THERMAL DENATURATION OF SUBCHROMOSOMAL PARTICLES

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SUMMARY

Monomer chromatin subunits prepared by micrococcal nuclease digestion showed a monophasic thermal denaturation transition with a Tm of about 77°C. By contrast, dimers and higher oligomers gave a biphasic melting profile, with Tms at 45-55°C and 77°C. The data support the hypothesis that about 2/3rds of the DNA within the subunit is tightly complexed with histones, and nuclease-resistant, while the remainder is bound more loosely, and is more susceptible to nuclease attack.

The existence of discrete chromatin subunits which can be released by nuclease digestion (1, 2, 3) or sonication (4) is now well established. There is also considerable evidence to suggest that these subunits are identical with the spherical particles observed in electron micrographs of whole (5, 6, 7) and nuclease-digested chromatin (8, 3). One of the remaining questions is that of the existence and nature of the 'interconnecting strands' between subunits which are seen in chromatin fibers (5, 7, 9) and in purified dimer and trimer preparations (3). We have inferred from the lengths of DNA molecules extracted from monomers, dimers, trimers and tetramers of subunits that there are micrococcal nuclease-sensitive portions of DNA about 60 base pairs in length between subunits (3). While the possibility that a significant portion of the DNA in chromatin is 'accessible' has been raised before (13), other evidence is in conflict with this view. On the basis of the electrophoretic mobility of DNA fragments extracted from micrococcal nuclease digests of chromatin, it has been inferred that oligomers of subunits contain exact multiples of monomer DNA (1, 2), and length measurements of DNase II digest fragments have

prompted similar conclusions (19) though the size of the monomer unit was considerably lower in the latter case. Both these results suggest that interconnecting strand DNA is either too short to affect the results, or, under some circumstances is not totally nuclease sensitive. However, if there are regions of chromatin in which the DNA is bound more loosely to (or to less) protein, and these regions comprise the interconnecting links between subunits, then differences in melting behavior between monomers and oligomers of subunits might be anticipated. In this communication, we show that this is indeed the case.

MATERIALS AND METHODS

Nuclei were prepared from chicken erythrocytes as previously described, and digested with micrococcal nuclease (1 enzyme unit/10 OD_{260} units of chromatin) at 37°C for 3 mins. in 0.2mM $CaCl_2$ 5mM sodium phosphate buffer pH 8.0 (1, 3). The reaction was stopped by adding 2.0mM Na_2EDTA, and the undigested material pelleted at 15,000g for 15 mins. The solubilized chromatin in the supernatant (20% to 50% of the starting DNA) was layered onto 5-20% linear sucrose gradients containing 0.5M NaCl 0.2mM EDTA pH 8.0 (3), and centrifuged at 35,000 rpm for 12 hrs. in an SW 41 rotor (Beckmann). It was previously shown (3) that the presence of 0.5M NaCl in the sucrose gradients which was required for good resolution of oligomers, removed only histone V (f2c) from erythrocyte chromatin. Histone I was also absent, being lost during the digestion (3). Gradient fractions corresponding to monomer, dimer, trimer and higher oligomer (n=4-8) were collected, and monitored for purity in the electron microscope. They were then exhaustively dialysed against 0.25mM Sodium EDTA pH 8.0 before thermal denaturation in a spectrophotometer modified for this purpose (Gilford Instruments).

RESULTS

The sucrose gradient profiles of digested chromation (Fig. 1) showed the usual separation into monomer, dimer, etc. bands (1, 3). Negatively stained preparations of each band showed that the peaks did indeed con-

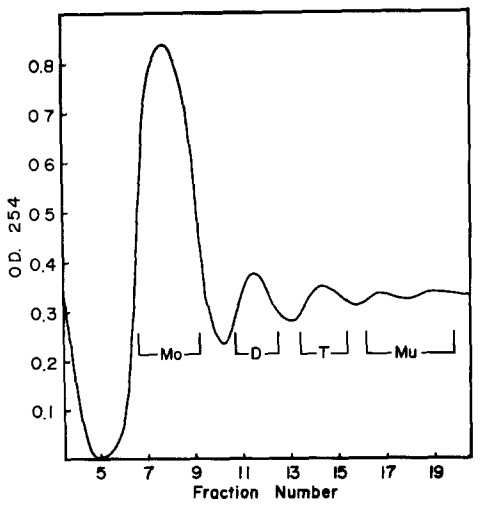

Fig. 1 Typical sucrose gradient profile of nuclease-digested chromatin. The fractions collected for melting are indicated as follows: Mo=monomer, D=dimer, T=trimer, Mu=multimer.

