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Synergistic Effect of Histone H1 and Nucleolin on Chromatin Condensation in Mitosis: Role of a Phosphorylated Heteromer

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ABSTRACT: Repeated motifs, rich in basic residues, are characteristic of both the N-terminal domain of the nucleolus-specific protein, nucleolin, and the second half of the C-terminal domain of histone H1. These repeats are also the target for phosphorylation by the mitosis-specific $p34^{cdc2}$ kinase. We have previously shown that synthetic peptides [$(KTPKKAKKP)_2$ for histone H1 and $(ATPAKKAA)_2$ for nucleolin] corresponding to these two repeated motifs are able to act in synergy to induce DNA hypercondensation (Erard et al., 1990). In order to determine the molecular basis of this synergistic interaction, we have studied the condensation of the homopolymer poly(dA)·poly(dT) in the presence of the two synthetic peptides. Circular dichroism has been used to monitor the ψ^+-type condensation and has revealed that phosphorylation enhances the synergistic effect of the two peptides. Analysis of different combinations of the two peptides suggests that there is a direct interaction between them which is stabilized by phosphorylation. Furthermore, there is a striking correlation between the degree of homopolymer condensation and the stability of the heteromeric complex. Phosphorylation takes place on the threonine residues on the repeat motifs within a region which is likely to adopt a β-turn structure. Circular dichroism and infrared spectroscopy provide evidence that phosphorylation stabilizes the β-turn structure of both peptides, and computer modeling shows that this may be due to steric hindrance imposed by the phosphate group. We suggest that phosphorylated nucleolin and histone H1 interact through their homologous domain structured in β-spirals in order to condense certain forms of DNA during mitosis.

Although it has been known for some time that histone H1 and its cell cycle dependent phosphorylation is involved in chromatin DNA condensation (Bradbury et al., 1973), the precise nature of the molecular interaction remains to be elucidated. We have recently shown (Erard et al., 1990) that it is the region of the histone H1 C-terminal domain comprising the highly conserved DNA-binding repeat KT(S)-PKKAKKP which performs the function, common to all of the histone H1 proteins, of condensing chromatin linker DNA. Indeed, the H1 repeat can adopt a rigid β-turn-containing structure which binds to the DNA minor groove, dislodging its spine of hydration. The effect on DNA conformation is therefore essentially equivalent to dehydration, which leads

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to its subsequent condensation. In the same report, we also described the intriguing behavior of a similar repeat, ATPAKKAA, found in the N-terminal domain of nucleolin, a protein associated with both nucleolar chromatin in interphase (Olson & Thompson, 1983a; Erard et al., 1988) and nucleolar organizer regions of metaphasic chromosomes in mitosis (Gas et al., 1985; Lischwe et al., 1981). This DNA-binding repeat, by itself unable to promote any alteration in DNA conformation, is able to enhance the DNA condensation induced by the histone H1 repeat. However, we could not, at that stage, provide a satisfactory explanation for the synergistic effect of the histone H1 and nucleolin repeats on DNA condensation. One aim of the present study is to shed light on this interesting phenomenon.

The recent demonstration that nucleolin is a substrate for the cell cycle dependent $p34^{cdc2}$ kinase in mitosis (Belenguer et al., 1990; Peter et al., 1990) indicates not only that histone H1 and nucleolin are both present during mitosis but also that they possess analogous sites which are the targets of concomitant phosphorylation. The nucleolin phosphorylation sites have been identified as XTPXK and are homologous to the H1 repeated sequence KT(S)PKK, which is also recognized by the $p34^{cdc2}$ kinase [for reviews, see Dorée (1990) and Draetta (1990)]. Moreover, these phosphorylation sites are parts of the DNA-binding repeats of the two proteins [KT(S)PKKAKKP for histone H1 and XTPXKKXX for nucleolin]. This has led us to hypothesize that phosphorylation of nucleolin and histone H1 during mitosis could modify their binding to DNA and hence subsequent DNA condensation, particularly bearing in mind the well-established correlation between H1 phosphorylation and chromosome condensation.

In order to determine whether it is the synergy between the two proteins and/or their mitosis-specific phosphorylation which is important in promoting ribosomal DNA condensation in mitosis, we have set up a model system to mimic the principal interactions taking place. This system consists of two previously studied peptides $(KTPKKAKKP)_2$ and $(ATPAKKAA)_2$, which correspond to typical repeats of histone H1 and nucleolin respectively (Erard et al., 1990) and which can be phosphorylated in vitro by the $p34^{cdc2}$ kinase (Belenguer et al., 1990), interacting singly or in combination with the homopolymer poly(dA)·poly(dT). This latter was chosen because we presumed that these peptides would have a preferential affinity for (dA)·(dT) stretches. This type of affinity is well documented for peptide-like minor-groove-binding drugs such as netropsin and Hoechst 33258 [for a review, see Neidle et al. (1987)] and has also been clearly established for related peptide motifs such as $(SPRK)_2$, found in sea urchin spermatogeneous histones H1 and H2B (Churchill & Suzuki, 1989), and TPKRPRGRPKK, the consensus sequence of HMG-I proteins (Reeves & Nissen, 1990). All these ligands share several key structural features: a bent shape caused by the presence of rings (pyrrole or benzimidazole for the drugs and proline for peptides) and the presence of available NH amide groups and charged residues. Moreover, a preferential affinity for AT base pairs has already been shown for both histone H1 and nucleolin. Thus, it has been reported by Sponar and Sormova (1972) that H1 interacts selectively with nucleic acids rich in AT base pairs and direct evidence for such base-pair specificity has been provided by ultraviolet Raman spectroscopy (Chinsky & Turpin, 1982). H1 also displays a highly preferential binding to scaffold-attachment regions (SARs) due to their arrays of (dA)·(dT) tracts (Käs et al., 1989). Nucleolin itself binds strongly to a region of the nontranscribed spacer of ribosomal DNA which is rich in AT base pairs (Olson et al., 1983b). It is important to underline that, in both cases, the repeat peptide motifs are excellent candidates to explain the AT base-pair specificity.

