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Spectral densities of nitrogen nuclei in *Escherichia coli* ribonuclease HI obtained by ^{15}N NMR relaxation and molecular dynamics

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Summary

Spectral densities of the ^{15}N amide in *Escherichia coli* ribonuclease HI, obtained from NMR relaxation experiments, were compared with those calculated using a molecular dynamics (MD) simulation. All calculations and comparisons assumed that the auto-correlation function describing the internal motions of the molecule was independent of the auto-correlation function associated with overall rotational diffusion. Comparisons were limited to those residues for which the auto-correlation function of internal motions rapidly relaxed and reached a steady state within 205 ps. The results show the importance of frequency components as well as amplitudes of internal motions in order to obtain a meaningful comparison of MD simulations with NMR data.

Fluctuations of protein structures at the atomic level have previously been investigated by examining the time-correlation functions or the spectral density functions calculated from molecular dynamics (MD) simulations (McCammon et al., 1979; Swaminathan et al., 1982; Brooks et al., 1983; Nadler et al., 1987). Generally, spectral densities have well-characterized frequency and amplitude components which accordingly reflect the internal motions of proteins. However, calculated spectral densities have not been directly compared with the results obtained using NMR, because of the limited time scales available. Recently, MD simulations of up to a nanosecond have been used successfully to estimate the complicated internal dynamics of protein molecules (Levitt and Sharon, 1988; Brunne et al., 1993). Moreover, NMR measurements of ^{15}N relaxation parameters have been performed and have been related to the internal mobility of proteins using model spectral density functions (Lipari and Szabo, 1982a,b; Kay et al., 1989,1992; Clore et al., 1990; Barbato et al., 1992; Peng and Wagner, 1992a; Schneider et al., 1992; Stone et al., 1992; Cheng et al., 1994; Kraulis et al., 1994; Tycko, 1994).

Recently, comparisons have been made between generalized order parameters resulting from MD simulations and those obtained experimentally from NMR (Chandrasekhar et al., 1992; Ericksson et al., 1993; Balasubramanian et al., 1994; Fushman et al., 1994; Yamasaki et al., 1995). The generalized order parameters were obtained using a model-free analysis of the ^{15}N relaxation data by assuming simple motional models (Palmer et al., 1991; Mandel et al., 1995). Good agreement between simulation and experiment was obtained for globular proteins, where the NMR spectral densities themselves can be completely reproduced by combining only a single rotational correlation time of a protein molecule and internal motions that are given by MD simulations. In such a case, the spectral densities obtained by NMR experiments should be directly comparable to those obtained from MD simulations without the use of a 'filter', i.e., a simple motional model. Additionally, systems whose auto-correlation function are not truncated due to insufficient time should be comparable to the NMR experimental data, otherwise the calculation cannot be assured to express accurate values of the spectral densities.

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In this report, we compare values of the spectral density function obtained from NMR experiments with those calculated from MD simulations. The auto-correlation function of an N-H vector was represented by a simple multiplication of the overall rotational diffusion of a molecule and internal motions of the protein (Balasubramanian et al., 1994). The auto-correlation function will provide a valid description of the motion of the N-H bond vector if it reaches a steady-state value within the time course of the simulation. The spectral density function was then obtained by Fourier transformation of the auto-correlation functions. Experimental spectral densities were obtained using quasi-spectral density function (QSDF) analysis. The QSDF analysis provides a simple method for the determination of three spectral densities, $J(0)$, $J(\omega_N)$, and $J(\omega_N + \omega_H)$, from three rates of relaxation: longitudinal, transverse and cross relaxation. ω_N and ω_H are the nitrogen and proton angular frequencies, respectively (Farrow et al., 1995; Ishima and Nagayama, 1995a, b). This is a modification of the method developed by Peng and Wagner (1992a,b).

