The NMR solution structure of the pheromone Er-2 from the ciliated protozoan *Euplotes raikovi*

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Abstract

The NMR structure of the pheromone Er-2 from the ciliated protozoan *Euplotes raikovi* has been determined in aqueous solution. The structure of this 40-residue protein was calculated with the distance geometry program DIANA from 621 distance constraints and 89 dihedral angle constraints; the program OPAL was employed for the energy minimization. For a group of 20 conformers used to characterize the solution structure, the average pairwise RMS deviation from the mean structure calculated for the backbone heavy atoms N, Cα, and C' of residues 3-37 was 0.31 Å. The molecular architecture is dominated by an up-down-up bundle of 3 short helices of residues 5-11, 14-20, and 23-33, which is similar to the structures of the homologous pheromones Er-1 and Er-10. Novel structural features include a well-defined N-cap on the first helix, a 1-residue deletion in the second helix resulting in the formation of a 310-helix rather than an α-helix as found in Er-1 and Er-10, and the simultaneous presence of 2 different conformations for the C-terminal tetrapeptide segment, i.e., a major conformation with the Leu 39-Pro 40 peptide bond in the trans form and a minor conformation with this peptide bond in the cis form.

Keywords: ciliate pheromone Er-2; cis-trans isomerism of Xxx-Pro peptide bonds; *Euplotes raikovi*; NMR structure; steric constraints and α-helix-310-helix transition

Cellular recognition and signal transduction processes mediated by polypeptides or small proteins, e.g., growth factors and hormones, are an important feature of intercellular communication in higher eukaryotes (James & Bradshaw, 1984; Sporn & Roberts, 1990). The pheromone regulatory system in *Euplotes raikovi* represents a similar, simpler system in a unicellular eukaryote. As such, it may represent an evolutionary predecessor of the more sophisticated regulatory mechanisms in multicellular species (Luporini et al., 1994). The apparent simplicity of the molecular basis of regulation makes the *E. raikovi* system an attractive paradigm for investigating the structural basis that underlies both "self" and "non-self" recognition processes, and the ability of these recognition processes to elicit different cellular responses. To establish a structural basis for further studies of this system on the molecular level, we previously determined the NMR solution structure of the pheromone Er-10 (Brown et al., 1993). This paper describes the determination of the NMR structure of the pheromone Er-2, and the structure of Er-1 is now also available (Mronga et al., 1994 [companion paper]). The amino acid sequence of Er-2 has previously been determined (Raffioni et al., 1992), and the residue pairing for all 3 disulfide bonds has also been established using chemical methods (Stewart et al., 1992).

Results and discussion

The aqueous solution conditions for the structure determination of Er-2 were fixed at pH 5.0 and 10 °C on the basis of the following observations. Circular dichroism spectra indicated that at pH 5.0 the fraction of helical secondary structure decreases approximately linearly with temperature over the range of 10-80 °C. CD and 1H NMR spectra recorded at 10 °C after heating the Er-2 sample to 80 °C for several minutes were indistinguishable from spectra recorded at 10 °C prior to heating.