Volume 239, number 1, 83–87 FEB 06432 October 1988

Nuclear Overhauser effects in aqueous solution as dynamic probes in short linear peptides

J.L. Nieto, M.A. Jiménez, M. Rico, J. Santoro and J. Herranz

Instituto de Estructura de la Materia, CSIC, Serrano 119, 28006 Madrid, Spain

Received 2 August 1988; revised version received 12 September 1988

The possibility of obtaining interresidue NOEs from short linear peptides in aqueous solution has been investigated from an experimental point of view using peptides of various lengths (namely GGRA, LHRH and RNase S-peptide). It is shown that, provided that long (~800 ms) NOESY mixing times are used, complete sets of sequential αN NOEs are obtainable. From the intensities and signs of the observed NOEs, the relative mobilities of different parts of the polypeptide chain can be determined.

NOE; Peptide flexibility; NMR assignment; Luteinizing hormone-releasing hormone; RNase S-peptide

1. INTRODUCTION

Proton nuclear Overhauser enhancements are the basis of NMR spectral assignments and structure determination in proteins [1]. However, interresidue NOEs from short flexible peptides are difficult to observe [2,3] due to conformational averaging [4] and an unfavourable correlation time [5]. The influence of the last parameter can be controlled using a solvent of high viscosity [6] or by rotating frame NOE experiments [7,8].

Nevertheless, the interest is normally centered on the conformational properties shown by the peptide in a water solution, which may be perturbed by the change or addition of a new solvent. Interresidue NOEs are necessary to characterize such properties, but unfortunately, they are generally absent [9–11] or only incompletely detected [12,13] for linear peptides in water solutions. We considered it of interest to investigate, from an experimental point of view, the possibility of obtaining NOEs from linear peptides of various sizes in aqueous solutions.

Our results show that, in contrast to general belief [5], complete sets of sequential αN NOEs [1] are detectable in such cases provided that long (≈800 ms) mixing times are used. An interesting conformational property, the relative mobility of different parts of the polypeptide chain, can be obtained from the magnitude and sign of the observed NOEs.

Correspondence address: J.L. Nieto, Instituto de Estructura de la Materia, CSIC, Serrano 119, 28006-Madrid, Spain

Abbreviations: NOE, nuclear Overhauser effect; NOESY, two-dimensional nuclear Overhauser and exchange spectroscopy; COSY, two-dimensional homonuclear correlated spectroscopy; LH-RH, luteinizing hormone-releasing hormone; RNase S-peptide, 1–19 fragment of bovine pancreatic ribonuclease A (EC 3.1.27.5)

2. EXPERIMENTAL

The LH-RH and GGRA peptides were obtained from Bachem AG, and RNase S-peptide from Sigma. The NMR samples were 20–30 mM in H_2O/D_2O, 90:10 (v/v), or in DMSO-d_6/H_2O mixtures (pH 3.0). NOESY spectra were obtained in the phase-sensitive mode [14] in a Bruker WM-360 spectrometer, using presaturation of the water signal. Size of data and transformed matrices were 2048 × 256 and 4096 × 1024 words in f_2 and f_1 dimensions, respectively. Shifted $\sin^2$ weighting functions in the f_2 and f_1 dimensions were applied before the two-dimensional Fourier transformation. Mixing times were 100, 400 or 800 ms, randomly variated by 5%. Experiment times ranged from 30 to 60 h and equidistant NOE intensity levels were selected for plotting.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

3. RESULTS AND DISCUSSION

Due to slow NOE build-up rates, long NOESY mixing times are expected to increase the intensities of NOEs arising from small molecules [15]. Fig.1 shows the influence that the mixing time has on the detection of the weak NOE intensities that short peptides originate in water solution. No detectable αN NOEs are seen in the NOESY spectrum of GGRA at 22°C, when 100 ms is used, a normal setting for protein samples. When 800 ms is used the result is very different since all sequential αN NOEs are then detected. An intermediate situation was found when a mixing time of 400 ms was used, where only partial detection of NOEs was achieved (fig.1).

The pattern of NOEs detected in GGRA, that is, the presence of sequential αN and the absence of sequential NN NOEs is in agreement with the existence of an extended polypeptide chain [1]. It should be noted that NOEs are detected despite the existence of conformational averaging, since this peptide is known to be in a random coil form [16]. To our knowledge, it is the first time that sequential NOEs are detected for random peptides of this size in aqueous solution.