By using circular dichroism to probe the hypercondensation of poly(dA)·poly(dT) induced by the histone H1 and nucleolin peptides, either unmodified or phosphorylated by the $p34^{cdc2}$ kinase, we have reached several major conclusions. First, we provide evidence that it is not the histone H1 mitosis-specific phosphorylation per se which brings about DNA hypercondensation but rather the synergy between H1 and nucleolin, enhanced by their concomitant phosphorylation. Second, since this synergy of the two proteins relies on the interaction between their homologous domains structured in β-turns, we propose that a β-spiral-containing heteromer may be a potent effector of DNA condensation. The stability of this new structure would then be enhanced by the mitosis-specific phosphorylation of the two proteins. Finally, we would like to emphasize that the interaction between this heteromer and poly(dA)·poly(dT) is not solely electrostatic but probably also results from Van der Waals contacts and hydrogen bonding within the narrow minor groove of the polymer. This suggests that it may be necessary to reevaluate the commonly held assumption that histone H1, and more generally basic peptides or proteins, is only capable of a nonspecific mode of binding to the anionic phosphate backbone of the DNA.

Experimental Procedures.

Peptide Phosphorylation and Purification. Synthesis and purification of the histone H1 peptide $(KTPKKAKKP)_2$ and of the nucleolin peptide $(ATPAKKAA)_2$ have been described previously (Erard et al., 1990). The $p34^{cdc2}$ kinase from starfish oocytes was purified according to Labbé et al. (1989a,b). $(KTPKKAKKP)_2$ and $(ATPAKKAA)_2$, which both contain two potential phosphorylation sites, were phosphorylated in vitro, using the assay conditions reported by Peter et al. (1990). In each case, the resulting mixtures of di-, mono-, and unphosphorylated species were loaded onto a CX Aquapore column, equilibrated in 50 mM sodium phosphate buffer, pH 6.0, and eluted with a linear gradient of 0–0.6 M NaCl in the same buffer. The first and the second fractions corresponded to the diphosphorylated and the monophosphorylated peptides, respectively, and were collected separately. Each fraction was then desalted by chromatography through a C18 column, eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid, and lyophilized.

All the experiments described in this article were carried out with either unphosphorylated or diphosphorylated peptides. The monophosphorylated peptide fractions were heterogeneous, containing peptides modified either on their first or on their second repeat.

Peptide–Poly(dA)·Poly(dT) Complex Preparation. Complexes were prepared by slow mixing of peptide solutions at various concentrations in 0.15 M NaCl/10 mM sodium phosphate, pH 7.4, with an equal volume of poly(dA)·poly(dT) (Pharmacia) in the same buffer, giving a final polynucleotide concentration of 0.03 mg/mL. Unless otherwise stated, these complexes were analyzed by spectroscopy immediately after their formation.

Circular Dichroic Measurements. Circular dichroic spectra were recorded at 20 °C with a Jobin-Yvon dichrograph V connected to an Apple II microcomputer. A cell of 1-cm optical path length was used to record spectra of poly(dA)·poly(dT) complexes in the near-ultraviolet region (320–220 nm). The results are presented as molar ellipticity values in $deg \cdot cm^2 \cdot dmol^{-1}$, on the basis of the nucleotide mean residue mass of 330 Da.

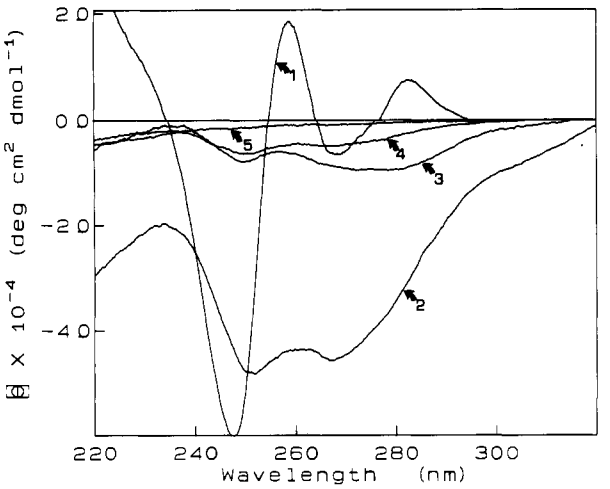

FIGURE 1: Circular dichroic analysis of the interaction between the unphosphorylated histone H1 peptide $(KTPKKAKKP)_2$ and poly(dA)·poly(dT). Curve 1, spectrum of free poly(dA)·poly(dT). Curves 2 and 3, spectra of complexes of poly(dA)·poly(dT) with $(KTPKKAKKP)_2$, for peptide/polynucleotide mass ratio (r) of 1 and 2, respectively, at zero time. Curves 4 and 5, spectra of the same respective complexes after 24 h.

