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Kinetic analysis of ψ-DNA structure formation induced by histone H1 and its C-terminal domain

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In this paper we have studied the kinetics of ψ-DNA structure formation induced by histone H1 and H1 peptides containing the C-terminal domain, namely the CTB peptide, obtained by thrombin digestion, and the CNBS peptide, derived from *N*-bromosuccinimide treatment of H1. The time course for the formation of the ψ structure has been followed by measuring the changes in ellipticity at 270 nm as a function of time under different experimental conditions. In all cases studied here, we have observed the existence of two elementary processes: one fast, the other slow. Kinetic experiments performed with high molecular weight DNA showed that the greater the salt concentration, the higher was the apparent rate of ψ structure formation. In complexes formed with sonicated DNA and H1, CNBS and CTB, we observed that the greater the content of the C-terminal domain, the higher was the apparent rate at which the final ψ structure was reached. Thus, the presence of increasing amounts of either salt or C-terminal domain facilitates the formation of the ψ structure. The molecular basis for these phenomena is discussed. The influence of the order of addition of the different components of the complex on the kinetics of ψ structure induction is also studied.

1. Introduction

Histone H1 has been shown to be involved in the salt-dependent folding of a polynucleosome chain into higher-order structures [1–5], as well as in chromatin condensation in nuclei [6,7]. At a functional level, selective H1-mediated chromatin condensation may play an important role in the regulation of gene expression [8–12].

In order to obtain more information on the action of H1 in chromatin, many studies have been performed using H1-DNA complexes as a model system. The interaction between H1 and DNA modifies the physical properties of the latter in several ways. One of the most dramatic changes observed under conditions of physiological salt is distortion of the DNA circular dichroism (CD) conservative spectrum. The positive ellipticity band at 275 nm disappears completely and a large negative band appears at 270 nm. This negative band characterizes the so-called ψ-DNA, first observed by Jordan et al. [13], which is believed to result from condensation of DNA molecules into ordered aggregates, as discussed by Fasman et al. [14] and Jordan et al. [13]. Induction of the spectrum of ψ-DNA has been determined to be a non-instantaneous process [13,14]. Previous kinetic studies have shown the existence of two steps: rapid disordered compaction and ulterior transition from disordered to ordered structure [14].

On the other hand, it is well established that the structural domains of histone H1 are involved in multifunctional roles in the structure of chromatin [15–18]. In this sense, we have previously

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reported [19] the prominent role played by the C-terminal domain in DNA condensation.

Knowledge of the elementary processes involved in ψ structure formation in complexes of DNA and H1 and its structural domains may contribute to understanding of the mechanism of DNA condensation as well as of the process through which H1 induces higher-order structures in polynucleosomes and chromatin.

In the present study, we have analysed the kinetics of formation of the ψ structure induced by histone H1 and some H1 peptides containing the C-terminal domain, namely, CTB, obtained by thrombin digestion, which matches precisely the C-terminal domain of the molecule, and CNBS, resulting from *N*-bromosuccinimide treatment of H1, which includes 50 residues more than the C-terminal domain. We have followed the process of DNA condensation by monitoring the time course of the ellipticity at 270 nm under different experimental conditions of both ionic strength and molecular size of DNA.

2. Materials and methods

2.1. Preparation of histone H1 and its fragments

Histone H1 was prepared from calf thymus as described elsewere [19] and further purified by carboxymethylcellulose chromatography [20,21]. The C-terminal fragment CTB (residues 123–C-terminus) was obtained by cleavage at Lys-122 with thrombin following the method of Chapman et al. [22]. The digest was loaded onto a carboxymethylcellulose column and eluted with a linear salt gradient [19]. The C-terminal fragment CNBS (residues 73–C-terminus) was prepared by cleavage at Tyr-72 with *N*-bromosuccinimide following the procedure described by Bustin and Cole [23]. The reaction products were separated by gel filtration on a column of Sephadex G-100 [17,24].