Signs of all GGRA NOEs in H_2O at 22°C are positive, which means a short molecular correlation time, τ_c. As τ_c is increased by temperature or solvent composition changes [5], the observed NOEs are expected to become negative, after passing through a zero value [15]. This is actually seen in the NOESY spectra of GGRA when recorded in solvents of increasing viscosity. In a DMSO/H_2O (1:1) mixture, positive αN NOEs are still detected between residues 1–2 and 3–4 and zero (or near zero) αN NOE between residues 2 and 3 (not shown). In DMSO/H_2O (4:1), the only non-zero αN NOE detected is between residues 2 and 3 but showing now the opposite sign, i.e. negative (see fig.1). It is reasonable to interpret these changes in GGRA NOE intensities as due to global molecular τ_c changes and not to solvent induced conformational changes since this tetrapeptide is accepted to be random either in water [16] or DMSO solution [17]. Even if the promediated $\langle d_{\alpha N} \rangle$ distances were affected by the modification of the experimental conditions (through perturbations in peptide local conformations), the effect will only be a change in the absolute intensity of the NOEs, but not a change in their sign [4,14] as experimentally observed here. A plot of the type shown in fig.4, intensity of the αN NOE vs residue number, can be used to identify regions of the chain with different τ_c values and therefore different local mobilities. It is clear that the central R residue of GGRA has reduced mobility (longer τ_c) as compared with the other residues.

To explore further the possibilities of NOE intensities (measured near the point where $\omega\tau_c \sim 1$)

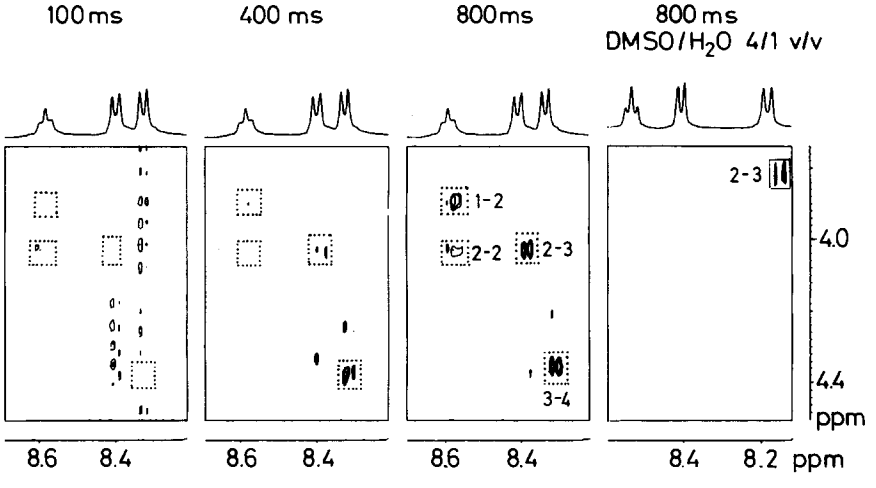

Fig.1. Influence of the mixing time and the solvent composition on the NOESY spectra of GGRA (20 mM, 22°C, pH 3.5). (——) Negative NOE; (···) positive NOE.

in detecting chain mobilities in a longer peptide, we have investigated LH-RH. No NOEs have been detected previously for this peptide in aqueous solution [9]. Fig.2 shows the LH-RH NOESY spectra obtained in H_2O at 52°C using 800 ms as mixing time, where almost a complete set of sequential αN NOEs can be seen. Signs are all positive indicating a short global molecular τ_c value. At 22°C (fig.2), sequential αN NOEs are also detected, but now they show negative signs, corresponding to longer τ_c values. At an intermediate temperature, 32°C, positive αN NOEs from terminal residues (1–2 and 9–10) are the only ones observed.

Fig.4 shows the corresponding LH-RH NOE intensity-sequence plot at the three temperatures. It can be seen that the change in τ_c values simply originated from temperature shifts up or down the corresponding trace, that can then be considered a peptide flexibility profile. The N- and C-terminal residues have again increased relative mobility, which is in agreement with ^{13}C relaxation time data reported for this peptide [18]. NOE intensities very close to zero have been detected at the three temperatures between residues G6 and L7 as shown in fig.4. A possible explanation for this fact is the existence of an increased local mobility at that point of the peptide chain, which is in agreement with the well known inherent flexibility that the amino acid residue G has.

Going further in peptide length, we have investigated NOE effects in RNase S-peptide, an interesting example of a short peptide that undergoes a temperature-induced conformational transition from random coil to α-helix [19]. Identification of αN correlations of their COSY spectra (not shown) at 5, 24 and 34°C was immediate with the help of their previously assigned [20,21] monodimensional ^1H NMR spectra. The S-peptide is mainly in random coil form in water solution at 5°C, pH 3.0 [19,21], so that NOEs typical of an extended chain are expected. This NOE pattern was actually seen in the S-peptide NOESY spectra taken in those conditions (see fig.3) where sequential αN NOEs are seen for all residues, except the one connecting S18 with A19 (see fig.3). All NOEs detected are negative, which corresponds to long molecular τ_c values.

