Conformational Analysis of the *Saccharomyces cerevisiae* Tridecapeptide Mating Pheromone by $^{13}C,^{15}N$ Rotational-Echo Double Resonance Nuclear Magnetic Resonance Spectroscopy†

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ABSTRACT: The solid-state conformation of [Nle^{12}]α-factor, the *Saccharomyces cerevisiae* tridecapeptide mating pheromone (WHWLQLKPGQPNleY), was investigated by $^{13}C,^{15}N$ rotational-echo double resonance (REDOR) nuclear magnetic resonance spectroscopy (NMR). Previous high-resolution NMR studies of [Nle^{12}]α-factor in solution revealed a transient Type II β-turn spanning residues 7–10 of the peptide. To investigate this region of [Nle^{12}]α-factor in the solid state, a series of four selectively $^{13}C,^{15}N$-enriched tridecapeptides were synthesized by solid-phase methods. Carbon–nitrogen distances between the labeled sites in lyophilized samples of [Nle^{12}]α-factor were accurately measured by REDOR NMR. Experimentally determined distances were compared with those from calculated models for Type I and Type II β-turns and for an extended chain. The measured distances indicate that, in a lyophilized powder, the central region of the [Nle^{12}]α-factor is *not* in an extended conformation. The experimental data was most consistent with distances obtained from a distorted Type I β-turn model.

The process of mating in the yeast *Saccharomyces cerevisiae* is mediated by extracellular peptide mating factors. The peptide pheromone α-factor (WHWLQLKPGQPMY) is secreted by MATα haploids of *Saccharomyces cerevisiae* (Marsh et al., 1991). It interacts with a receptor on MATa haploids and induces changes necessary for cell fusion. A central goal in peptide and protein research is the determination of the conformation of a peptide hormone and its receptor at the inception of and/or during the transduction of a biological response. The amino acid sequence of α-factor and its receptor has been defined (Burkholder & Hartwell, 1985), but the conformation of the mating factor, either free or bound to its receptor, is still under investigation. Much effort has been focused on the creation of new bioactive α-factor analogs by substitutions in the amino acid sequence (Levin et al., 1993; Naider & Becker, 1986) and/or by constraint of the conformation of the pheromone (Xue et al., 1989). Unfortunately, the lack of information on the structure of α-factor bound to its receptor negatively influenced this design and none of the synthetic analogs had a higher activity than the parent mating factor.

High-resolution proton NMR[1] analysis has been a powerful tool for investigating the conformation of α-factor, both free in solution and bound to phospholipid vesicles. Previous results, based primarily upon the analysis of NOESY spectra, indicate that the preferred conformation of α-factor in DMSO and water (Jelicks et al., 1988) as well as in phospholipid vesicles (Jelicks et al., 1989) is a transient Type II β-turn spanning residues 7–10. Structure/activity studies of a series of α-factor analogues have established a strong correlation between the appearance of NOESY cross-peaks characteristic of this Type II turn and peptide activity (Jelicks et al., 1988, 1989; Naider et al., 1992; Gounarides et al., 1993).

$^{13}C,^{15}N$ rotational-echo double resonance (REDOR) NMR (Gullion & Schaefer, 1989a,b) is a modern solid-state NMR experiment which permits accurate measurement of carbon–nitrogen distances of up to 5 Å in solid samples. $^{13}C,^{15}N$-REDOR has been used recently to map the backbone conformation of the tripeptide melanostatin (Garbow & McWherter, 1993) and to study the conformation and dynamics of gramicidin A in multilamellar dispersions (Hing & Schaefer, 1993). Intertryptophan distances in rat cellular retinol-binding protein II have been measured using ^{13}C–^{19}F REDOR (McDowell et al., 1993), and the ^{13}C–^{31}P REDOR version of the experiment has been used to characterize glyphosate bound to the protein enolpyruvateshikimate-3-phosphate synthetase (Christensen & Schaefer, 1993). In this work, we synthesized a series of four α-factor analogs labeled at specific sites with ^{13}C or ^{15}N. The positions of the labels were based on an examination of the folded and extended conformations of the tridecapeptide by computer modeling. Carbon–nitrogen distances were measured via REDOR NMR and the results were compared with those calculated for a variety of different models of α-factor.

