CONFORMATION AND REACTIVITY OF DNA
V. pH-DEPENDENT CONFORMATIONAL CHANGES OF DNA IN COMPLEXES WITH POLY-L-HISTIDINE: TRANSITIONS FROM B- TO A-FORM AND TO A CONDENSED STATE

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1. Introduction

The interactions between DNA and proteins or enzymes involve conformational changes of the DNA-double helical molecule which are important for the functional state of the genetic material. DNA undergoes structural changes into a compact state in complexes with f1 histone [1], complexes with f2a1 histone [2], complexes with polylysine [3–5] and in synthetic polymer solution, such as polyethylene oxide [6,7] or polyethylene glycol [8]. In many studies concerning DNA–protein and polypeptide interactions [9–12] the special effects and the role of lysine and arginine residues have been intensively discussed. Recently the possible role of aromatic amino acid in protein binding to nucleic acids was inferred [13] from binding data of tryptamine [14]. No data exist on DNA–polypeptide complexes containing minor amino acids such as tryptophane or histidine. The latter has a pK of 6.0 and is therefore of special interest in changing the positive charges along the polypeptide chain in a physiological pH region.

2. Materials and methods

Calf thymus DNA was that described by Sarfert and Venner [15]. Poly-L-histidine was a commercial product from Miles Laboratories Inc., Elkhart, Indiana, with a molecular weight of 6,000 to 7,000. DNA–polypeptide complexes were prepared by salt gradient dialysis [16] with minor modifications [17]. After salt gradient dialysis at pH < 6 the complexes were titrated discontinuously by 0.1 M HCl or NaOH to the desired pH using a method which minimizes local acid effects [18]. The amount of polypeptide material which could dialyze through the bag was found to be negligible under the experimental conditions. CD measurements were made in a Cary 60 spectropolarimeter with 6001 CD attachment using 1 cm cells.

3. Results and discussion

Poly-L-histidine exhibits a dissociation constant of pK_a of 5.9 for the imidazolic group of the polymer [19–21] and undergoes possibly a transition from random coil to right-handed α-helix between pH 6.0 to 3.0 [22,23]. Information on conformational changes of DNA were obtained from ORD and CD spectra. Typical changes of the ORD of DNA upon complex formation with poly-L-histidine are shown in fig. 1. At 1 M $NaClO_4$ the ORD profile of DNA plus poly-L-histidine is identical with that of DNA alone (curves 2 and 3). Dialysis to 0.02 M Na^+ exhibits a pronounced decrease of the peak at 290 nm (curve 4) accompanied by a shift of the crossover point to a longer wavelength. The complete

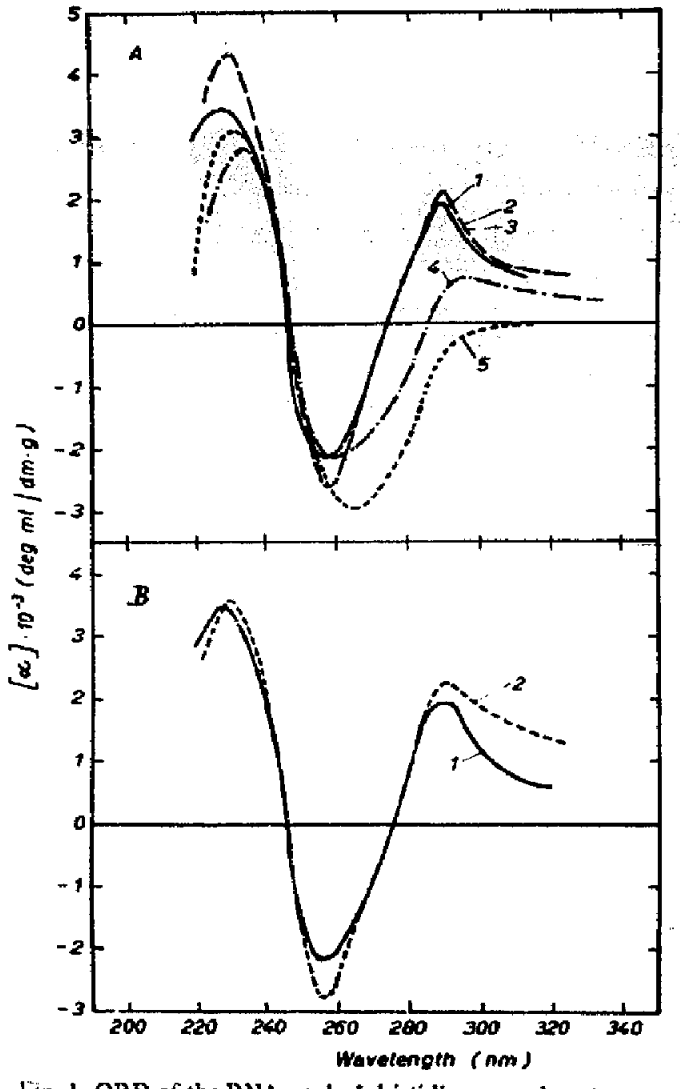

Fig. 1. ORD of the DNA–poly-L-histidine complex at approx. 0.5 histidine to DNA-phosphate. A) Complex prepared by salt gradient dialysis; curves indicate as follows: 1, DNA in 10^{-3} M and 2×10^{-2} M $NaClO_4$; 2, DNA alone and 3, complex in 1.0 M $NaClO_4$; 4, complex in 2×10^{-2} M $NaClO_4$; 5, in 10^{-3} M $NaClO_4$; pH ~ 6. B) Complex prepared by mixing at 10^{-3} M $NaClO_4$, pH ~ 6.