2.2. Protein concentrations

Protein concentrations were determined spectrophotometrically in 0.01 M HCl at 205 and 210 nm using $E^{1\%}_{205} = 310$ and $E^{1\%}_{210} = 205$ according to Morán et al. [19].

2.3. DNA preparation

DNA (Sigma, type I) was further purified by chloroform/isoamyl alcohol extractions after digestion with proteinase K (0.1 mg enzyme/mg DNA) in the presence of 0.5% SDS. This preparation was estimated to have an average molecular weight of $4–5 \times 10^6$. DNA was sonicated as described previously [19], resulting in an average molecular weight of 7×10^5 (approx. 1000 base-pairs). DNA of 146 base-pairs was obtained from chromatin core particles prepared as described elsewhere [25]. Molecular weights were estimated by electrophoresis on agarose gels.

2.4. Protein-DNA complexes

Complexes with both sonicated and 146 base-pair DNA were prepared directly by the addition of a protein solution in 0.14 M NaCl, 2×10^{-3} M phosphate buffer (pH 7.0) to an equal volume of DNA solution in the same buffer. The resulting DNA concentration was 0.02 mg/ml. Unsonicated DNA complexes (high molecular weight) were prepared under the same conditions but 2.0 M NaCl was added and further stepwise dialysis against phosphate buffer with gradually decreasing NaCl concentration was carried out until no NaCl remained. Finally, the same amount of NaCl solutions of different concentrations was added to the complexes to reach the desired salt concentrations.

2.5. Circular dichroism

CD spectra were recorded on a mark II dichrograph (Jobin-Yvon), in cells of 1 cm optical path, at 20 °C. The 270 nm region of the CD spectrum has been specified as the best area for observation of the most characteristic changes using the transition from conservative to ψ-DNA spectra. The time courses of ψ structure formation were obtained by measuring the ellipticity at 270 nm as a function of time. When sonicated and 146 base-pair DNAs were used, the protein solution was

added to the DNA solution in the CD cell and the CD changes immediately recorded. In this way, only the first 10 s of the kinetics were lost. In the case of complexes of high molecular weight DNA, the CD spectral transition was induced by addition of NaCl to complexes previously formed under low salt conditions. In all cases, the CD spectrum was monitored throughout the the first hour after complex formation.

2.6. Kinetic analysis

We have considered the overall kinetic process to be a linear combination of elementary first-order kinetic processes. The variation in ellipticity at 270 nm as a function of time can then be expressed as:

$$\Delta[\theta]_{270}(t) = [\theta]_{270}(t) - [\theta]_{270}(t_0) = -\sum_{i=1}^{n} E_i(1 - e^{-k_i t}) \quad (1)$$

where $[\theta]_{270}(t)$ and $[\theta]_{270}(t_0)$ denote the ellipticity values of the complex at time t and time t_0 (just before addition of the component, either salt or protein, that brings about formation of the ψ structure), respectively; k_i is the apparent first-order kinetic constant for elementary process i, E_i its contribution at infinite time, n the number of elementary processes and t time (in min). The minus sign in eq. 1 is due to the fact that the ellipticity decreases as a consequence of ψ structure formation. The $[\theta]_{270}$ values range from 4000 to $-360\,000$ degree cm^2 dmol^{-1} nucleotide. Thus, in order to facilitate comparison of different kinetics, the time courses have been normalized assuming an ellipticity value at 24 h of 100%, since the changes in ellipticity had been completed by this time. Therefore, eq. 1 could be transformed into:

$$D(t) = \sum_{i=1}^{n} D_i(1 - e^{-k_i t}) \quad (2)$$

$D(t)$ being the time course of the overall transformation, and D_i the contribution (%) of each elementary process.

In order to determine the values of the parameters in eq. 2 a BMDPAR-derivative-free nonlinear regression program (BMDP Program Loader Version 4.2) [26], run on an IBM-AT personal computer, was used to fit the experimental time courses to eq. 2. In each case, values of n ranging between 1 and 4 were tested. The criteria for selecting the best fit were both minimization of the sum of square residuals for the same value of n, and the minimum root mean square (r.m.s.) to choose among different values of n.