MATERIALS AND METHODS

Materials. All chemicals and solvents were HPLC grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI), VWR Scientific (Piscataway, NJ), and Fisher Scientific (Springfield, NJ). Protected amino acids were purchased from Bachem Inc. (Torrance, CA) and from Advanced ChemTech (Louisville, KY). *N-t*-Boc[α-^{15}N]Gln, [^{15}N]Pro, and [2-^{13}C]Gly were obtained from MSD Isotopes (Montreal,

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[1] Abbreviations: NMR, nuclear magnetic resonance, REDOR, rotational-echo double resonance; CPMAS, cross-polarization magic-angle spinning; NOESY, nuclear Overhauser spectroscopy; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; FAB, fast-atom bombardment; DCHA, dicyclohexylamine; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acid; DMS, dimethyl sulfate.

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Table 1: ^{13}C, ^{15}N Analogs of α-Factor

1	WHWLQLK-[1-^{13}C]PG-[α-^{15}N]QPNleY
2	WHWLQLK-[^{15}N]P[1-^{13}C]G-QPNleY
3	WHWLQLK-[^{15}N]P[2-^{13}C]G-QPNleY
4	WHWLQLKP[2-^{13}C]G-[α-^{15}N]QPNleY

Canada); [1-^{13}C]Pro was from Cambridge Isotope Laboratories (Woburn, MA); and *N-t*-Boc[1-^{13}C]Gly was from Isotec (Miamisburg, OH).

Selection of Labeling Sites. All analyses were carried out on samples of [Nle^{12}]α-factor (Nle = L-norleucine). Nle is isosteric with Met and the [Nle^{12}]α-factor was found to have biological activities indistinguishable from those of native α-factor (Raths et al., 1988). As described in the introduction, previous conformational studies of α-factor in solution and phospholipid vesicles revealed the presence of a transient Type II β-turn spanning residues Lys^7-Pro^8-Gly^9-Gln^{10}. Because we were interested in a solid-state study of the region of the peptide spanned by the transient β-turn, we prepared the four selectively ^{13}C,^{15}N-enriched peptides shown in Table 1. The choice of sites for selective ^{13}C and ^{15}N labels in the α-factor samples was based upon the following considerations. A bend can be loosely defined as a conformation in which a sequence of four consecutive amino acid residues folds back on itself (Zimmerman, 1985). The conformation of the bend is defined by the backbone dihedral angles ϕ and ψ, which in turn determine specific interatomic distances. For α-factor, the dihedral angles of Pro^8 and Gly^9 are crucial for the identification of a chain reversal involving residues 8 and 9. The labeled sites in samples **1**, **2**, and **3** were selected to provide information about these dihedrals. Assuming planar peptide bonds and constant bond angles and bond lengths, the distance between the carbonyl carbon of Pro^8 and the α-nitrogen of Gln^{10} (**1**) depends on ϕ_{Gly} and ψ_{Gly}, the distance between the nitrogen of Pro^8 and the carbonyl carbon of Gly^9 (**2**) depends on ψ_{Pro} and ϕ_{Gly}, and the distance between the nitrogen of Pro^8 and the α-carbon of Gly^9 (**3**) depends on ψ_{Pro} alone. Accurate determination of the carbon–nitrogen distances between these labeled sites greatly restricts the allowed values of the dihedrals of Pro^8 and Gly^9. Sample **4**, in which the labeled positions are separated by 2.40 Å, served as a fixed-distance reference.

Synthesis. Boc-[1-^{13}C]Pro and Boc-[^{15}N]Pro were synthesized by conventional procedures using di-*tert*-butyl dicarbonate with yields of 92% and 89%, respectively. Both compounds were pure by TLC and HPLC analyses and coeluted with authentic material. Boc-[2-^{13}C]Gly was also prepared using di-*tert*-butyl dicarbonate and was characterized by TLC, FAB mass spectrometry, 300-MHz ^1H NMR, and 75-MHz ^{13}C NMR. Boc-[^{15}N]Gln, whose original purity as determined by HPLC was only ~80%, was purified by HPLC prior to peptide synthesis.