The current study used results from the MD simulations and ^{15}N relaxation measurements of *Escherichia coli* ribonuclease HI (RNase HI) described previously by Yamasaki et al. (1995). The MD simulation was carried out for 430 ps in water, without the truncation of long-range Coulomb interactions, in order to avoid artificial fluctuations (Saito, 1994). After an equilibration time of 100 ps, the auto-correlation function for each N-H vector resulting from internal motions was calculated using the remainder of the data in the range from 1 to 205 ps (Yamasaki et al., 1995). To include the effect of rotational diffusion, the 205 ps auto-correlation function was multiplied by the auto-correlation function describing the rotational diffusion of the molecule. The time dependence of the auto-correlation function between 206 ps and 130 ns was assumed to depend only upon the rotational diffusion of the molecule, i.e., in this period the auto-correlation function for internal motions was assumed to have the same value as that at 205 ps. The auto-correlation function describing the overall rotation of the molecule was assumed to be a single exponential decay. The correlation time for the overall rotation was determined by minimizing the difference in the spectral densities derived from the NMR experiments and the MD calculations, using a grid search from 8 to 17 ns with steps of 0.1 ns.

The time course used to estimate the auto-correlation function was determined with a resolution of 1 ps; internal motions faster than 1 ps were assumed to make a negligible contribution to values of the spectral densities, because the rotation of the molecule contributes more to the spectral densities than these internal motions. For the same reason, the noise between 206 ps and 130 ns was estimated to be negligible. Of the 155 residues in RNase

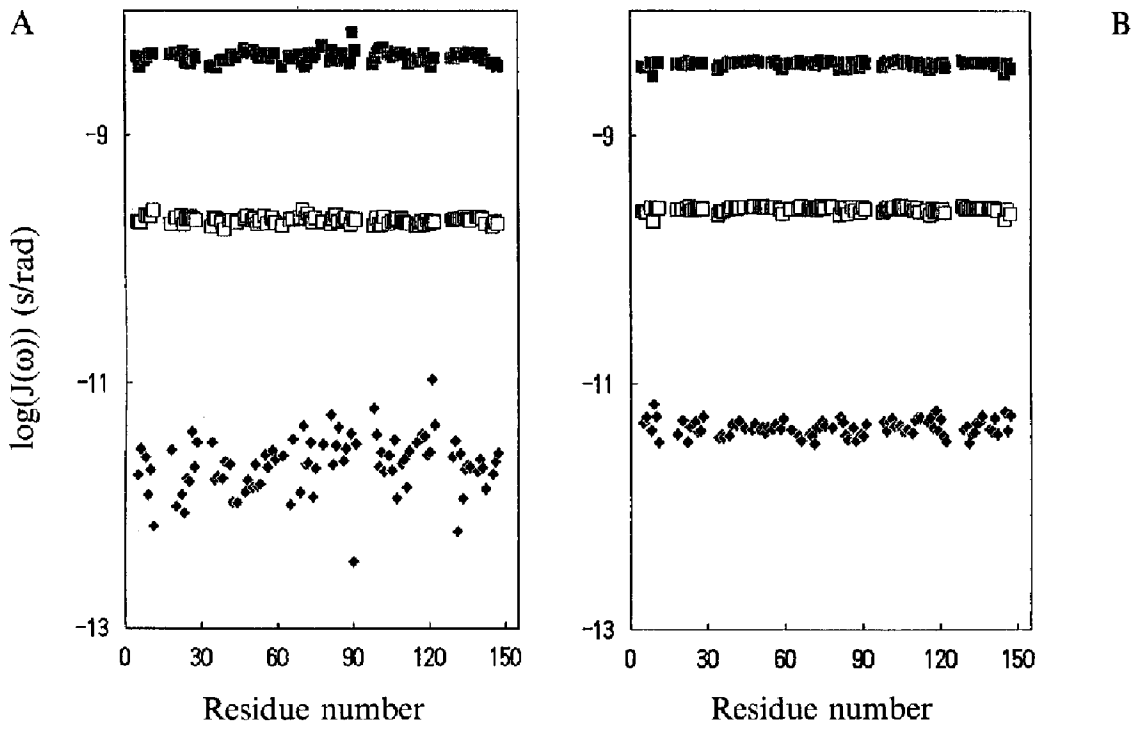

Fig. 1. Values for J(0) (■), J(60 MHz) (□) and J(540 MHz) (◆) plotted against residue number for RNase HI, obtained from (A) NMR experiments and (B) MD simulations. Results from 600 MHz NMR measurements were taken from Yamasaki et al. (1995). Errors in the spectral densities were less than 10% for J(0) and J(60 MHz) and are not shown in the figure. Errors for J(540 MHz) are shown in Fig. 3. The data at 60 and 540 MHz correspond to the Larmor frequencies of ^{15}N and (^{1}H plus ^{15}N), respectively.