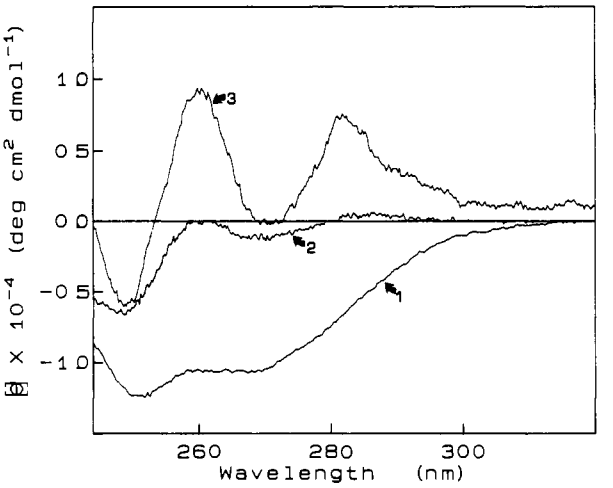

FIGURE 2: Circular dichroic spectra of complexes between poly(dA)·poly(dT) and different mixtures of the two peptides. Curve 1, spectrum of a complex in which both peptides are unphosphorylated. Curves 2 and 3, spectra of complexes when only one peptide is phosphorylated, the histone H1 peptide (curve 2) or the nucleolin peptide (curve 3). For clarity, the spectrum corresponding to the complex in which both peptides are phosphorylated has not been represented since it is virtually identical to curve 3.

A cell of 1-mm optical path length was used to record spectra of peptides or mixtures of peptides in the far-ultraviolet region (260–190 nm) at a total concentration of 0.2 mg/mL in peptidic material, in 0.15 M NaCl/10 mM sodium phosphate, pH 7.4. During scanning, we chose an integration time of 4 s. The results are presented as molar ellipticity values on the basis of the amino acid mean residue mass of 110 Da.

Infrared Spectroscopy Measurements. Infrared spectra were recorded at 20 °C with a Perkin-Elmer Model 1760 Fourier transform spectrophotometer connected to a PE Series 7000 computer and equipped with a high-sensitivity narrow-range mercury cadmium telluride detector. An attenuated total reflectance cell was used, the characteristics of the ZnSe crystal giving it an equivalent optical path length of 15 μm. For each spectrum from 2000 to 800 cm^{-1}, 1000 interferograms were co-added and Fourier-transformed to give a final resolution of 4 cm^{-1}. Peptides, at a concentration of 2 mg/mL, were dissolved in pure D_2O.

Molecular Modeling. Computer modeling was carried out using the Desktop Molecular Modeler software (version 1.2) developed by Crabbe and Appleyard. The energy minimization procedure used to refine peptide structures is based on the algorithm and data given by Vinter et al. (1987).

RESULTS

Assessing Poly(dA)·Poly(dT) Condensation. It is now well established that DNA can condense, under the appropriate conditions, into liquid-crystalline phases [for a review, see Tinoco and Bustamante (1980)]. These so-called "ψ DNA phases" give rise to characteristic circular dichroic spectra, the sign of which reflects the packing arrangement of DNA helices. The preferred tertiary organization is dictated both by the solution conditions and by the type of DNA secondary structure (Shin & Eichhorn, 1984). In particular, a recent study has shown that poly(dA)·poly(dT) is unusual in that it can adopt the ψ^+ form in solutions that normally favor the formation of ψ^- DNA (Chaires, 1989). This intriguing behavior has been ascribed to the peculiar secondary structure of poly(dA)·poly(dT) as discussed later.

Figure 1 shows the CD spectra of poly(dA)·poly(dT), either free (curve 1) or complexed with the unphosphorylated histone H1 peptide $(KTPKKAKKP)_2$ (curves 2–5) at different peptide/DNA mass ratios (r) and at different times (t). The large negative spectrum observed at $t = 0$ and $r = 1$ (curve 2) would seem to indicate that a certain proportion of poly(dA)·poly(dT) molecules form a ψ^--type condensate in the presence of H1 peptide, as previously reported in the case of standard B DNA (Erard et al., 1990). However, the evolution of the spectrum after 24 h (curve 4) would suggest that this form of condensation is only transient. The same spectral transition toward less negative values of molar ellipticity after 24 h is also apparent for $r = 2$ (compare curves 3 and 5). Moreover, if poly(dA)·poly(dT) molecules were indeed condensing into a ψ^- phase, the proportion of molecules in this phase should increase in line with the quantity of added peptide. By comparing curve 2 ($r = 1$, $t = 0$) and curve 3 ($r = 2$, $t = 0$) it is clear that this is not the case. Taken together, these data show that poly(dA)·poly(dT) undergoes a transition toward a ψ^+ form in the presence of the histone H1 peptide and that the kinetics of this transition can be speeded up by the amount of peptide added. This is a further example of the aforementioned unusual condensation of poly(dA)·poly(dT), since the H1 peptide usually induces the formation of ψ^- DNA.