The solid-phase synthesis of the α-factor samples containing ^{13}C and ^{15}N amino acids was carried out starting from 300 mg of BocNleTyr(2-BrZ)-OCH$_2$-PAM resin in each synthesis. The capacity of the resin was determined to be 0.43 mmol/g of resin by bromine analysis. The usual synthetic cycle (Xue et al., 1989) was modified for Boc group cleavage with 1% indole being added to the 45% TFA/2% DMS solution in CH_2Cl_2 to avoid side reaction with tryptophan. A mixture of *p*-cresol/*p*-thiocresol (3:1 v/v) served as scavenger during the HF cleavage. BocHis(Tos) for the coupling was prepared from its DCHA salt. Crude peptides from the HF cleavage were purified by HPLC on a Waters μBondapak C_{18} column (19 × 300 mm) using a linear gradient of water and acetonitrile from 0 to 60% acetonitrile in 90 min with a flow rate of 6 mL/min. Both eluents contained 0.025% trifluoroacetic acid. The overall yield of peptides was 20–35% and the purity of all peptides was greater than 98% as judged by analytical HPLC on a C_{18} column. All ^{13}C,^{15}N-labeled peptides were identical with authentic unlabeled [Nle^{12}]α-factor as judged by coinjection on HPLC and had identical bioactivity in a growth arrest assay using *Saccharomyces cerevisiae* RC 629.

Computer Modeling. α-Factor models were built using the SEQUENCE BUILDER module of the QUANTA 3.3 software. Energy minima in vacuo were found by CHARMm22 (Brooks et al., 1983) steepest descents and conjugate gradients minimization combined with Boltzmann searches. Parameters for the Boltzmann jump search were as follows: number of samples = 20; torsion angle window = 15°–30°; temperature = 20 000 K. Minimizations of [Nle^{12}]α-factor structures were performed starting with three different initial models: (i) a Type II β-turn involving residues 8 and 9, (ii) a Type I β-turn involving residues 8 and 9, or (iii) a fully extended conformation (all ϕ, ψ, ω = 180°, except for $\phi_{Pro\text{-}8}$ and $\phi_{Pro\text{-}11}$). For models (i) and (ii), dihedral angles for all residues except Pro^8, Pro^{11}, and Gly^9 were set equal to 180° prior to minimization. To estimate the distance between the carbonyl carbon of Pro^8 and the peptide nitrogen in Gln^{10} in a random population of conformers, a grid search (in 2° steps) of the ϕ and ψ angles of Gly^9 was performed. The algebraic average of distances for all conformers not excluded by van der Waals contacts was then computed.

Sample Preparation. Samples of [Nle^{12}]α-factor for solid-state NMR analysis were lyophilized from solutions having 10 mg/mL peptide in a mixture of acetonitrile/water (1/4 v/v) containing 0.025% TFA. To verify the accuracy of the REDOR measurements and check for the possible influence of intermolecular interactions on these measurements, some of the samples were diluted with unlabeled [Nle^{12}]α-factor prior to lyophilization.

REDOR NMR. ^{13}C,^{15}N Rotational-echo double resonance (REDOR) (Gullion & Schaefer, 1989a,b) is a solid-state NMR technique that permits the accurate measurement of through-space carbon-to-nitrogen distances between selectively ^{13}C- and ^{15}N-enriched sites. REDOR distances, r_{CN}, are calculated from measured heteronuclear dipolar coupling constants, D_{CN}, according to the following equation (Abragam, 1961):

$$r_{CN} = \left(\frac{\gamma_C \gamma_N h}{D_{CN} 2\pi}\right)^{1/3}$$

where γ_C and γ_N are the gyromagnetic ratios of ^{13}C and ^{15}N, respectively.