3. Results

3.1. Complexes with unsonicated DNA

Complexes with unsonicated DNA and H1 were obtained at a fixed protein/DNA (w/w) ratio of 0.5. Kinetic experiments were performed at three different salt concentrations, viz., 0.11, 0.17, and

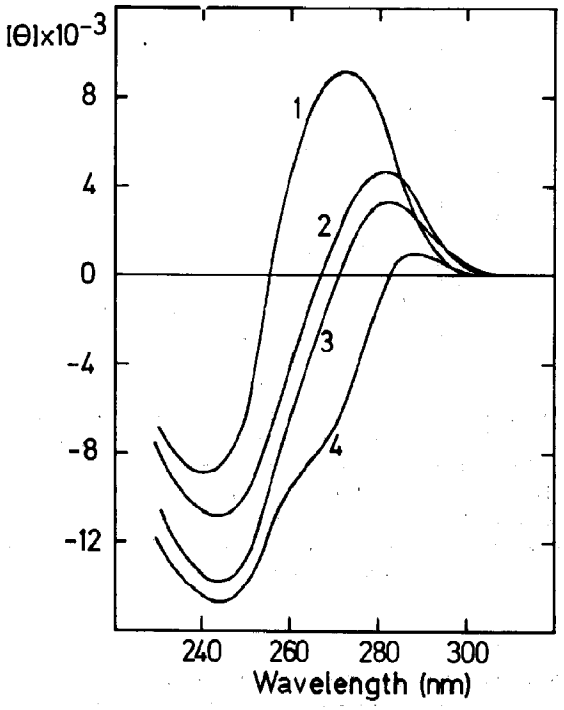

Fig. 1. CD spectra of H1-unsonicated DNA complexes, as a function of salt concentration at 0.05 mg/ml DNA, r (protein/DNA ratio, w/w) 0.5, in 2 mM phosphate buffer (pH 7.4). Salt concentration (curve): 0 (1), 0.11 (2), 0.17 (3) 0.35 M NaCl (4).

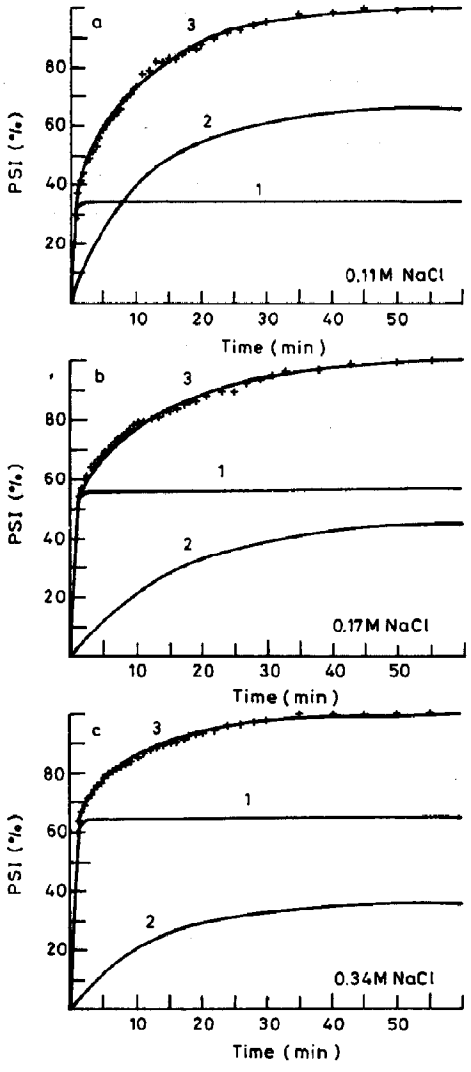

Fig. 2. Time courses of ψ structure formation for H1-unsonicated DNA complexes as a function of salt concentration (+): 0.11 (a), 0.17 (b), 0.35 M NaCl (c). Experimental conditions as given in fig. 1. (Solid lines) Curves 1 and 2 represent the two components of the kinetics calculated by exponential fitting as described in the text. Curve 3 represents the sum of these elementary components.

