger than the location of the first minimum in the O···O rdfs of the Monte Carlo simulation of methanol in water because use of the latter value gives poor agreement for the hydration free energies of ethers. The optimized value was used in determining the hydration shell radii of other polar atoms. So the large coordination numbers may be caused by these large values of the hydration shell radii.
33. V. Madison and K. D., Kopple, *J. Am. Chem. Soc.*, **102**, 4855 (1980).

H. A. Scheraga, *ibid.*, **88**, 6231 (1984).

EPR Study of the High T_c Superconductor YBa₂Cu₃O₇₋ᵧ Doped with Palladium or Zinc

Hag Chun Kim, Hyunsoo So*, and Ho Keun Lee†

Department of Chemistry, Sogang University, Seoul 121-742
†*Korean Standards Research Institute, Taejeon 302-340. Received April 15, 1991*

EPR spectra of the high T_c superconductor YBa₂Cu₃O₇₋ᵧ (YBCO) doped with Pd^{2+} or Zn^{2+} have been measured at several temperatures and dopant concentrations. The spectral intensity of YBa₂(Cu₁₋ₓPdₓ)₃O₇₋ᵧ is proportional to the dopant concentration. The behavior of YBa₂(Cu₁₋ₓZnₓ)₃O₇₋ᵧ is quite different: the spectral intensity remains almost constant up to $x = 0.10$ and then increases rapidly above $x = 0.10$. The results are interpreted in terms of localized and antiferromagnetically spin-paired d holes in both CuO chain and planes. The Pd^{2+} ion substitutes on the CuO chain consisting of "CuOCu dimers", and a Cu^{2+} ion with an unpaired spin is generated for each Pd^{2+} ion substituted. On the other hand, Zn^{2+} substitutes on the CuO planes, and all or most of the spins in the two-dimensional plane manage to pair up in the region of low dopant concentration. When the dopant concentration exceeds a certain limit, it becomes more difficult for the spins to find partners, and the number of unpaired spins increases rapidly with increasing dopant concentration. The Zn^{2+} ion is more effective than the Pd^{2+} ion in suppressing the superconductivity of YBCO. This is attributed to the fact that Zn^{2+} substitutes on the CuO planes which are mainly responsible for the superconductivity, while Pd^{2+} substitutes on the CuO chain which is of secondary importance in the superconductivity.

Introduction

Various experimental techniques have been used to study the high T_c superconductor YBa₂Cu₃O₇₋ᵧ (YBCO) first prepared by Wu *et al.*[1] We are using electron paramagnetic resonance (EPR) spectroscopy to study high T_c superconductors.