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THE FOUR-STATE EQUILIBRIUM UNFOLDING OF A scFv ANTIBODY FRAGMENT

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INTRODUCTION. Single chain Fv fragments (scFv) are interesting alternatives to the use of whole antibody molecules[1]. We describe here an equilibrium intermediate of the thermal and urea unfolding that may compromise the stability of this class of molecules and constitute the main target for scFv stabilisation.

METHOD. The single-chain Fv antibody fragment against a hepatitis B surface antigen (anti-HBsAg scFv) was a generous gift from the Centro de Ingeniería Genética y Biotecnología, la Habana, Cuba. The equilibrium unfolding and spectroscopic characterisation of the scFv was performed essentially as described for other proteins studied in our laboratory[2].

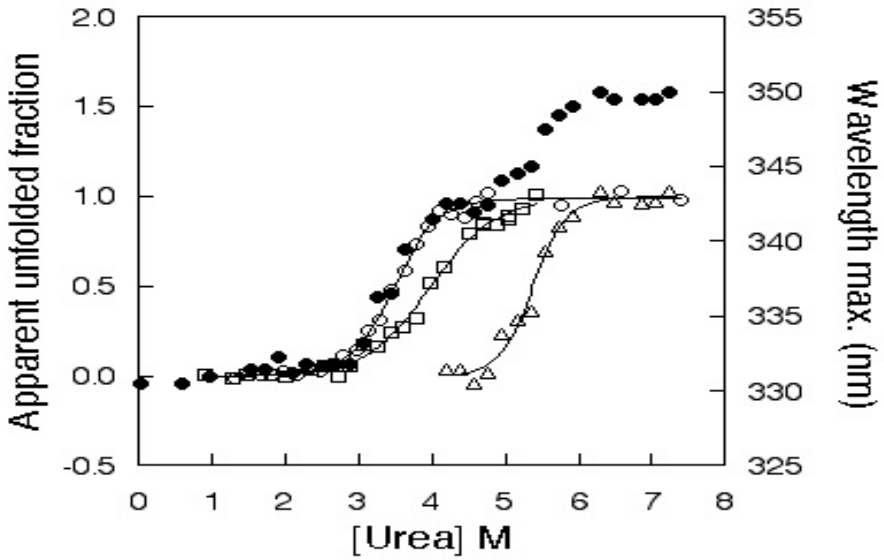

FIGURE 1. Urea denaturation of scFv at pH 7.0, followed by fluorescence intensity (o), far-UV CD (□) and wavelength of maximal fluorescence emission (•). The wavelength red-shift at higher urea concentration is additionally represented for better comparison (Δ).

RESULTS. At neutral pH and low protein concentration the scFv is a well-folded monomer and its urea and thermal denaturations are fully reversible. The noncoincidence of the fluorescence and circular dichroism transitions indicates the accumulation of an intermediate (I_1) not previously

described in scFv molecules. In addition, at higher temperatures or higher urea concentrations, a red-shift in the fluorescence emission maximum reveals a second intermediate (I_2), similar to one already reported for other scFvs. The equilibrium unfolding of the anti-HBsAg scFv is thus four-state.

DISCUSSION. Our minimal model for both the urea and the thermal unfolding of the scFv molecule is thus:

N<—> I_1<—> I_2<—>U

In this model N, I_2 and U represent the species already characterised by the Plückthun group[3] while I_1 is a new intermediate. We have globally analysed the urea (and the temperature) unfolding data (as explained in [2]). The global analysis of the thermal unfolding suggests I_1 displays substantial secondary structure and some well-defined tertiary interactions. Its fluorescence properties are consistent with a disruption of the V_L/V_H interface and a compact non-native conformation of the V_L domain. The second intermediate probably contains a well-folded V_H domain and a fully denatured V_L domain, and unfolds at higher temperature in a noncooperative fashion. Global analysis of the urea unfolding data allows to calculate the N-I_1, I_1-I_2, and I_2-D free energy differences. Although the N-D free energy difference is very large, the N-I_1 one, representing the 'relevant' conformational stability of the scFv, is small. Stabilising the native state relative to the I_1 state thus requires an improvement to the ´relevant´ conformational stability of this scFv molecule.

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