0.35 M NaCl. Fig. 1 shows the CD spectra of the complexes 24 h after formation. One can observe that in the absence of salt the spectrum of the H1-DNA complex is similar to that of free DNA. Addition of salt produced an increasing negative band at 270 nm, i.e., an increment in ψ structure. Moreover, the time course for induction of the ψ structure was affected by the presence of salt. As demonstrated in fig. 2, the greater the NaCl concentration, the higher was the apparent rate of formation of the ψ structure. Thus, increasing salt produced not only an increment in ψ structure, but also an increase in its apparent rate of formation. However, the apparent first-order kinetic constants for the elementary processes involved in the transition do not appear to be influenced by the increase in salt (table 1). These results were obtained by fitting the experimental profiles as described in section 2.6. In each case, the best fitting presents two kinetic components. The rapid process has a kinetic constant of about 2.4 min^{-1}, that of the slow component being about 0.07 min^{-1}. On the other hand, the contribution of each process to the formation of ψ structure depends on salt concentration: the greater the salt concentration, the higher is the contribution of the rapid process and thus the faster the overall transformation. Hence, the increased apparent rate of ψ structure formation is due to the larger contribution by the rapid process, not to modification of the values of the kinetic constants.

3.2. *Complexes with sonicated and 146 base-pair DNA*

Kinetic experiments with both sonicated and 146 base-pair DNA were performed at a protein/DNA (w/w) ratio of 0.4. Fig. 3 shows the

Table 1

Kinetic analysis of formation of the ψ structure in H1-unsonicated DNA complexes

Case	[NaCl] (M)	K_1 (min^{-1})	D_1 (%)	K_2 (min^{-1})	D_2 (%)	r.m.s.
a	0.11	2.2±0.38	35±0.9	0.09±0.003	66±0.8	22.7
b	0.17	2.1±0.19	55±0.8	0.06±0.004	44±0.8	22.7
c	0.35	2.4±0.13	64±0.5	0.08±0.003	36±0.5	29.7

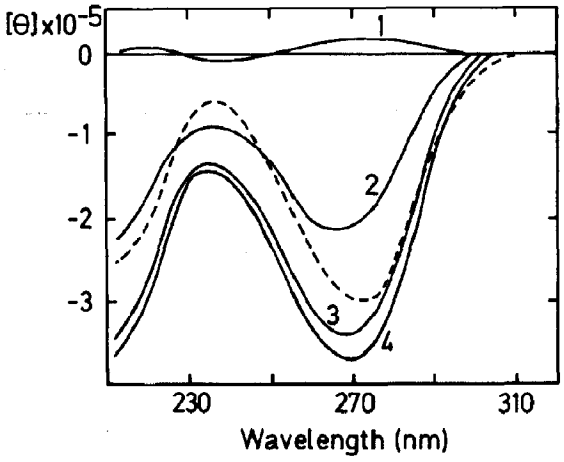

Fig. 3. CD spectra of protein-sonicated DNA complexes, at 0.02 mg/ml DNA, r (protein/DNA, w/w) 0.4, in 0.14 M NaCl, 2 mM phosphate buffer (pH 7). Native DNA (curve 1), H1-DNA (2), CNBS-DNA (3), CTB-DNA (4). (------) CNBS-146 base-pair DNA complex under the same conditions.

CD spectra of free sonicated DNA and H1-, CNBS- and CTB-sonicated DNA complexes 24 h after formation. The spectrum of CNBS with 146 base-pair DNA (CNBS-146bpDNA) is also shown. The negative band at 270 nm, characteristic of ψ structure, appears in each case. As reported previously [19], this negative band increases with increasing relative contribution of the H1 C-terminal domain in the complex. Fig. 4 illustrates the time courses of ψ structure formation during the first hour after complex formation. In the complexes of sonicated DNA, the apparent rate of ψ-structure formation increases in the following order: H1 < CNBS < CTB. Kinetic analyses indicate the existence of two main differentiated subprocesses (table 2): a rapid process (kinetic constants: 0.9, 0.8 and 0.9 min^{-1} for H1-, CNBS- and CTB-DNA complexes, respectively) and a slow one (corresponding kinetic constants: 0.05, 0.04