Synergistic Effect of Histone H1 and Nucleolin Peptides on Poly(dA)·Poly(dT) Condensation. Enhancement after Phosphorylation by the p34^{cdc2} Kinase. Despite its own incapacity to promote DNA condensation, the nucleolin peptide $(ATPAKKAA)_2$, is, on the other hand, able to enhance the effect of the histone H1 peptide $(KTPKKAKKP)_2$ in generating hypercondensed DNA (Erard et al., 1990). This synergistic effect of the two peptides is also apparent for poly(dA)·poly(dT) condensation, as illustrated in Figure 2, which represents the CD spectra of poly(dA)·poly(dT) after addition of different mixtures of the two peptides (curves 1–3). All spectra were taken at $t = 0$, and each peptide was present at a peptide/DNA mass ratio of 2, either in unphosphorylated or diphosphorylated form (see Experimental Procedures). From an examination of curve 1, it is possible to deduce that there is already a concerted effect on poly(dA)·poly(dT) condensation when the two peptides are unphosphorylated. If the two peptides were acting independently, one would predict a CD spectrum which is a simple combination of the CD spectra of the two separate complexes, namely $(KTPKKAKKP)_2$–poly(dA)·poly(dT) and (ATPAK-

Table I: Circular Dichroic Analysis of the Interaction between Nucleolin and H1 Peptides

peptide(s)	$[\theta]_{198nm}$[a] (measured)	$[\theta]_{198nm}$[b] (calculated)
N	−19400	
N*	−14500	
H	−13000	
H*	−10000	
NH	−13200	−16200
NH*	−10000	−14700
N*H*	−7700	−12250
N*H	−4800	−13750

[a] Measured molar ellipticity values at 198 nm (in deg·cm²·dmol⁻¹). [b] Calculated molar ellipticity values at 198 nm assuming that the peptides do not interact (see text). N = nucleolin peptide; H = histone H1 peptide; two letters indicate a mixture of the two peptides in equal amounts. Asterisks indicate that the respective peptide is phosphorylated on its two threonine residues.

$KAA)_2$–poly(dA)·poly(dT). The fact that curve 1 cannot be simulated by such computations suggests that the two peptides might interact with each other, giving rise to a heteromer which is more efficient in condensing the polynucleotide than the histone H1 peptide alone. The CD spectrum of this hypothetical ternary complex between the two peptides and the poly(dA)·poly(dT) (curve 1 of Figure 2) is indeed closer to a ψ^+-type CD spectrum: in particular, the molar ellipticity value of −6700 at 282 nm is less negative than that of the H1 peptide–poly(dA)·poly(dT) complex (−9000), shown on curve 3 of Figure 1.

The synergistic effect of the two peptides on poly(dA)·poly(dT) condensation is markedly enhanced when they are phosphorylated, as can be seen from the gradual increase in molar ellipticity values of the spectra shown in Figure 2. By comparing curves 2 and 3, it appears that the most critical factor is the phosphorylation of the nucleolin peptide. If this peptide is phosphorylated, then the synergy is optimal, even if the histone H1 peptide is unphosphorylated. It should be underlined that the nucleolin peptide, either unphosphorylated or phosphorylated, is by itself unable to promote poly(dA)·poly(dT) condensation (data not shown).

Phosphorylation Stabilizes Heteromeric Complexes of Nucleolin and Histone H1 Peptides. Circular dichroic spectra of both the nucleolin peptide $(ATPAKKAA)_2$ and the histone H1 peptide $(KTPKKAKKP)_2$ suggest the presence of β-turn structures, since they have the typical characteristics of class C spectra (Erard et al., 1990). This class of spectra, which is characterized by the presence of a minimum at about 200 nm, is often found for proline-containing peptides organized in β-turns (Hollosi et al., 1985). We have also shown that there is a strict correlation between a decrease in the intensity of the dichroic minimum and a stabilization of the β-turn structure. This stringent quantitative criterion had allowed us to conclude that the tendency to form rigid structures containing repetitive β-turns is always much more marked in the case of the histone H1 peptide than in the case of the nucleolin peptide. In the present study, we will again use these variations of the dichroic minimum to assess the stability of a given structure, with a lower absolute value of this minimum indicating a more stable structure. The structures we have examined comprise mixtures of equal amounts of the two peptides, either unmodified or phosphorylated on their two threonine residues by the $p34^{cdc2}$ kinase.

The first four lines of Table I show the measured ellipticity values at 198 nm of the nucleolin peptide and of the histone H1 peptide, either unphosphorylated or diphosphorylated. In both cases, phosphorylation induces a significant decrease in the intensities of the dichroic minima, from 19400 to 14500 for the nucleolin peptide and from 13000 to 10000 for the histone H1 peptide. We interpret this to mean that the potential β-turn structure of each peptide is stabilized by its phosphorylation.

The measured $[\theta]_{198nm}$ values for different combinations of the phosphorylated and unphosphorylated species of the two peptides are indicated in the lower half of Table I. Each measured $[\theta]_{198nm}$ value is compared with a calculated $[\theta]_{198nm}$ value, the basis for this calculation being the assumption that the two peptides do not interact (each calculated $[\theta]$ is half the sum of the $[\theta]$ values of the corresponding individual peptides). This assumption is quite clearly false, and in all four cases, the two peptides interact with each other to give rise to a heteromeric complex as will be discussed later. Here again, the most critical factor is the phosphorylation of the nucleolin peptide, which is present in the two most stable complexes, with $[\theta]_{198nm}$ values of −7700 and −4800. It is surely not a coincidence that these complexes, containing the phosphorylated nucleolin peptide, along with either the unphosphorylated or the phosphorylated histone H1 peptide, are also the most potent effectors of poly(dA)·poly(dT) condensation as described in the previous section.