The pulse sequence used to collect REDOR ^{15}N NMR data is shown in Figure 1. ^{15}N π pulses applied at the completion of each rotor period refocus isotropic chemical shifts. On alternate scans of the experiment, the π pulses on the carbon channel are either applied or omitted and signals from these alternate scans are accumulated and Fourier-transformed separately. The rotor-synchronous ^{13}C π pulses reintroduce ^{13}C–^{15}N dipolar couplings removed by sample rotation in standard CPMAS experiments. The ^{13}C π pulses cause a partial dephasing of the ^{15}N signal due to the ^{13}C–^{15}N dipolar coupling, leading to a loss in ^{15}N signal intensity. The magnitude of this signal loss, ΔS, is determined from the difference between spectra from the experiments with and without ^{13}C π pulses. The ratio of this difference to the signal from the isotopically enriched site alone (S_0), $\Delta S/S_0$, depends on the product of D_{CN}, the spinning period, T_r ($=1/\nu_r$), and the number of rotor periods of ^{13}C–^{15}N dipolar coupling

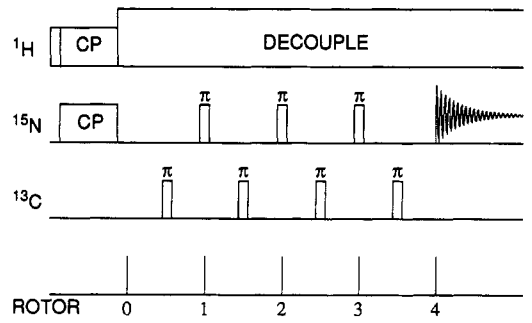

FIGURE 1: Pulse sequence for ^{15}N-observe REDOR NMR. On alternate scans of the REDOR experiment, the ^{13}C π pulses are either applied or omitted, and signals from these alternate scans are accumulated and Fourier-transformed separately. The magnitude of the difference in signals collected with and without the ^{13}C π pulses, ΔS, depends on the product of the dipolar coupling constant, D_{CN}, the spinning period, T_r ($=1/\nu_r$), and the number of rotor periods of ^{13}C–^{15}N dipolar coupling evolution, N_C. This figure illustrates the REDOR pulse sequence with $N_C = 4$; N_C can be increased (in increments of 2) by adding rotor periods and pairs of ^{13}C and ^{15}N π pulses between the end of the cross-polarization preparation (CP) and the start of data acquisition.

evolution, N_C (Gullion & Schaefer, 1989b).

Experimental NMR. REDOR ^{15}N NMR experiments were performed on a home-built spectrometer operating at a ^{1}H Larmor frequency of 127.0 MHz. Samples were spun at the magic angle (54.7°) with respect to the static magnetic field in a double-bearing rotor system (Schaefer et al., 1987) at a rate of 3 kHz. Electronic feedback circuits on the spectrometer permit us to control ν_R to within ±3 Hz and the field strengths, H_1, on each of the three radiofrequency channels to within ±0.25%. This control circuitry helps to provide excellent long-term spectrometer stability, allowing us to produce reliable difference spectra that are necessary for accurate distance measurements. Nitrogen NMR spectra were obtained at 12.9 MHz following 2-ms matched, 35-kHz ^{1}H–^{15}N cross-polarization contacts. Proton decoupling fields were 110 kHz during the dipolar evolution period and 80 kHz during data acquisition. The amplitude of the ^{13}C radio-frequency field was 50 kHz. Phases of both the ^{13}C and ^{15}N π pulses were cycled according to the XY-8 (*xyxyyxyx*) scheme (Gullion et al., 1990a) to minimize off-resonance effects (Gullion et al., 1990b; Gullion & Schaefer, 1991). $\Delta S/S_0$ values were computed as the ratios of peak heights in the REDOR spectra.

RESULTS AND DISCUSSION

Figure 2 shows REDOR ^{15}N NMR spectra of two ^{13}C,^{15}N-enriched [Nle^{12}]α-factor samples collected with the pulse sequence of Figure 1. In this figure, the bottom spectrum in each column is the full echo spectrum collected in the absence of ^{13}C π pulses, while the top spectrum is the REDOR difference spectrum, formed by subtracting spectra collected with and without ^{13}C π pulses. These difference spectra are due directly to ^{13}C–^{15}N dipolar coupling, and carbon–nitrogen dipolar coupling constants, D_{CN}, can be calculated from the ratios of REDOR difference and echo signals.