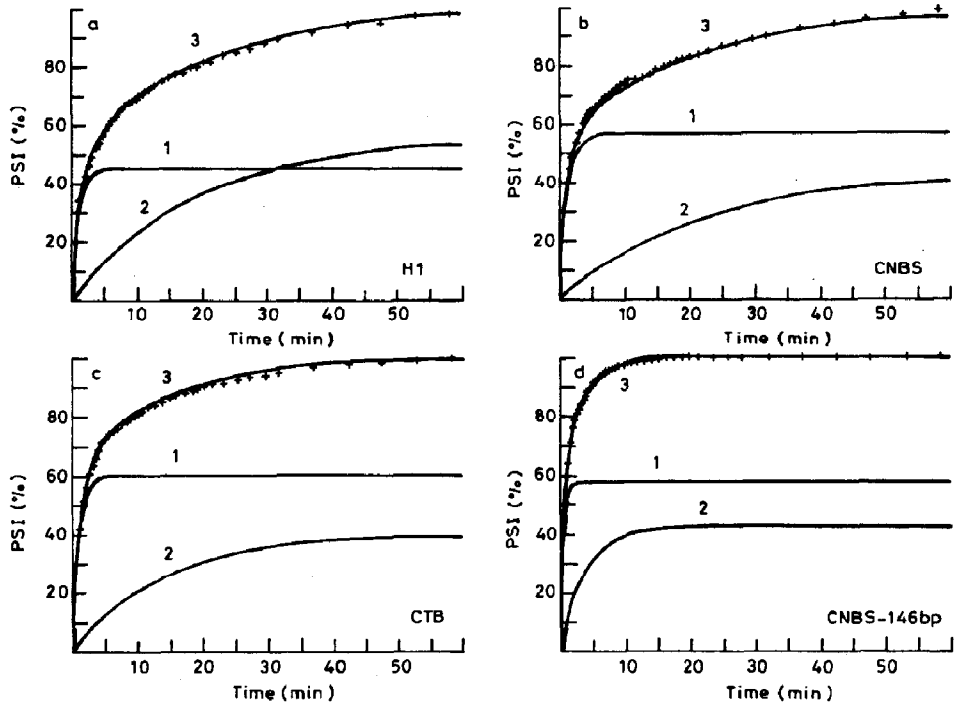

Fig. 4. Time course (+) for formation of ψ structure for complexes between H1 (a), CNBS (b), CTB (c) and sonicated DNA, and between CNBS and 146 base-pair DNA (d). Experimental conditions as given in fig. 3. (Solid lines) Curves 1 and 2 represent the two components of the kinetics calculated by exponential fitting as described in the text. Curve 3 denotes the sum of these elementary components.

Table 2

Kinetic analysis of formation of the ψ structure in complexes with 146 base-pair and sonicated DNAs

Case	Complex	K_1 (min^{-1})	D_1 (%)	K_2 (min^{-1})	D_2 (%)	r.m.s.
a	H1-DNA	0.9±0.06	45±1.2	0.05±0.003	55±1.0	178.1
b	CNBS-DNA	0.8±0.04	56±1.1	0.04±0.004	44±1.0	535.5
c	CTB-DNA	0.9±0.03	60±0.7	0.07±0.003	40±0.6	163.1
d	CNBS-146bpDNA	2.3±0.1	58±1.0	0.29±0.006	42±1.0	37.7
e	CNBS-DNA+S	1.8±0.1	84±0.9	0.06±0.01	16±0.8	451.6

and 0.07 min^{-1}). The contribution of the fast process increases from 45.2 to 56.5%, and finally to 60.2%, for H1-, CNBS- and CTB-DNA complexes, respectively. When complexes are formed with 146 base-pair DNA, much faster kinetics are observed. In this case, both constants are one order of magnitude greater than the respective values for sonicated DNA (i.e., 2.5 and 0.31 min^{-1}).