As the temperature is raised to 24°C, some of the αN NOEs previously detected now become zero (residues 2, 16–18) while the NOE connecting residues 18–19 changes from zero to positive as shown in fig.4. The only NOEs that still maintain negative sign (longer local τ_c) arise from residues 3–14, a region that matches the one that is helical in the native protein [22]. These αN NOEs cannot arise from the small proportion (~13%) of the helical S-peptide molecules that exist in those experimental conditions [21] because sequential NN NOEs are then expected [1].

At a higher temperature, 34°C, very few helical

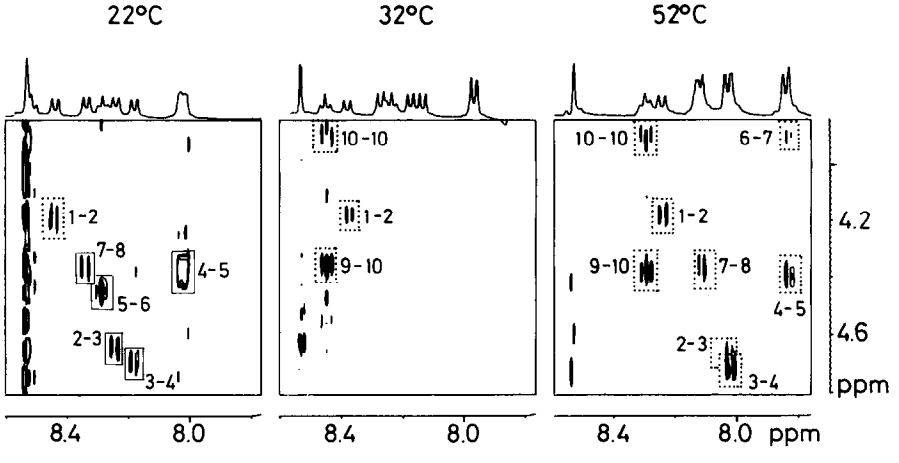

Fig.2. Effects of temperature changes on the NOESY spectra of LH-RH (30 mM, pH 3.0, 800 ms). (——) Negative NOE; (···) positive NOE.

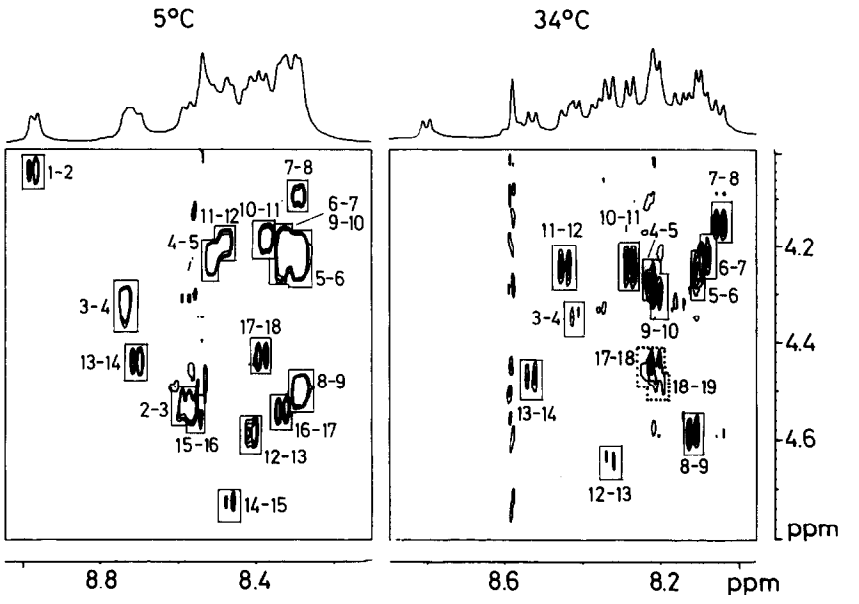

Fig.3. NOESY spectra of RNase S-peptide recorded at two temperatures (20 mM, pH 3.0, 800 ms). (——) Negative NOE; (···) positive NOE.

S-peptide molecules exist [19], but still a relative rigidity is manifested in the native helical region, as evidenced by negative αN NOEs detected for residues 5–14 (fig.3). Residues near N- and C-terminal ends have an increased mobility because they show zero (2–4, 16–17) or positive NOEs (18–19) which corresponds to shorter local τ_c values. The tendency of the S-peptide to be helical seems to be manifested as a reduced mobility on the same chain region that will become helical under particular conditions, which is an interesting result that merits further examination.