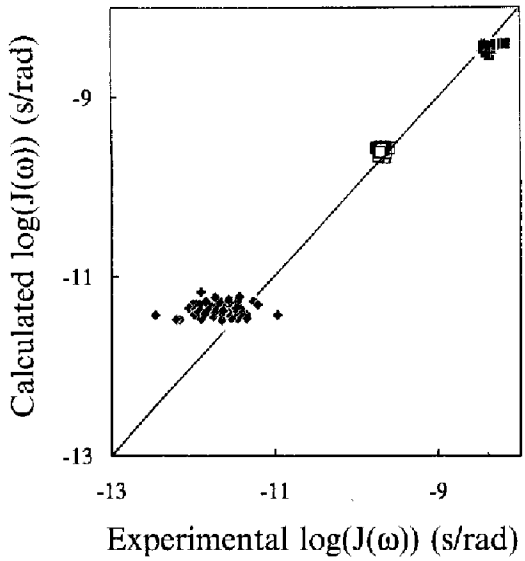

Fig. 2. Correlation between the calculated (MD simulation) and experimentally determined values of the spectral density function at 0 (■), 60 (□) and 540 (◆) MHz.

HI, 92 had auto-correlation functions describing internal motions which reach a steady state within 205 ps. Residues were judged to have reached their steady-state values when the standard deviation of their auto-correlation function between 150 and 205 ps was less than 0.02.

Figure 1 shows values of the spectral density function from NMR experiments (Fig. 1A) and MD simulations (Fig. 1B). The rotational correlation time for the calculated spectral densities was estimated to be 12 ns, which is close to the value of 10.9 ns determined by Yamasaki et al. (1995) using the model-free analysis. To determine the rotational correlation time, the experimental values for J(0), as well as J(60 MHz) and J(540 MHz) were used. The calculated J(0) and J(60 MHz) had almost identical values for all residues shown in Fig. 1. This indicates that the calculated spectral densities are determined almost completely by the rotational correlation time. This is reasonable, because most of the auto correlations of the represented residues reached steady state within 10 ps.

The correlation coefficient between the values of two spectral density functions derived from NMR experiments (Fig. 1A) and MD simulations (Fig. 1B) was 0.995 (Fig. 2). This indicates that the simulated values of the three spectral densities are of the same order as the NMR-derived values. However, the individual correlation coefficients between the experimental and the simulated values for J(0), J(60 MHz) and J(540 MHz) were very poor: 0.54, 0.077 and 0.11, respectively. In particular at 540 MHz, the experimentally determined values were more scattered than the simulated values (Fig. 1), and the average value was smaller.

There are a number of possible explanations for the observed difference between the experimental and simulated values of J(540 MHz). First, the assumption used in the QSDF, that the values for $J(\omega_H)$ and $J(\omega_H - \omega_N)$ are equivalent to the $J(\omega_H + \omega_N)$ values (Ishima and Nagayama, 1995a,b), may not be valid for many of the residues, as shown by the scatter in the experimental J(540 MHz) values. We compared the experimental J(540 MHz) values

