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TEMPERATURE INDUCED PROTEIN UNFOLDING AND FOLDING OF RNase A STUDIED BY TIME-RESOLVED INFRARED SPECTROSCOPY

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When a protein finds its native three-dimensional structure from the unstructured amino-acid chain various processes spanning a large time range are relevant. To understand the mechanism of protein folding one needs to cover the entire folding/refolding (U ↔ N) reaction on a structural level. In the case of RNase A, the main structural changes occur in the ms time range, that can be monitored with rapid-scan-FTIR spectroscopy combined with rapid mixing techniques. To induce unfolding we inject aqueous protein solution into a hot IR cuvette and record the time course of the spectral changes. A lag phase is found when the unfolding conditions are relatively weak, suggesting an unfolding intermediate.

Keywords: Protein folding; time-resolved infrared spectroscopy; RNase A; stopped-flow

Unfolding studies on RNase A revealed three major kinetic phases. In addition to the main unfolding transition (ms) and slow proline isomerization steps (sec. to minutes), a very fast phase has been found by Phillips *et al.*, at pH* 5.7 [1], as well as by our group at pH* 2.0 [2]. Nevertheless, the medium phase seems to comprise the main unfolding transition and will be focused on in this study.

Deuterated Ribonuclease A (RNase A) was heated to defined temperatures and IR absorption spectra were recorded (insert in Fig. 1).

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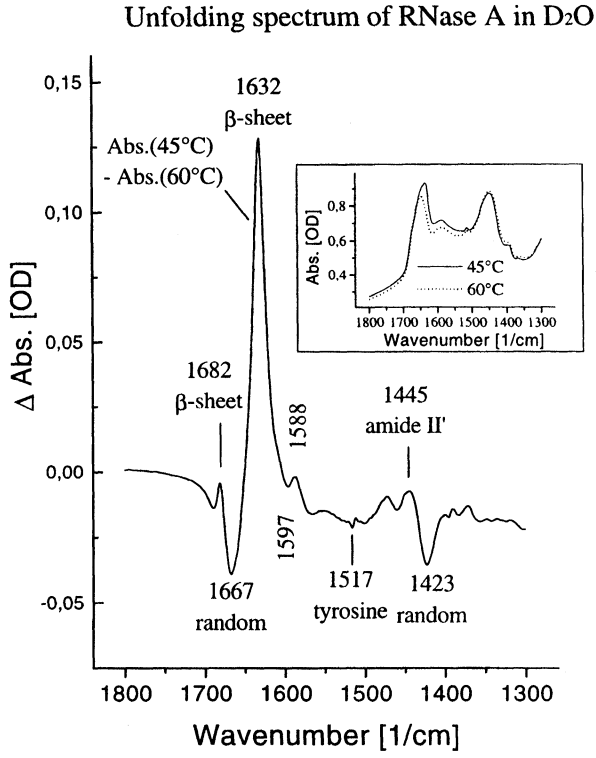

FIGURE 1 Temperature dependent IR absorption difference of 1 mM RNase A at pH* 3.9, ΔAbs. = Abs.(45°C)–Abs.(60°C); insert: IR absorption spectra at 45°C (solid line) and at 60°C (dotted line).

The calculated absorption difference is shown in Figure 1 and shows the assignment of distinct absorption bands to structural elements of RNase A. Injecting the native protein at 25°C by a stopped-flow apparatus into a hot (T_{final} = 56(50)°C) IR-cuvette, a ms temperature jump is generated inducing the unfolding of the protein [3]. Rapid-scan-FTIR spectra are recorded every 16 ms. By rearranging the data the kinetic response of RNase A can be extracted for any wavenumber of interest. Figure 2 shows the unfolding kinetic under strong (T_{final} = 56°C) or weak (T_{final} = 50°C) unfolding conditions at 1632 cm^{-1} and 1667 cm^{-1}, representing the decay of β-sheet structure and the growth of "random" structure, respectively. Under the weak

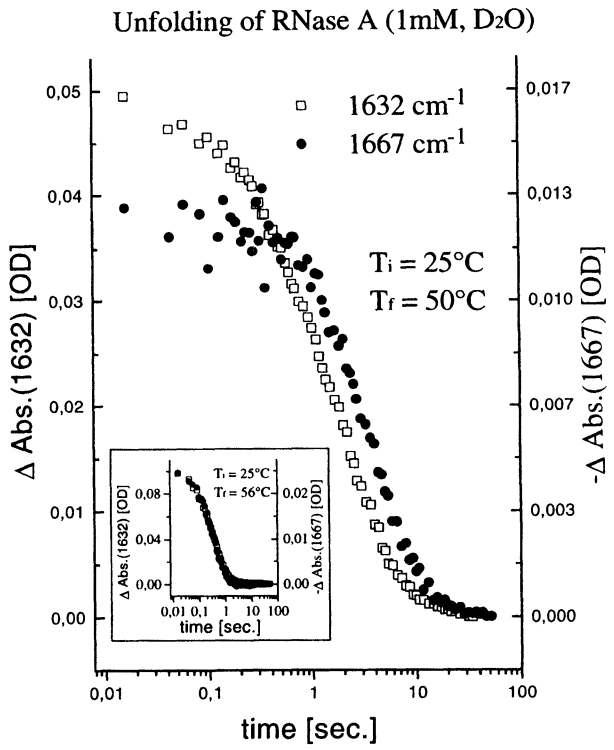

FIGURE 2 Temperature jump induced IR absorption changes of 1 mM RNase A at pH* 3.9 for 1632 cm^{-1} (open squares) and 1667 cm^{-1} (filled circles) and a final temperature of 50°C; insert: Final temperature of 56°C.

unfolding condition the growth of the "random" structure relative to the decay of the β-sheet structure suggests an intermediate that accumulates under these conditions.

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