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tain the expected subchromosomal particles (Figs. 2-4). Both DNA (extracted from erythrocyte chromatin) and monomer particle preparations melted as single components with Tms of 45°C and 77°C respectively (Figs. 5, 6). The melting profile and Tm of the monomers is very similar to that reported for the nuclease resistant 'PS' particles isolated from calf thymus chromatin (10). In constrast, dimers, and higher oligomers all showed partial melting at temperatures much lower than 77°C (Fig. 5). Derivative plots (Fig. 7) showed that as the size of the oligomer increased, an increasing proportion of the DNA melted at the lower temperature. This general pattern of a monophasic melting profile for monomers, and a biphasic profile for oligomers was repeated in four separate experiments using different digest preparations. Whole, sheared erythrocyte chromatin melted over a broad temperature range (Fig. 6), and yielded a typically complex multiphasic derivative plot (11).

DISCUSSION

The biphasic melting of oligomers of chromatin subunits compared with the monophasic pattern of monomers indicates a fundamental difference

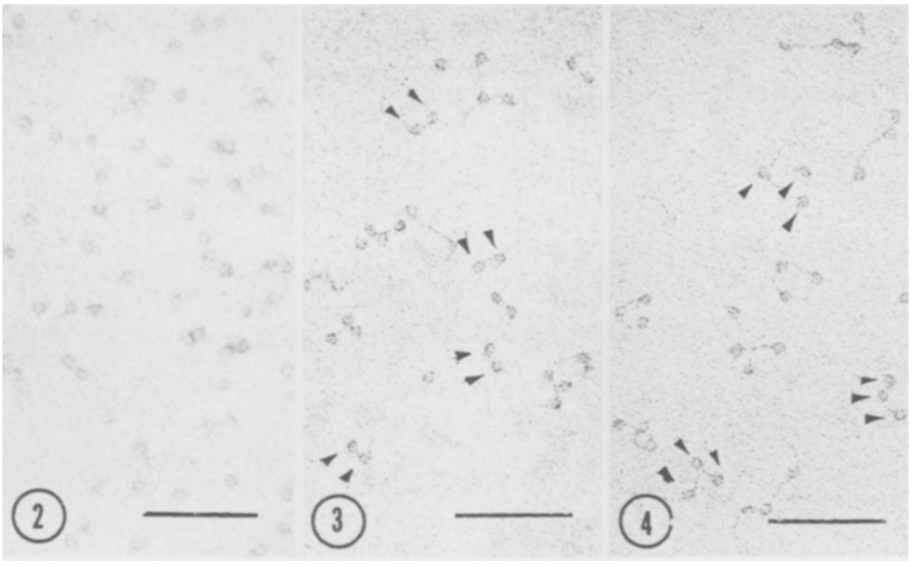

Fig. 2-4 Electron micrographs of three of the fractions indicated in Fig. 1 after staining with 0.5% uranyl formate. Fig. 2, monomer, Fig. 3, dimer, Fig. 4, trimer. Scales=1,000Å.

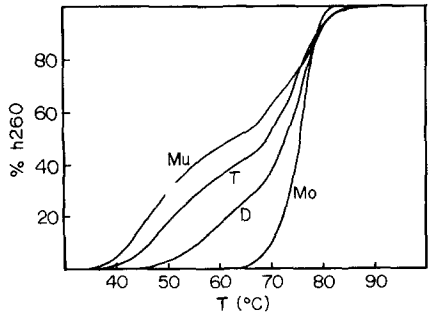

Fig. 5 Typical melting curves for monomer (Mo), dimer (D), trimer (T), and multimer (Mu) fractions. Plotted as % hypochromicity.

between these two classes (Figs. 5, 6). Evidently, all oligomers contain a portion of DNA which melts at a temperature close to that of naked DNA. This is consistent with the hypothesis that the DNA of the connecting links seen in oligomers, but absent in monomers (Figs. 2-4) is complexed with less (or more loosely) bound protein than the DNA within the subunit and therefore has a lower Tm. Since the ratio of interconnecting

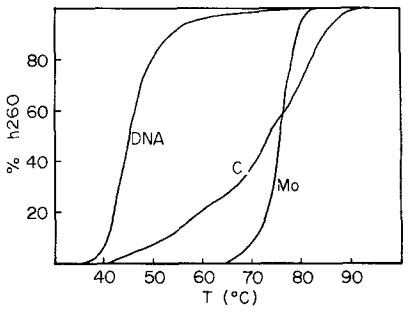

Fig. 6 Melting curves for the monomer fraction (Mo), extracted DNA from whole chromatin (D), and whole, sheared chromatin (C).

strand DNA to subunit DNA will increase as oligomer size increases, the proportion of DNA with a low Tm should be at a minimum in dimers, and increase with oligomer size. This pattern is observed (Figs. 5, 7).

The increasing proportion of early melting DNA with oligomer size is paralleled by an increasing amount of poly-L-lysine needed to precipitate the particles (data not shown). These titrations, while difficult to interpret quantitatively (14), also indicate an increased proportion of 'accessible' DNA as oligomer size increases (13).

