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INTERACTION OF THE C-TERMINAL DOMAIN OF THE HISTONE H1 WITH DNA

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The interaction of calf thymus H1 and its C-terminal domain (residues 123 - C-terminal) with sonicated DNA (average size 1000 b.p.) has been studied by analysing sedimentation curves and thermal denaturation profiles of complexes in a salt free medium (1 mM Na_2EDTA, 1mM phosphate pH 7). C-terminal domain was obtained by cleavage at lysine 122 with thrombine (Chapman,G.E., Hartman,P.G. and Bradbury,E.M. Eur. J. Biochem.61 69-75 (1976)). It was termed CTB fragment. The addition of the CTB fragment did not produce appreciable precipitation of DNA up to r(w/w)=0.8 (CTB/DNA), which is the charge neutralization ratio,as the sedimentation experiments showed (Fig 1A). A progressive precipitation of DNA occurred above this value, which was completed at r=1.3. When the overall DNA had been precipitated the excess of added protein remained in the supernatant (Fig 1A). Thus, the DNA in the precipitated complex was completely saturated by the protein, resulting in a binding size of 12 b.p. for the CTB fragment. The stoichiometry of the precipitated complexes remained constant along the

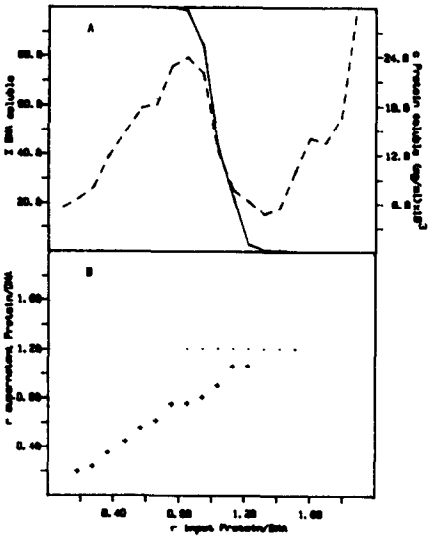

Figure 1. Precipitation of DNA by the CTB fragment of H1. A (—) Percentage of DNA in the supernatants (–•–) CTB concentration in the supernatants. B stoichiometry of soluble (x) and precipitated (••) CTB-DNA complexes as a function of the input protein/DNA ratio.

sedimentation profile (r'= 1.2) whereas the stoichiometry of the soluble complexes increased (Fig 1B). Both soluble and precipitated complexes presented a positive charge excess. Precipitated complexes (r'=1.2) have a charge ratio (+/-) of 1.5. Soluble complexes of stoichiometry ranging from r'=0.76 to r'=1.07 have charge ratios (+/-) from 0.95 to 1.33, respectively. The coexistence of soluble and precipitated complexes along the sedimentation profile, the last ones having a stoichiometry much higher than any of the soluble complexes indicated that a cooperative association between CTB and DNA must take place. This might be a consecuence of its electrostatic binding to DNA as was established by Manning (Manning,J.S. Biopolymers 18 2929-2942 (1979)) for the binding of polyelectrolites to DNA.

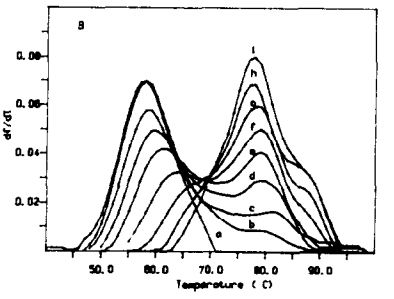

Figure 2. Derivative melting profiles of CTB-DNA soluble complexes.(a)free DNA, (b)r=0.166, (c)r=0.198, (d)r=0.239, (e)r=0.351, (f)r=0.556, (g)r=0.618, (h)r=0.753 and (i) r=0.811

Similar experiments have been carried out with H1 for comparison. The H1-mediated DNA precipitation is less efficient than the CTB-mediated one. It was necessary to add H1 up to r=1.1 to promote appreciable DNA precipitation, which was completed at r=1.8. In this case no coexistence of precipitated and soluble complexes of different stoichiometry was observed. Thus, cooperativity in the interaction of intact H1 and DNA in a salt free medium is discarded, as previously described (Renz,D. and Day,L.A. Biochemistry 15 3220-3228 (1976); Clark,D.J. and Thomas,J.O. J. Mol. Biol. 187 569-580 (1986)).

The first derivative melting profiles of CTB-DNA soluble complexes from r'=0.166 to r'=0.35 were biphasic, and those of complexes from r'=0.56 to r'=0.81 were triphasic (Fig 2). In all the cases the first transition corresponded to free DNA and the others to DNA occupied by the CTB fragment. Tm_1 ranged from 58 ^{o}C to 69 ^{o}C, Tm_2 from 70 ^{o}C to 77 ^{o}C and Tm_3 from 87 ^{o}C to 88 ^{o}C, depending on the r' value.

The appearance of two transitions, due to CTB (Tm_2 and Tm_3), suggests the existence of two kinds of DNA interactions in CTB. The number of base pairs occupied (n) by CTB and by the different interaction domains showed variability depending on the r' values. So, for CTB, n ranged from 25 b.p. (r'=0.166) to 16 b.p. (r'=0.811). For the CTB domain corresponding to the second transition n ranged from 25 b.p. (r'=0.166) to 11 b.p. (r'=0.811). Finally for the domain corresponding to the third transition n ranged from 0.76 b.p. (r'=0.56) to 4.5 b.p. (r'=0.811). The emergence of the strong interaction at a critical CTB/DNA ratio and the observed variability of the binding site size of CTB and of both interaction domains suggest that some intramolecular cooperative association among different regions of CTB molecule could exist.

When similar experiments were carried out with intact H1, new transitions appeared, probably due to other different molecular regions (globular region and/or N-terminal tail).

These studies could contribute to understand the role of the different domains of the histone H1 in its interaction with DNA.