Peptide Phosphorylation as a Stabilizing Factor of β-Turn Structure. The influence of threonine phosphorylation on peptide structure has also been investigated by Fourier transform infrared spectroscopy, which provides us with a second stringent criterion for the existence of repetitive β-turns (Erard et al., 1990). The so-called amide I band (I′ in D_2O) in the 1600–1700-cm^{-1} frequency range is composed of the vibrational modes of the CO groups of the peptide backbone which are a function of its secondary structure. The changes in structure of the nucleolin and the H1 peptides with increasing ionic strength can be visualized by the gradual emergence of a band at around 1675 cm^{-1}, particularly marked in the case of the histone H1 peptide. As bands at similar frequencies have been reported for proteins and polypeptides comprising contiguous β-turns (Renugopalakrishnan et al., 1986; Fasman et al., 1990), we have concluded that these peptides adopt structures which contain repeated β-turns.

Figure 3A represents the infrared spectra between 1600 and 1700 cm^{-1} in pure D_2O of the histone H1 peptide $(KTPKKAKKP)_2$, either unphosphorylated (curve 1) or phosphorylated on its two threonine residues (curve 2). As expected, the peptide is very flexible in the absence of added salt, and hence numerous conformers with different vibrational frequencies are present (curve 1). Quite strikingly, the phosphorylation of the peptide is, by itself, sufficient to promote stabilization in a rigid β-turn-containing structure, as assessed by the emerging band near 1675 cm^{-1} (curve 2). Evidence for threonine phosphorylation is provided by the presence of two absorption peaks at 980 and 1140 cm^{-1} (curve 2 of Figure 3B) which are characteristic of the vibrational modes of $[PO_3]^{2-}$ groups and of phosphate-esterified alcohol groups, respectively (Tsuboi, 1974). Similar results have been obtained for the nucleolin peptide (data not shown).

Computer Modeling of the Histone H1 and Nucleolin Phosphorylation Sites. From a survey of different protein phosphorylation sites, Small et al. (1977) have demonstrated that many of the phosphorylated residues lie within predicted β-turn structures. Typical of such a feature is the H1 and nucleolin consensus sequence XTPXK, recognized by the $p34^{cdc2}$ kinase (Belenguer et al., 1990). Indeed, the TPXK tetrapeptide is likely to adopt a β-turn structure according to the Chou and Fasman prediction (1977) and by analogy with the hormone peptide TPRK, whose turn structure was pre-

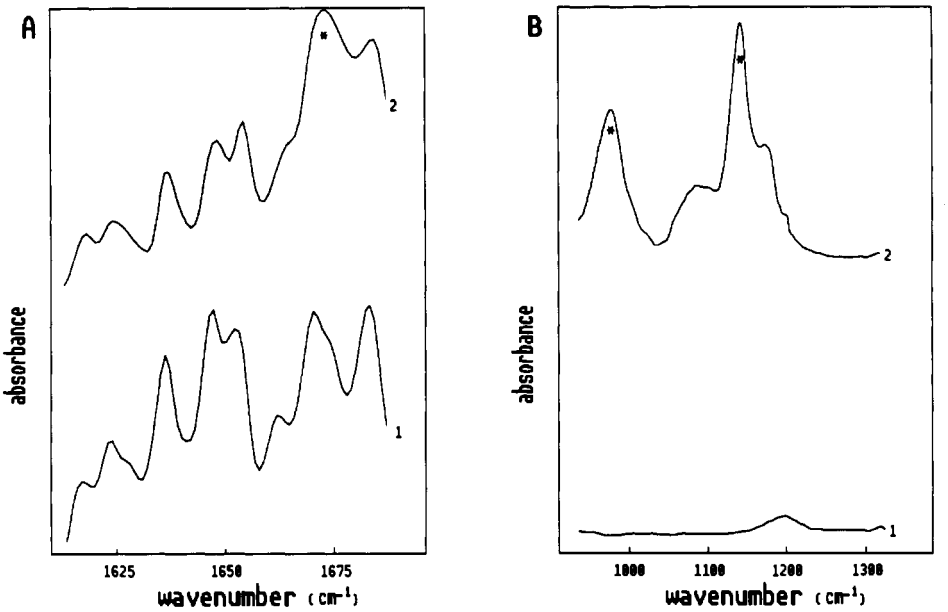

FIGURE 3: Fourier transform infrared spectroscopy in the amide frequency range (A) and in the phosphate frequency range (B) of the histone H1 peptide. In each case (A and B), curves 1 and 2 represent the spectra of the unphosphorylated and phosphorylated peptides, respectively. The significance of the bands marked with an asterisk is given in the text.

dicted by energy calculations (Anderson et al., 1978). These four amino acids probably make a major contribution to the β-turn signal seen by circular dichroism and infrared spectroscopy. We have built computer models of the two pentapeptides KTPKK and ATPAK, corresponding to the phosphorylation sites of the H1 and nucleolin peptides, respectively, in order to evaluate the influence of threonine phosphorylation on their β-turn structure.