The influence of the order of addition of the various components of the complex on formation of the ψ structure has been investigated by forming complexes with CNBS according to two different experimental procedures: (1) addition of fragment CNBS to DNA in 0.14 M NaCl (CNBS-DNA); (2) addition of salt up to 0.14 NaCl to a previously prepared low-salt CNBS-DNA complex (CNBS-DNA + S). The results of these experiments are demonstrated in fig. 5. As reported previously [19], in low salt complexes no appreciable amount of ψ structure was found. However, complexes with salt display the characteristic negative band independently of the order of addition of the components. The time course for ψ structure formation in the complex CNBS-DNA + S and its kinetic analysis are shown in fig. 6. Two elementary processes with constants of 1.8 and 0.068 min^{-1}, and contributions of 83.9 and 16.1%, respectively (see table 2, case e), were noted.

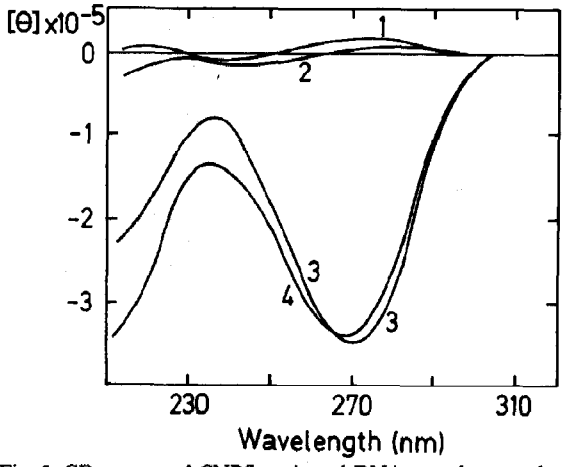

Fig. 5. CD spectra of CNBS-sonicated DNA complexes under the same experimental conditions as for fig. 3. Native DNA (1), complex before salt addition (2), complex after salt addition up to 0.14 M NaCl (3), and complex CNBS-DNA formed by addition of fragment CNBS to DNA in 0.14 M NaCl (4).

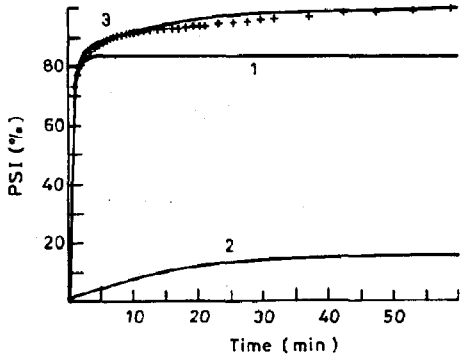

Fig. 6. Time course for formation of ψ structure for CNBS-DNA + S complex (+), obtained by salt addition up to 0.14 M NaCl to a previously prepared low-salt CNBS-sonicated DNA complex. Experimental conditions as in fig. 3. (Solid lines) Curves 1 and 2 represent the two components of the kinetics calculated by exponential fitting as described in the text. Curve 3 indicates the sum of these elementary components.

4. Discussion

It has been previously reported that the interaction of some ligands with DNA modifies the CD conservative spectrum of the nucleic acid, yielding a negative band at 270 nm. This fact has been considered to be a consequence of the ordered aggregation of DNA molecules [13,14,27]. How-

ever, different kinds of ligand and medium conditions (such as the ionic strength) produce ψ spectra of different magnitude, indicating that the transition from a conservative to a nonconservative CD spectrum is not of the 'all-or-none' type between two extreme states. This assumption is also supported by the absence of isodichroic points in the spectra of complexes formed under different conditions. Thus, ψ structure could be introduced as a family of final structures. The final state depends on the degree of compaction reached by DNA molecules.