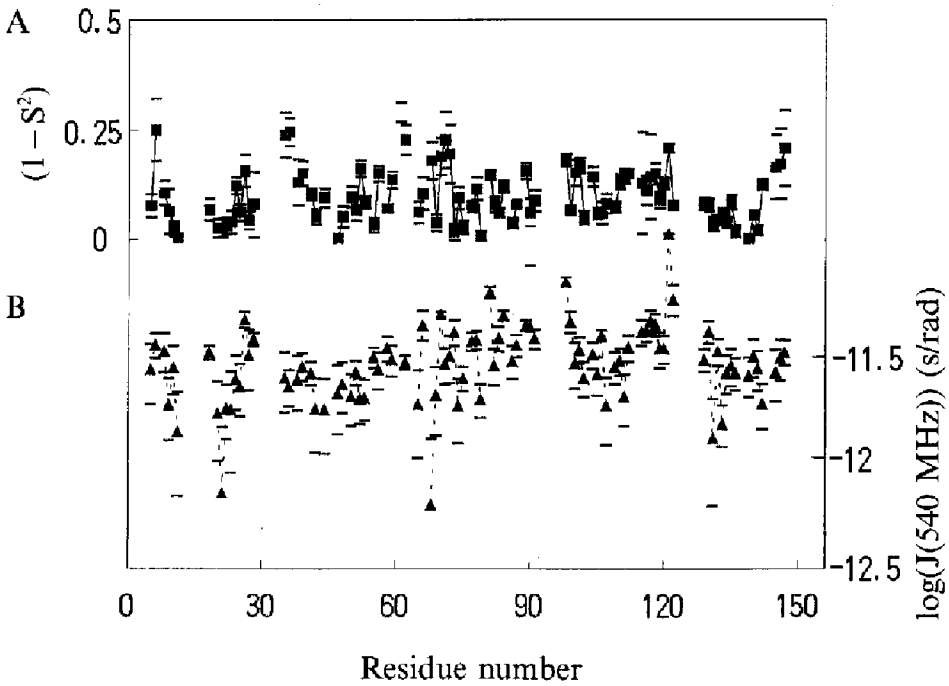

Fig. 3. Plot of (A) $1-S^2$ values; and (B) the experimentally determined value of J(540 MHz). The $1-S^2$ values were taken from the data of Yamasaki et al. (1995). Data are shown for the residues as illustrated in Fig. 1.

with the NMR order parameters (S^2) in the model-free analysis (Yamasaki et al., 1995) in order to examine whether or not the QSDF assumption was one of the reasons for the observed discrepancy. Figure 3 shows values for J(540 MHz) and the $(1-S^2)$ terms. The parameter S^2 indicates the restriction of internal motions in proteins. Figure 3 shows that there is a correlation in the residue profile between the values for J(540 MHz) and those for $(1-S^2)$. The correlation is positive, which indicates that the measured J(540 MHz) values are mainly increased by internal motions. The existence of this correlation does not support the possibility that the assumption in the QSDF is not valid for many residues.

Second, experimental errors may increase the scatter in the NMR-derived J(540 MHz) values relative to those determined from the simulation. There are many factors that affect the experimental data of individual residues. Evaluation of cross-peak intensities is complicated by low S/N ratio, problems with base-line distortion, and peak overlap. In particular, water suppression was used for a short period (100 ms) in order to minimize the water signal. We have to take these factors into account when we compare small differences in the values.

Third, low-frequency motions, which are out of the range of MD simulations, may increase the scatter in the experimental values of J(540 MHz). The obtained poor correlation between the experimental and calculated J(60 MHz) values supports this idea, because low-frequency motions also contribute to J(60 MHz). Simple calculations show that even a small amplitude against the total amplitude of the auto-correlation can contribute to the large variation in J(540 MHz) when the correlation time is sufficiently large. For example, the value of $\tau/(1+\omega^2\tau^2)$ at 540 MHz is 1 ps/rad when $\tau = 1$ ps, while this value is 230 ps/rad when $\tau = 1$ ns. Therefore, significant contributions from small amounts of low-frequency motions to the spectral density values are expected. We conclude that the scatter observed in the experimentally derived J(540 MHz) values reflects both the low-frequency internal motions in proteins and the experimental errors.

Experimentally determined spectral densities have been compared with those from the MD simulations for residues whose calculated auto-correlation functions reached steady states within the time range of the simulations. For this class of residues, it has been demonstrated that: (i) the calculated spectral densities accurately reproduce those determined experimentally, although effects of low-frequency motions in the protein are suggested even for residues reaching steady states; (ii) the values for J(540 MHz) reflect internal motions, as contributions from internal motions dominate those resulting from the overall rotation of the molecule at this frequency; and (iii) in order to obtain a meaningful comparison of MD simulations with NMR experimental data, both the amplitude and the frequency of the motion have to be considered.