The image on the left-hand side of Figure 4A represents the space-fill model of the KTPKK pentapeptide. This model has been built on the assumption that the tetrapeptide TPKK is structured in a type I β-turn, the tight core of which can be seen in the center of the picture. The oxygen atom of the threonine hydroxyl group is indicated with a star. The right-hand side of Figure 4A displays the space-fill model of the same peptide after phosphorylation of its threonine, with the phosphate group in the center of the structure and covering the oxygen of the threonine. It is already clear that the phosphorylation of the KTPKK pentapeptide does not seriously alter its overall structure.

The pentapeptide ATPAK can similarly be modeled in such a way that the tetrapeptide TPAK is in a type I β-turn as illustrated in Figure 4B. The main constraints in the model building process were the theoretical sets of dihedral angles (ϕ, ψ) of the second and the third amino acids forming the turn, according to Ramachandran and Sasisekharan (1968). After energy minimization of the structure, these values were (−69°, −22°) and (−112°, −24°) for proline and alanine, respectively, and hence were in the range defined by Lewis (1973) and more recently adopted by Wilmot and Thornton (1990) in a more empirical approach. The distance and angle requirements for hydrogen-bond formation with an oxygen atom as acceptor are satisfied twice in this structure: in addition to the classical 1–4 hydrogen bond between the CO of the turn's first residue (threonine) and the NH of the fourth residue (lysine) there is also the possibility of a main chain–side chain interaction between the oxygen of the threonine hydroxyl group and the NH of the alanine, as has been proposed by Suzuki (1989) and Reeves et al. (1990).

A stereogram of the same ATPAK pentapeptide with its threonine phosphorylated is shown in Figure 4C. From their relative spatial position, it is clear that the 1–4 hydrogen bond is unaffected by the presence of the phosphate. As far as the second hydrogen bond is concerned, it is conceivable that one of the unesterified oxygens of the phosphate group could play a role similar to that of the oxygen of the threonine hydroxyl group. This phosphate oxygen, ionized at physiological ionic strength, is situated at about 1.7 Å from the hydrogen of the alanine NH group, with a PO···H angle of about 106°. These are quite acceptable conditions for hydrogen bonding if one takes into account the reduced angular constraints on oxygen acceptors and the shorter interatomic distances for hydrogen bonds comprising charged atoms (Baker & Hubbard, 1984). Moreover, the bulky phosphate group is likely to hinder the movement of the neighboring atoms and therefore to locally diminish peptide flexibility. Taken together, these observations could explain why the phosphorylation of the peptides should stabilize their β-turn structure.

Discussion

Because of the organization of its N-terminal domain in long acidic stretches interspersed with basic repeats, nucleolin, a nucleolus-specific protein, can bind to histone H1, displace it from linker DNA, and induce chromatin decondensation (Erard et al., 1988). We now show that nucleolin may indeed have another function, antagonist to the first one, that is to say the enhancement of H1-induced chromatin condensation during mitosis. The basic repeats present in the N-terminal domain of nucleolin define a subdomain which is homologous to the second half of the histone H1 C-terminal domain, and direct sequence alignment can be made on the basis of the $p34^{cdc2}$ kinase phosphorylation consensus sequence XT(S)PXK. A comparative study of the structures and the modes of interaction with DNA of two synthetic peptides $(KTPKKAKKP)_2$ and $(ATPAKKAA)_2$, corresponding to the histone H1 and nucleolin repeat motifs, respectively (Erard et al., 1990), led us to the surprising conclusion that the nucleolin peptide was able to act in synergy with the histone H1 peptide to induce DNA hypercondensation. Two main questions remained unresolved: what was the basis of this synergy between the two peptides, and what was the role of their phosphorylation by the mitosis-specific $p34^{cdc2}$ kinase? And, more generally, what could this mean in terms of the