Figure 2 (left) shows REDOR ^{15}N NMR data for the fixed-distance sample, **4**, collected with $N_C = 16$. The ratio of REDOR difference to echo signal, $\Delta S/S$, for this sample is 0.703. This ratio must be corrected for natural-abundance signal contributions to both S and ΔS before a dipolar coupling can be accurately determined. From consideration of the primary sequence of [Nle^{12}]α-factor, we note that in addition to the Gln^{10} peptide nitrogen, which is isotopically enriched to 99 atom % ^{15}N in its α-nitrogen position in this sample, there are a total of 11 nitrogen atoms in peptide bonds and two side-chain amide nitrogens (Gln^4 and Gln^{10}). Each of these 13 natural-abundance amide nitrogens contributes to the observed signal at 90 ppm. Taking ^{15}N's 0.37% natural-abundance level into account, we calculate that natural-abundance ^{15}N contributes 4.6% of the total echo signal.[2] Adjusting the ratio $\Delta S/S$ for these natural-abundance contributions yields a $\Delta S/S_0$ value of 0.737. The second effect of natural-abundance spins is their contributions to the REDOR difference signal and is composed of two components: natural-abundance ^{13}C spins that are proximate to the labeled [α-^{15}N]Gln position and natural-abundance ^{15}N spins close to the [2-^{13}C]Gly. As described previously, these natural-abundance spins contribute known, calculable amounts to the observed $\Delta S/S_0$ (Garbow & McWherter, 1993). Following the previously established procedure, we calculate a natural abundance contribution of 0.038 to $\Delta S/S_0$ for this sample.[3] We thus determine the true $\Delta S/S_0$ value for this sample to be 0.699 (0.737–0.038).

Table 2: ^{15}N REDOR NMR of [Nle^{12}]α-Factor

sample	$\Delta S/S_0$	N_C	D_{CN} (Hz)	r_{CN} (Å)
1	0.304	32	53	3.61
2	0.151	48	24	4.70
3	0.231	48	30	4.35
4	0.699	16	182	2.40

A $\Delta S/S_0$ value of 0.699, collected with $N_C = 16$ and $\nu_r = 3.0$ kHz, translates into a ^{13}C–^{15}N dipolar coupling of 182 Hz. This dipolar coupling is approximately 10% smaller than the value calculated in the rigid-lattice limit. A similar reduction in coupling has been observed previously in measurements of ^{13}C–^{15}N (Marshall et al., 1990; Garbow & McWherter, 1993) and ^{13}C–^{1}H (Munowitz & Griffin, 1982; Schaefer et al., 1983) dipolar interactions and is attributed to high-frequency molecular vibrations and librations. In practice, we take this scaling of the coupling for the known, fixed distance α-factor sample as an experimental measure of these high-frequency motions. Thus we report in Table 2 a carbon–nitrogen distance of 2.40 Å for sample **4** and use the relationship between coupling constant and distance in this sample to calculate the three conformationally dependent distances reported in Table 2.

Figure 2 (right) shows REDOR ^{15}N NMR data for [^{15}N]-Pro^8-[2-^{13}C]Gly^9 [Nle]α-factor (**3**), one of the three samples in which the internuclear distance between labeled sites is conformationally dependent. The spectra in this figure were collected following an evolution period of 48 rotor periods ($N_C = 48$). The measured $\Delta S/S$ value from these spectra, 0.297, must be corrected for natural-abundance effects as

[2] As noted in the text, a total of 13 amide-nitrogen positions contribute at natural abundance (0.37%) to the ^{15}N signal at 90 ppm. The total intensity of this resonance equals the signal from the labeled ^{15}N position (1×0.99) + the natural-abundance signal (13×0.0037) = 1.038. Of this signal, 0.99/1.038 = 95.4% arises from the labeled position. To convert $\Delta S/S$ to $\Delta S/S_0$, we multiply the value of $\Delta S/S$ by (1/0.954) = 1.049, yielding a $\Delta S/S_0$ value of 0.737.

[3] Natural-abundance contributions to $\Delta S/S_0$ arise from natural-abundance ^{13}C spins near the [α-^{15}N]Gln and natural-abundance ^{15}N spins near the [2-^{13}C]Gly. From the perspective of [α-^{15}N]Gln^{10}, four natural-abundance carbon positions, all at known fixed distances from the α-^{15}N label, must be considered: [C$_i$]Gln^{10} (2.40 Å), [C$_\alpha$]Gln^{10} (1.48 Å), [C$_i$]Gly^9 (1.33 Å), and [C$_\beta$]Gln^{10} (2.40 Å). From the perspective of [2-^{13}C]Gly^9, natural-abundance ^{15}N at Gly^9, at a distance of 1.48 Å, must be considered. The $N_C = 16$ contributions to $\Delta S/S_0$ from these sources are readily calculated (Garbow & McWherter, 1993) and total 0.038; thus $\Delta S/S_0$ from the labeled spin pair is 0.737–0.038 = 0.699.

