Contributions of Terminal Peptides to the Associative Behavior of α_{s1}-Casein*

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ABSTRACT

The N- and C-terminal segments of bovine α_{s1}-casein-B (f1-23 and f136-196) were characterized under conditions that promoted or inhibited self-association to determine the relative contributions of each fragment to the interaction of α_{s1}-casein with itself or with other caseins. In earlier studies of f1-23, nuclear magnetic resonance (NMR) data and circular dichroism (CD) spectra showed that its conformation was thermostable between 10° and 25°C. In contrast, NMR studies of f136-196 indicated temperature sensitivity between 10 and 60°C, as did near-UV and far-UV CD data, suggesting a molten globule-like structure at higher temperatures. To compare the effects of temperature on conformational attributes of α_{s1}-casein and its terminal peptides, additional CD studies were conducted over a broader temperature range (10 to 70°C). The far-UV CD spectra indicated little temperature sensitivity for α_{s1}-casein, and the N-terminal peptide remained thermostable. During molecular dynamics simulations, the N-terminal peptide conformation did not change significantly, but the conformation of the C-terminal peptide (f136-196) was dramatically altered. These changes are correlated with the thermal instability observed by both CD and NMR in f136-196. Analytical ultracentrifugation studies of the self-association reactions of genetic variants A, B, and C of α_{s1}-casein showed that at 37°C the associative state is primarily dimeric; the amounts of higher order polymers significantly decreased when temperature was increased from 20 to 37°C. In all 3 genetic variants, the C-terminal portion of the whole molecule showed thermal instability with respect to aggregation to higher polymers, confirming the predictions of CD data and molecular dynamics simulations. The temperature dependency of these conformational changes suggests a possible function for α_{s1}-casein in facilitating casein-casein interactions in casein micelle formation.

(**Key words:** α_{s1}-casein, casein micelle, circular dichroism, molecular modeling)

Abbreviation key: CD = circular dichroism, **EM** = electron microscopy, **FTIR** = Fourier transform infrared spectroscopy, **MGSA** = melanoma growth stimulatory activity, μ = ionic strength, **NMR** = nuclear magnetic resonance, **PIPES** = piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), **PPII** = polyproline II conformation (a left-handed 3_{10} helix), **SAXS** = small-angle X-ray scattering.