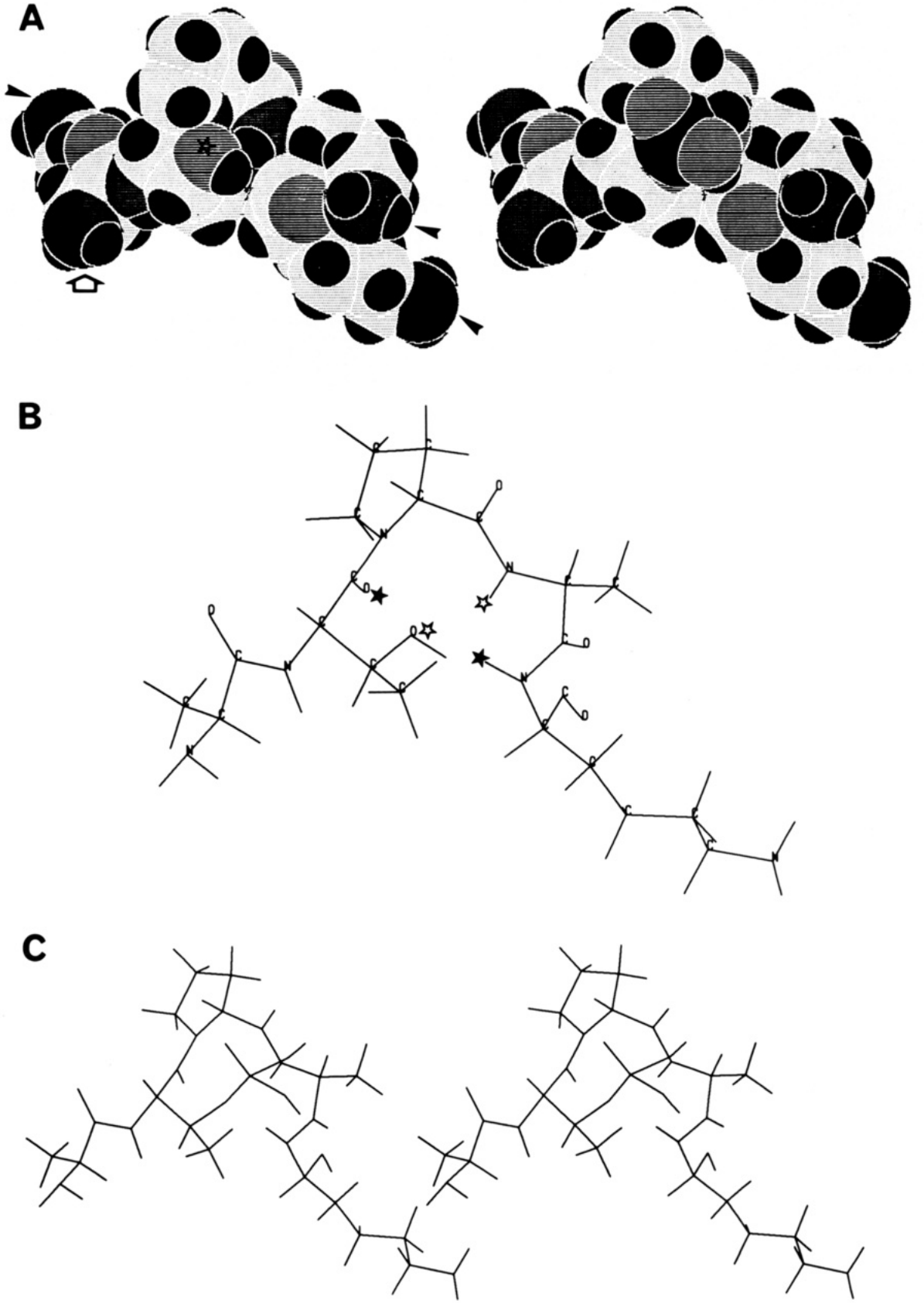

FIGURE 4: Computer modeling of the phosphorylation sites of the two peptides. (A) Space-fill model of the pentapeptide KTPKK. The oxygen of the threonine hydroxyl group is indicated by a star. The large arrow points to the α-NH2 terminus of the peptide, while arrowheads point to the ϵ-NH2 of the three lysine residues. (B) A bonds-only model of the ATPAK pentapeptide. All the atoms except hydrogen are labeled. The two potential hydrogen bonds are marked, with filled stars for the main chain–main chain interaction and with open stars for the side chain–main chain interaction (see text for more details). (C) Stereogram of the phosphorylated ATPAK pentapeptide. The phosphate group is visible at the end of the esterified threonine.

function of the two corresponding proteins with regard to nucleolar chromatin?

The model system we have chosen to tackle these questions comprises the two aforementioned peptides, phosphorylated or not, and the homopolymer poly(dA)·poly(dT), the rationale for this choice being detailed earlier in this paper. By using circular dichroism to assess the appearance of a ψ^+-type condensation of poly(dA)·poly(dT), we show that the nucleolin peptide $(ATPAKKAA)_2$ and the histone H1 peptide $(KTPKKAKKP)_2$ exert a synergistic effect on homopolymer condensation and that the phosphorylation of either one or both of these peptides by $p34^{cdc2}$ kinase enhances this synergy (Figure 1 and 2). The circular dichroic analysis of different combinations of the unphosphorylated and phosphorylated species of the two peptides provides evidence for a direct interaction between these two peptides which is stabilized by phosphorylation (Table I). Moreover, there is an excellent correlation between the stability of the heteromeric complex and its ability to trigger the hypercondensation of poly(dA)·poly(dT). Results from both circular dichroism and infrared spectroscopy (lines 1–4 of Table I and Figure 3) indicate that phosphorylation stabilizes the potential β-turn structure of each individual peptide. The phosphorylated residue is in both cases the threonine contained within the consensus motif XTPXK recognized by $p34^{cdc2}$ kinase, precisely the same motif which is a good candidate to adopt a β-turn structure. Modeling of the KTPKK and ATPAK pentapeptide, which represent the phosphorylation sites of the histone H1 and nucleolin peptides respectively, illustrates that phosphorylation does not impede turn formation and may even lock the corresponding atoms in this conformation by inducing a steric hindrance to their mobility (Figure 4).