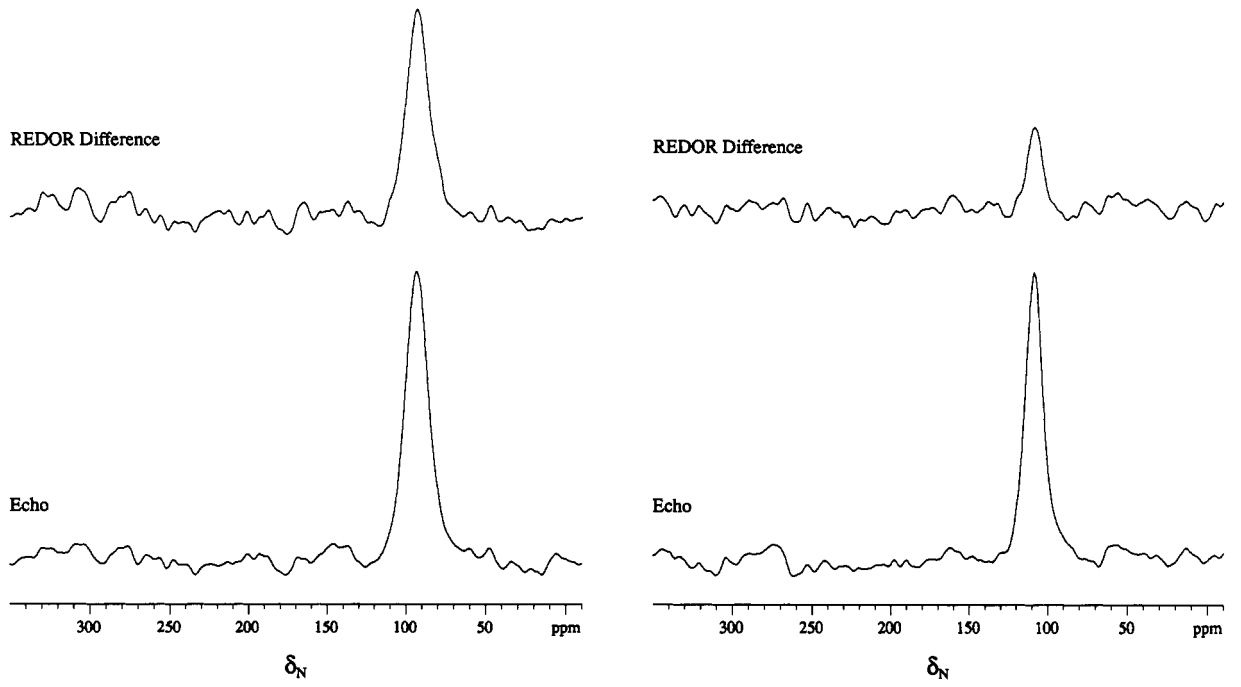

FIGURE 2: REDOR ^{15}N NMR spectra of ^{13}C,^{15}N-enriched [Nle^{12}]α-factor samples: (left) fixed-distance WHWLQLKP[2-^{13}C]G-[α-^{15}N]-QPNleY (sample **4**, 43 mg; 12 000 accumulated scans) and (right) WHWLQLK-[^{15}N]P[2-^{13}C]G-QPNleY (sample **3**, 17 mg; 70 000 accumulated scans). (Bottom) Echo spectra of full sample; (top) REDOR difference. Spectra were collected using the pulse sequence of Figure 1 with ν_R = 3 kHz, (left) N_C = 16; (right) N_C = 48.