Monomer particles show a remarkably sharp melting transition compared with whole sheared chromatin (Fig. 6). Ideally, it would be expected that whole chromatin should behave as an infinitely large oligomer showing a pronounced biphasic pattern, but as reported by others (10, 15, 18) this is not observed in sheared preparations. The melting of chromatin does encompass the same range of temperatures as oligomers, but the pattern indicates considerably more heterogeneity than present in the nuclease-derived subchromosomal particles. (The melting profile of erythrocyte chromatin was unaffected by prior treatment with 0.5M NaCl.) This heterogeneity may be due to the destructive action of shearing: after shearing, nuclease digestion no longer gives a high yield of subunits (2), and the characteristic subunit structure of the fibers can no longer be seen with the electron microscope (7). In cases where

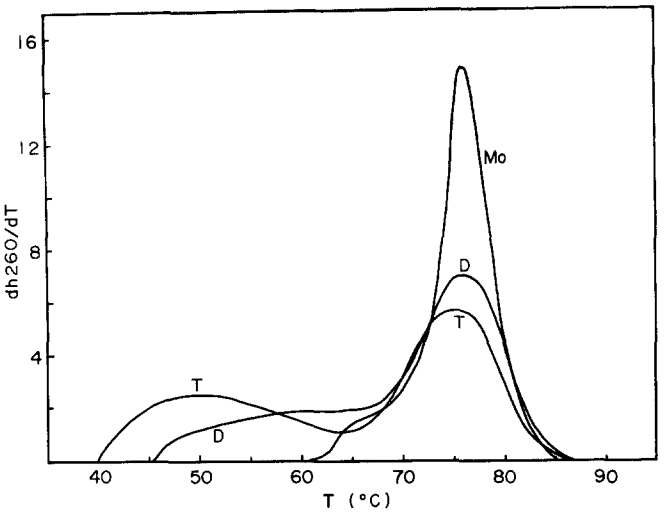

Fig. 7 Derivative melting profiles for purified monomer (Mo), dimer (D), and trimer (T) fractions.

biphasic melting profiles have been observed in artificial nucleoproteins (16, 17), the phenomenon was also attributed to the occurrence of distinct DNA-protein complexes interspersed with free DNA.

The argument that the 70Å particles, or 'nu' bodies seen in electron micrographs of whole chromatin (5, 6, 7, 9) are identical with the nuclease resistant 11-12s chromatin fragments (1, 8, 10) is based primarily on their structural similarity. Isolated particles have the same size and shape, including a central electron-opaque spot, as do the subunits on chromatin fibers (3, 8, 12), and oligomers have the expected configuration of particles separated by interconnecting strands (Figs. 3, 4). This would not be expected if, for example, nuclease attack occurred primarily within the particles. We have proposed that the effect of nuclease digestion is not only to cleave the chromatin fiber between subunits, but also to digest away a large proportion of the 'free ends' of cleaved interconnecting strands (3). On the basis of this model, we have estimated that in a unit consisting of a monomer particle plus one connecting strand, about 30% of the DNA

(about 60 base pairs) comprises the nuclease-sensitive connecting strand (3). Dimers should contain two monomers and one connecting strand, the latter comprising about 20% of the DNA, etc. If it is assumed that the lower melting portions of oligomers represent connecting strand DNA, then the melting data can be used to test these predictions. By estimating areas in derivative melting profiles (Fig. 7), it is found that 20-25% of dimer preparations melt between 35°C and 60°C. This compares well with the 20% predicted earlier (3) from length measurements on DNA. However, for trimers and higher oligomers, the proportions melting early were highly variable, ranging from 30% to 50%. The variability of these data for which there seems no obvious explanation, does not permit satisfactory test of the predictions, and further work is clearly necessary to increase the accuracy of the data.

The problem remains of reconciling our results with the mobility of DNA extracted from nuclease digests on polyacrylamide gels (1, 2). This mobility has been interpreted as showing that oligomers contain direct multiples of monomer DNA (1). This would mean that the same proportions of interconnecting strand DNA and particle DNA must be present in monomers as in oligomers, and the melting profiles of all subchromosomal particles should be identical. The apparent loss of free ends of interconnecting strand DNA in our experiments does not appear to be the result of over-digestion; reducing the nuclease digestion time from 3 mins. to 15 seconds does not appreciably alter the size of DNA extracted from the monomer (unpublished results). A detailed study of the relationship between electrophoretic mobility and length of small DNA fragments is clearly indicated.

The differential melting behavior of subunit and interconnecting strand DNA gives rise to the intriguing possibility of partially melting whole chromatin, preserving the melted regions, and observing the result in the electron microscope. This would provide a further

test of our model and also allow the overall distribution of chromatin subunits in nuclei to be determined.

In this communication, we have used the term 'interconnecting strand' as a convenient way of describing the proposed nuclease-sensitive, early-melting DNA. However, it is likely that in vivo, this portion of the subunit DNA is associated with the 70Å particle itself, perhaps loosely bound to the surface. In such an arrangement, this DNA would be both open to nuclease attack, and readily pulled from the subunit to give the 'connecting strand' seen in electron micrographs.

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