The main significance of our results is that histone H1 and nucleolin can interact with each other through homologous domains comprising similar basic repeat motifs organized in β-turns. This interaction between the two proteins constitutes the underlying mechanism of the synergistic effect regarding DNA condensation. In mitosis, both proteins are phosphorylated by $p34^{cdc2}$ kinase [Belenguer et al., 1990; for a review, see Draetta (1990)]. Our results indicate that phosphorylation of either one or both of these homologous domains stabilizes the interaction between the two proteins, yielding a heteromer which is a potent effector of DNA condensation. The fact that this synergy is optimal even if the H1 repeat is unphosphorylated shows that, as already suggested in a review by Crane-Robinson et al. (1984), mitosis-specific phosphorylation of histone H1 cannot, by itself, be the sole cause of DNA hypercondensation. In line with this, Hanks et al. (1983) have proposed that the maintenance of the mitotic chromosomes in a highly condensed state does not require the continuous presence of H1 in a highly phosphorylated form. The ability of H1, phosphorylated or not, to interact with other non-histone proteins organized in β-turns becomes the major factor in mitotic DNA hypercondensation. In fact, there is probably a whole range of mitotic condensation stages generated by such interactions and modulated by a differential phosphorylation or the corresponding proteins. The discovery of an ever-increasing number of $p34^{cdc2}$ kinase substrates makes this a quite conceivable possibility.

Quite a few examples of heterodimeric DNA-binding activities have now been brought to light [for a review, see Johnson and McKnight (1989)]. Similarly, we would suggest that nucleolin and H1 might form a stable heteromer through their homologous domains structured in β-spirals (helical structures made of repeating β-turns). Interaction between β-spirals has been reported by Urry et al. (1983) in the case of elastin peptides. In the presence of H1, nucleolin enters into a heterotypic protein–DNA complex that is more efficient in inducing DNA condensation than the complex formed by H1 alone. In the absence of extensive structural information, it is difficult to determine whether nucleolin participates in the complex via protein–protein contacts with H1 only or whether it also interacts more directly with DNA. However, we must take into account the fact that the difference between the types of DNA condensation induced by H1 and by the heteromer is only quantitative. In other words, the nature of the condensation depends only on the polynucleotide—i.e., ψ^--type condensate for random DNA (Erard et al., 1990) or ψ^+-type condensate for poly(dA)·poly(dT) (this report). It would therefore seem reasonable to assume that the same DNA/H1 interface is present in both cases, with nucleolin intervening, according to its phosphorylation state, to stabilize this active H1 domain.

In any case, the interaction between the H1/nucleolin complex and DNA cannot be solely electrostatic since heteromer phosphorylation enhances DNA condensation, which is an excellent index of stable binding. Contributions of Van der Waals contacts and hydrogen bonding should not be overlooked in these interactions, especially in the context of the AT specificity. One of the major characteristics of AT-rich DNA sequences is the narrowness of their minor grooves, and this is particularly marked for (dA)·(dT) stretches, ensuring a tighter binding to their ligands through Van der Waals contacts. As H1 is able to recognize a characteristic minor groove width and conformation generated by (dA)·(dT) tracts (Käs et al., 1989), the hypothesis of a unique H1/DNA interface is even more likely when the DNA in question is poly(dA)·poly(dT).

The H1/nucleolin heteromer and its phosphorylation can thus efficiently modulate nucleolar chromatin condensation in mitosis, particularly at the level of homopolymeric (dA)·(dT) sequences. Such sequences exist in nucleolar organizer regions (NORs) (Schweitzer et al., 1987) and, in particular, in the form of autonomously replicating sequences (ARSs) in the nontranscribed ribosomal DNA spacer from many organisms, including *Physarum*, *Tetrahymena*, and *Xenopus* [for a review, see Umek et al. (1989)]. Two recent reports have shown that the most efficient ARSs are found on DNA fragments with the strongest scaffold-binding activity (Amati & Gasser, 1990; Brun et al., 1990). Scaffold-associated regions (SARs) contain many (dA)·(dT) runs and are proposed to form the base of chromatin loops in eukaryotic nuclei and metaphase chromosomes [for a review, see Gasser and Laemmli (1987)]. As mentioned earlier in this discussion, H1 manifests a preferential binding to SARs which relies on the presence of the oligo(dA)·oligo(dT) tracts (Käs et al., 1989). We would like to suggest that the H1/nucleolin heteromer might be responsible for mediating scaffold attachment of those regions of the nontranscribed ribosomal DNA spacers previously characterized as ARSs.

Finally, the nucleolar system presented here could constitute a more general model of chromatin condensation modulation. Other nuclear proteins comprising repeats organized in β-turns such as, for example, HMG-I(Y) group proteins (Johnson et al., 1988) and datin (Winter & Varshavsky, 1989), also have a preferential affinity for homopolymeric (dA)·(dT) sequences. Interestingly, HMG-I proteins possess the same dual function as nucleolin: they are the object of a differential phosphorylation in interphase and mitosis (Lund et al., 1983) and have been found both in highly proliferative cells and at the level

of metaphasic chromosome G/Q- and C-bands, typically rich in A-T residues (Disney et al., 1989). More generally, the presence of repeat motifs structured in β-turns provide the corresponding proteins with three properties: the ability to bind to DNA and especially to oligo(dA)·oligo(dT) sequences, the capacity to form heterologous complexes with each other, and the possibility of being recognized by kinases such as $p34^{cdc2}$.

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Registry No. $(KTPKKAKKP)_2$, 127770-00-7; $(ATPAKKAA)_2$, 127769-98-6; poly(dA)·poly(dT), 24939-09-1; $p34^{cdc2}$ kinase, 70431-11-7.

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