described above. Taking into account natural-abundance contributions to both S and ΔS, we calculate a true $\Delta S/S_0$ value for the labeled spin pair in this sample of 0.231,[4] corresponding to a dipolar coupling of 30 Hz. On the basis of the relationship between dipolar coupling and internuclear distance established for the fixed-distance sample (*vide supra*), we calculate a carbon–nitrogen distance of 4.35 Å for this sample. Results of the REDOR ^{15}N NMR experiments for all of the [Nle]α-factor samples are tabulated in Table 2. We estimate uncertainties of ±0.1 Å for the REDOR-determined distances reported in Table 2. These error estimates are derived directly from estimated uncertainties in the measured values of $\Delta S/S_0$ and are consistent with error limits reported in previous REDOR studies (Marshall et al., 1990; Garbow & McWherter, 1993). To measure the possible effects of intermolecular dipolar couplings, ^{15}N REDOR experiments were performed on sample **3** diluted 1:2 and 1:4 in natural abundance. The measured carbon–nitrogen distances for these diluted samples were within 0.1 Å of the value reported in Table 2, demonstrating that dipolar coupling between labeled sites on adjacent chains are not significant.

REDOR ^{13}C NMR data, in which carbon signal is observed and ^{15}N π pulses reintroduce the carbon–nitrogen dipolar coupling, were also performed on each of these samples. In general, the agreement with the distances reported in Table 2 was good, with the largest discrepancy being ~0.3 Å for sample **3**. However, we regard the ^{13}C-observe REDOR data as less reliable than the ^{15}N data and do not report it in Table 2. One of the inherent assumptions of the standard REDOR analysis is that the observed signals arise *uniformly* from a sample of randomly oriented molecules. Intensities in the REDOR ^{15}N echo spectra were 85–95% of those in CPMAS ^{15}N NMR spectra, validating this assumption for the nitrogen-observe data. By contrast, in the ^{13}C experiments, N_C = 32 REDOR echo spectra had only 10–20% of the total signal intensity of corresponding CPMAS ^{13}C NMR spectra. Although the source of the signal loss has not been identified (experiments have shown that losses are not due to incomplete proton decoupling), it is most likely an orientation-dependent effect. In this case, the observed signal no longer arises uniformly from a randomly distributed set of molecules, the REDOR analysis breaks down, and the distances extracted are no longer meaningful. For this reason, REDOR ^{13}C NMR data are not reported.

To interpret the REDOR distances reported in Table 2, we carried out a minimization of the [Nle^{12}]α-factor structure using the CHARMm22 program. It should be noted that the tridecapeptide pheromone is a highly flexible molecule. For such molecules, molecular modeling produces a multitude of local minima and the final, minimized molecular conformation is often highly dependent on the initial set of dihedral angles. With previous solution NMR studies suggesting a β-turn centered about residues Pro^8-Gly^9 and with the value of the Pro^8 φ angle highly restricted by the geometry of the proline ring, the dihedral angles of Gly^9 are of particular importance in characterizing a possible bend in this region of [Nle^{12}]α-factor.

We modeled the conformation of the tridecapeptide starting with three different initial models: (i) a Type II β-turn centered about residues Pro^8-Gly^9, (ii) a Type I β-turn centered about Pro^8-Gly^9, or (iii) a fully extended conformation. In choosing these initial conditions, we were aided by the presence of L–Pro^8, which excludes all of the prime-type turns. Results of the energy minimizations are reported in Table 3. It is clear that the minimized extended structure does not contribute significantly to the structure of α-factor in the lyophilate. The observed distance in peptide **2** (4.70 Å) differs from the

[4] The experimentally determined value of $\Delta S/S$ (0.297) is first multiplied by 1.049 to yield a $\Delta S/S_0$ value of 0.311. Natural-abundance corrections to $\Delta S/S_0$ in this sample are similar to those described in footnote 3. However, additional terms must be included for natural-abundance carbons in the proline ring, all of which are within 2.40 Å of the labeled [^{15}N]Pro^8 spin, and the calculated contributions for N_C = 48 differ somewhat from the N_C = 16 numbers. The natural-abundance corrections to $\Delta S/S_0$ total 0.080 for both this sample and sample **2**.