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References

Balasubramanian, S., Nirmala, R., Beveridge, D.L. and Bolton, P.H. (1994) *J. Magn. Reson. Ser. B*, **104**, 240–249.

Barbato, G.B., Ikura, M., Kay, L.E., Pastor, R.W. and Bax, A. (1992) *Biochemistry*, **31**, 5269 5278.

Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S. and Karplus, M. (1983) *J. Comput. Chem.*, **4**, 187–217.

Brunne, R.M., Liepinsh, E., Otting, G., Wüthrich, K. and Van Gunsteren, W.F. (1993) *J. Mol. Biol.*, **231**, 1040 1048.

Chandrasekhar, I., Clore, G.M., Szabo, A., Gronenborn, A.M. and Brooks, B.R. (1992) *J. Mol. Biol.*, **226**, 239–250.

Cheng, J.W., Lepre, C.A. and Moore, J.M. (1994) *Biochemistry*, **33**, 4093–4100.

Clore, G.M., Driscoll, P.C., Wingfield, P.T. and Gronenborn, A.M. (1990) *Biochemistry*, **29**, 7387–7401.

Ericksson, M.A.L., Berglund, H., Härd, T. and Nilsson, L. (1993) *Proteins*, **17**, 375–390.

Farrow, N.A., Zhang, O., Forman-Kay, J.D. and Kay, L.E. (1995) *Biochemistry*, **34**, 868–878.

Fushman, D., Weisemann, R., Thüring, H. and Rüterjans, H. (1994) *J. Biomol. NMR*, **4**, 61–78.

Ishima, R. and Nagayama, K. (1995a) *J. Magn. Reson.*, in press.

Ishima, R. and Nagayama, K. (1995b) *Biochemistry*, **34**, 3162–3171.

Kay, L.E., Torchia, D.A. and Bax, A. (1989) *Biochemistry*, **28**, 8972–8979.

Kay, L.E., Nicholson, L.K., Delaglio, F., Bax, A. and Torchia, D.A. (1992) *J. Magn. Reson.*, **97**, 359–375.

Kraulis, P.J., Domaille, P.J., Campbell-Burk, S.L., Aken, T.V. and Laue, E.D. (1994) *Biochemistry*, **33**, 3515–3531.

Levitt, M. and Sharon, R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7557–7561.

Lipari, G. and Szabo, A. (1982a) *J. Am. Chem. Soc.*, **104**, 4546 4559.

Lipari, G. and Szabo, A. (1982b) *J. Am. Chem. Soc.*, **104**, 4559–4570.

Mandel, A.M., Akke, M. and Palmer III, A.G. (1995) *J. Mol. Biol.*, **246**, 144–163.

McCammon, J.A., Wolynes, P.G. and Karplus, M. (1979) *Biochemistry*, **18**, 927–942.

Nadler, W., Brunger, A.T., Schulten, K. and Karplus, M. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 7933–7937.

Palmer III, A.G., Rance, M. and Wright, P.E. (1991) *J. Am. Chem. Soc.*, **113**, 4371–4380.

Peng, J.W. and Wagner, G. (1992a) *Biochemistry*, **31**, 8571–8586.

Peng, J.W. and Wagner, G. (1992b) *J. Magn. Reson.*, **98**, 308–332.

Saito, M. (1994) *J. Chem. Phys.*, **101**, 4055–4061.

Schneider, D.M., Dellwo, M.J. and Wand, A.J. (1992) *Biochemistry*, **31**, 3645–3652.

Stone, M.J., Fairbrother, W.J., Palmer III, A.G., Reizer, J., Saier Jr., M.H. and Wright, P.E. (1992) *Biochemistry*, **31**, 4394–4406.

Swaminathan, S., Ichiye, T., Van Gunsteren, W.F. and Karplus, M. (1982) *Biochemistry*, **21**, 5230–5241.

Tycko, R. (Ed.) (1994) *Nuclear Magnetic Resonance Probes of Molecular Dynamics*, Kluwer, Dordrecht.

Yamasaki, K., Saito, M., Oobatake, M. and Kanaya, S. (1995) *Biochemistry*, **34**, 6587–6601.