We have carried out a kinetic analysis on the induction of the ψ spectrum following the interaction with DNA of H1 and some H1 peptides containing the C-terminal domain, in physiological salt (i.e., 0.14 M NaCl). Kinetic analyses showed that two elementary processes took place in every case studied. Both processes reflect those described previously by Jordan et al. [13] in the case of ψ structure induction by polyethylene oxide (PEO): rapid compaction followed by slow reordering. The relative contribution of the two processes depends on the experimental conditions, such as chain size of the DNA, protein/DNA ratio, ionic strength, or the peptide used to form the complex. The results obtained are summarised in fig. 7 as a comparative representation of the various kinetics on the appropriate scale. Fig. 7 depicts the negative ellipticity at 270 nm due to each process, the width and length of the arrows being proportional to the values of the kinetic constants and the contribution of each process, respectively.

Kinetic experiments performed with unsonicated DNA and intact H1 showed that the higher the NaCl concentration, the greater was the apparent rate of formation of the ψ structure. It was observed that the increase in salt produced an increment not only in formation of the ψ structure but also in the apparent rate at which this structure was reached. The kinetic analysis showed that this increment resulted from an increased contribution from the fast process (table 1), rather than as a consequence of modification of values of the kinetic constants. This is clearly demonstrated in the inset to fig. 7, where the length of arrows corresponding to the rapid process increases with

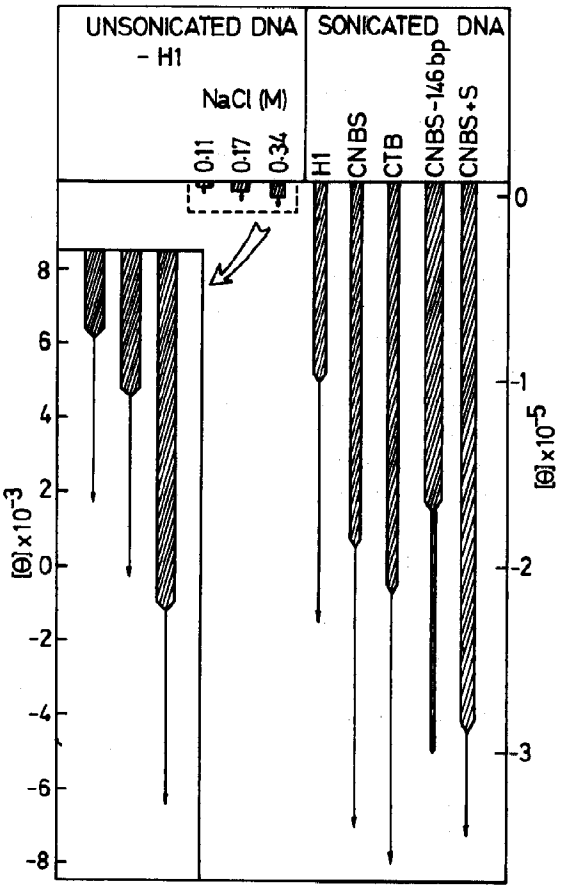

Fig. 7. Comparative representation of the different kinetics. Arrows represent the production of negative ellipticity at 270 nm for each elementary process. The width and length of the arrows are proportional to values of the kinetic constants and contribution made by each process, respectively. (Inset) Representation of the kinetics for H1-unsonicated DNA complexes on an expanded scale.

salt concentration, whereas those for the slow process have similar lengths. The presumed role played by salt in formation of the ψ structure would be shielding of the negative charges of the phosphates allowing a more compact structure to be assumed in the complex. The same amount of protein would produce a greater degree of compaction at higher salt concentration (note that the upper limit of this process is dissociation of the complex at about 0.6 M NaCl).