Table 3: Interatomic Distances: Experimental and Calculated in Models

	minimized conformations[a] (Å)			ideal conformations (Å)			
	type I β-turn	type II β-turn	extended	type I β-turn[b]	type II β-turn[c]	extended[d]	REDOR ^{15}N distance (Å)
1	3.56	3.31	4.69	3.08	2.92	4.77	3.61
2	4.70	5.18	5.82	4.62	4.93	6.10	4.70
3	4.21	4.75	4.76	4.10	4.67	4.87	4.35
4	2.40	2.40	2.40	2.40	2.40	2.40	2.40

[a] Minimized conformations were obtained from the corresponding ideal conformations using the minimization procedures described in the text in Materials and Methods, Computer Modeling. [b] ϕ(Pro) = −60°, ψ(Pro) = −120°, ω(ProGly) = 180°, ϕ(Gly) = 80°, ψ(Gly) = 0°. [c] ϕ(Pro) = −60°, ψ(Pro) = −30°, ω(ProGly) = 180°, ϕ(Gly) = −30°, ψ(Gly) = 0°. [d] All torsion angles *except* ϕ(Pro) = 180°; ϕ(Pro) = −60°.

distance calculated for an extended structure by approximately 1 Å. By contrast, the minimized structures obtained from Type I and Type II β-turns give interatomic distances within 0.4 Å of the REDOR distances. The experimental data are most consistent with distances observed for the minimized Type I β-turn, whose calculated values differ by only 0.1–0.2 Å from the ^{15}N REDOR distances. For completeness, we have included in Table 3 distances for idealized Type I and Type II β-turns and for a fully extended chain. Comparison of the ^{15}N-REDOR distances with these idealized distances would still support the conclusion that the lyophilized [Nle^{12}]α-factor forms a turn.

Additional insights into the meaning of the REDOR distances can be gained by comparing these with distances calculated for a random distribution of structures in the Lys^7–Gln^{10} region. A grid scan search of the ϕ, ψ angles of Gly^9 was first performed; all sets of dihedrals that violated van der Waals radii were then excluded. Assuming all nonexcluded conformations to be equally probable, we then computed an average C_1 of Pro^8–N_α of Gln^{10} distance of 4.08 Å. Since the REDOR value for **1** is approximately 0.4 Å shorter than the distance calculated for a random distribution of conformations, we conclude that folded structures predominate in the lyophilate. Thus, it is reasonable to conclude that β-turns may play a major role in the distribution of the pheromone in a lyophilized powder. A distorted Type I β-turn appears to be quite probable.

The results of our REDOR/computational analysis indicate that even in a lyophilized powder the [Nle^{12}]α-factor shows a preference for turn structures. This finding is similar to previous conclusions, based on ^1H NMR studies, that the pheromone assumes a transient Type II β-turn in organic and aqueous media and in the presence of lipid vesicles (Jelicks et al., 1988, 1989; Gounarides et al., 1993). A question might be raised as to the biological relevance of studies on lyophilized peptides. Our long-range goal is the determination of the structure of the [Nle^{12}]α-factor bound to its receptor. To accomplish this significant, yet technically difficult aim, we must establish whether the REDOR procedure can be applied to peptides in various environments. The study on lyophilized [Nle^{12}]α-factor was undertaken from this perspective.

In the lyophilate, the peptide retains a significant amount of water, as judged from DSC analysis (unpublished results). As the same time, we can anticipate more interpeptide interactions than in aqueous solution. (As described above, we have ruled out the influence of such interpeptide interactions on our REDOR measurements.) The fact that we still see a preference for turn structures in the lyophilate suggests that the peptide probably has a strong tendency to form a bend. Most importantly, the studies reported herein provide evidence that REDOR can be applied to lyophilized powders and would allow extension to studies on lyophilates of the [Nle^{12}]α-factor bound to its receptor.

CONCLUSIONS

REDOR measurements provide an important tool for studying the biologically active conformation of peptides. The method is amenable to crystalline and amorphous solids and, as shown herein, to lyophilized powders. In principle, one can apply this procedure to hormone–receptor complexes in lyophilized lipid films or in the frozen state. At present the major limitation of this approach is obtaining sufficient receptor (3–5 μmol; 100–250 mg) to perform the measurements. The results of this study demonstrate that REDOR measurements can be carried out on lyophilized samples of the tridecapeptide pheromone [Nle^{12}]α-factor and can provide meaningful estimates of interatomic distances. The findings on [Nle^{12}]α-factor support previous conclusions, derived from solution NMR studies, that the central region of this pheromone assumes a bent structure.

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