The experimental data reported here have shown the potential utility of NOE effects in investigating the conformational properties of flexible peptides in water solutions. Apart from the simplicity of NOESY with respect to ROESY [23,24], these results would be hardly obtained with ROESY since this experiment gives only positive peaks (no clear cut distinction between τ_c and $\langle d_{\alpha N} \rangle$ changes is possible), the intensities of which are in addition quite unsensitive to τ_c [14]. Two conclusions can be drawn from this paper. The first one is that complete or almost complete sets of sequential αN NOEs can be obtained in appropriate conditions which permit a sequential assignment of NMR spectra of linear peptides devoid of structure. The second one is that information on peptide relative chain mobilities is available from the intensities and signs of the observed NOEs.

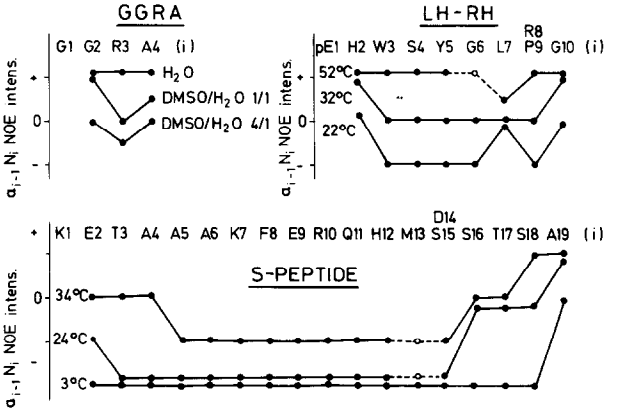

Fig.4. αN NOE intensity-sequence profiles of GGRA, LH-RH and RNase S-peptide under various experimental conditions. The NOE connecting residues 14–15 of S-peptide was not seen in any condition due to the proximity of the D14 H_α signal to the irradiated solvent line. NOEs showing reduced intensity for the same reason are shown by open circles.

REFERENCES

[1] Wüthrich, K., Billeter, M. and Braun, W. (1984) J. Mol. Biol. 180, 715–740.
[2] Motta, A., Tancredi, T. and Temussi, P.A. (1987) FEBS Lett. 215, 215–218.
[3] Picone, D., Temussi, P.A., Marastoni, M., Tomatis, R. and Motta, A. (1988) FEBS Lett. 231, 159–163.
[4] Shenderovich, M.D., Nikiforovich, G.N. and Chipens, G.I. (1984) J. Mag. Res. 59, 1–12.
[5] Motta, A., Picone, D., Tancredi, T. and Temussi, P.A. (1987) J. Mag. Res. 75, 364–370.
[6] Motta, A., Picone, D., Tancredi, T. and Temussi, P.A. (1988) Tetrahedron 44, 975–990.
[7] Bax, A. (1988) J. Mag. Res. 77, 134–147.
[8] Kessler, H., Gemmecker, G. and Hoase, B. (1988) J. Mag. Res. 77, 401–408.
[9] Chary, K.V.R., Srivastava, S., Hosur, R.V., Roy, K.B. and Govil, G. (1986) Eur. J. Biochem. 158, 323–332.
[10] Mammi, S., Mammi, N.J. and Peggion, E. (1988) Biochemistry 27, 1374–1379.
[11] Carver, J.A. (1987) Eur. J. Biochem. 168, 193–199.
[12] Gooley, P.R., Plaisted, S.M., Brems, D.N. and MacKenzie, N.E. (1988) Biochemistry 27, 802–809.
[13] Williamson, M.P., Hall, M.J. and Handa, B.K. (1986) Eur. J. Biochem. 158, 527–536.
[14] Kessler, H., Matthias, G. and Griesinger, C. (1988) Angew. Chem. Int. Ed. Engl. 27, 490–536.
[15] Macura, S. and Ernst, R.R. (1980) Mol. Phys. 41, 95–117.
[16] Bundi, A. and Wüthrich, K. (1979) Biopolymers 18, 285–297.
[17] Gratwohl, C. and Wüthrich, K. (1974) J. Mag. Res. 13, 217–225.
[18] Deslauriers, R. and Somorjai, R.L. (1976) J. Am. Chem. Soc. 98, 1931–1939.
[19] Rico, M., Santoro, J., Bermejo, F.J., Herranz, J., Nieto, J.L., Gallego, E. and Jiménez, M.A. (1986) Biopolymers 25, 1031–1053.
[20] Gallego, E., Herranz, J., Nieto, J.L., Rico, M. and Santoro, J. (1983) Int. J. Peptide Protein Res. 21, 242–253.
[21] Nieto, J.L., Rico, M., Santoro, J. and Bermejo, F.J. (1985) Int. J. Peptide Protein Res. 25, 47–55.
[22] Borkakoti, N., Moss, D.S. and Palmer, R.A. (1982) Acta Crystallogr. B38, 2210–2217.
[23] Farmer, B.T., Macura, S. and Brown, L.R. (1987) J. Mag. Res. 72, 347–352.
[24] Bothner-By, A.A. and Shukla, R. (1988) J. Mag. Res. 77, 524–535.