We have previously reported [19] that, with increase in the content of C-terminal domain in

the peptide used to form the complexes an increasing degree of ψ structure is observed. These studies pointed out the prominent role played by the C-terminal domain of histone H1 in formation of the ψ structure. Kinetic analysis of this process in complexes formed between sonicated DNA and H1, CNBS and CTB, carried out in the present article, reveals another effect: the apparent rates of ψ structure formation for the different types of molecule follow the order CTB > CNBS > H1 (see fig. 4). The reason why such an effect is observed in the kinetics is the fact that the contribution of the fast process increases according to the above-mentioned order (table 2), whereas the values of the kinetic constants remain practically invariant. The present experiments have been carried out using equal ratios, on a weight to weight basis, of peptide or protein to DNA for the various protein- or peptide-DNA complexes. This means that a similar ratio, in mol/mol (amino acids/DNA bases), has been used in each case. Thus, the fact that the contribution of the rapid process is greater in CTB than intact H1 must be due to the greater capacity of the amino acids of this region, i.e., the greater capacity of this region of the H1 molecule for inducing the disordered-compaction step. On the other hand, if the D_1 values in table 2 are normalised with respect to the molar concentration of the protein or peptide, these become 45, 37 and 26% for H1, CNBS and CTB, respectively. This signifies that the other non-CTB regions of the H1 molecule are neither without effect nor inhibitory to formation of the disordered intermediate states, but, in contrast, give rise to enhancement in the capacity of the C-terminal tail to form this structure.

The induction of states of higher compaction by the C-terminal domain would appear to be based on two mechanisms: (a) the lack of a globular domain could facilitate cross-linking of different DNA molecules; and (b) the higher proportion of charged residues in this fragment, in comparison to intact H1, would make it more effective in shielding the negative charge of the DNA. In fact, if the D_1 values obtained for H1, CNBS and CTB are normalised with respect to the content of basic residues (29, 36 and 42%, respectively), values of 45, 45 and 41% are obtained, respectively. This strongly supports the idea that the kinetics of complex formation is a function of the basic residue composition of the peptides. Moreover, the presence of increasing amounts of salt in unsonicated DNA complexes produces effects on the kinetics of ψ structure formation qualitatively similar to those observed with increasing proportions of C-terminal domain. Thus, mechanism b seems to explain most of the behavior of the C-terminal fragment, although the possibility of some contribution by mechanism a cannot be ruled out.

The C-terminal domain enrichment of the complexes produces not only a higher contribution of the compaction step, but also an increment in the final proportion of ψ structure, as pointed out above. As before, if the final ellipticity values at 270 nm for the different complexes are normalised with respect to the basic residue content, values of 2.3×10^5, 2.6×10^5 and 2.4×10^5 are obtained for H1, CNBS and CTB, respectively. These values do not differ greatly from each other and again the basic residue composition of the protein or peptides appears to be the main factor governing the formation of the ψ structure. However, in addition to the importance of the proportion of basic residues, the sequence is also a significant factor. As has been previously reported, polylysine has less ability to induce the ψ structure than alternating peptides [28].

When 146 base-pair DNA was used, kinetic analysis showed that both the rapid and slow processes had greater values of the kinetic constants. The smaller size of the DNA molecules facilitates the earlier process of compaction and the further reordering during formation of the ψ structure. However, the negative band of this complex at 270 nm, i.e., the final ψ structure contribution, was slightly less than that observed with sonicated DNA under the same experimental conditions (fig. 7). This could be due to an end-chain effect which would be much more apparent with nucleosomal DNA.

In the case of salt addition to a previously formed CNBS-DNA complex, kinetic analysis demonstrated an increasing rate of formation of the ψ structure. This effect is the result of two causes: firstly, the kinetic constant for the rapid

process increased, and secondly, the contribution of this process became more significant, reaching 84%. This implies that the compaction step is not only faster but also makes a greater contribution (see fig. 7). Prior formation of the complex in the absence of salt somehow facilitates formation of the ψ structure.

Comparison of the values listed in tables 1 and 2 shows that, whereas the degree of ψ structure is quite different between complexes of unsonicated or sonicated DNA, the kinetic profiles are very similar. With the sole exception of complexes of 140 base-pair DNA, the kinetic constants remain unaltered, but differences are observed in the contribution of each step.

The present results confirm the prominent role that the C-terminal domain of H1 plays in the induction of the ψ-DNA structure and point out its influence in the kinetics of the process.

Acknowledgements

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