depression of this ORD peak (curve 1) appears at 0.001 M Na^+ (curve 5) indicating perturbation of the DNA secondary structure due to binding between DNA and poly-L-histidine. In the pH region from neutral to pH 6 there is almost no influence of the polypeptide itself on the ORD-shape from 300 nm to 250 nm, but in the acidic region poly-L-histidine shows significant rotation at higher wavelengths

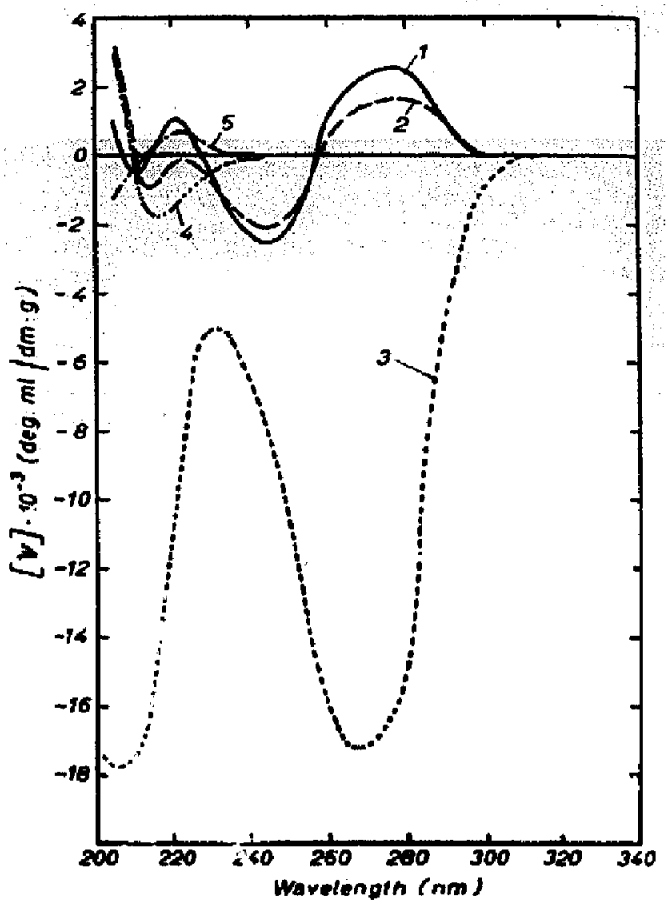

Fig. 2. CD spectra of the DNA–poly-L-histidine complex at the input ratio histidine to DNA–phosphate of 1.0. Curves indicate as follows: 1, DNA in 10^{-2} and 10^{-1} M Na^+; 2, complex at pH 7.2 in 10^{-1} M and 10^{-2} M Na^+; 3, complex at pH 5.2 in 10^{-1} M Na^+; 4 and 5, poly-L-histidine at pH 5.8 and 5.0, respectively.

[22]. CD spectra of poly-L-histidine solutions exhibit Cotton effects in neutral and acidic pH region below 235 nm [22] only. Thus for the positive and negative CD band of DNA the contribution of CD of the polypeptide is negligible above 240 nm. In addition to that DNA–poly-L-histidine complexes at acidic pH are slightly turbid and light scattering effects have no influence on the CD measurements in this case as also reported by other authors [4]. The DNA–poly-L-histidine complex formed by dialysis shows at neutral pH a decrease of the positive CD maximum at 275 nm while the negative maximum is almost unaltered as demonstrated in fig. 2 (curve 1 and 2). The CD of the polypeptide itself (curve 4

and 5) clearly has no influence in this region. Increasing protonation of poly-L-histidine to approx. 70–80% at pH 5.2 [21] causes a characteristic negative band at 268 nm in the DNA complex which has a large amplitude at 1.0 histidine to DNA phosphate (curve 3). This negative maximum we also observed at lower pH.

The appearance of this large negative CD maximum is strikingly similar to that observed upon complex formation of DNA with polylysine [4,5], with nucleohistone [1, 24] or in the presence of certain organic polymers [7, 8]. All these polymers change the DNA conformation considerably. They may alter the DNA-double helix structure in the B-form to a more compact conformation as first has been pointed out for DNA in polyethylene oxide [7]. This form was termed by Lerman as ψ-state [6] which belongs to the B fibre structure. A compact DNA structure has been visualized in polyethylene glycol by electron microscopy [8]. On the other hand the change from the CD spectrum of DNA (curve 1) to that of the complex in which polyhistidine is weak protonated (curve 2) closely resembles that of free DNA at high salt concentration such as NaCl or LiCl [25–30]. From the similarity of the CD changes in solution to films of Li^+ DNA a C-like conformation has been suggested in concentrated salt solutions [25] and in ethylene glycol [31]. The C-like structure represents a more condensed form regarding the change of the winding angle from B- to the C-form [32]. The depression of the positive CD maximum of DNA in concentrated salt solutions [25–30] were explained by a conformational change of the B helix structure which involves an increase in winding angle between base pairs. This implies an increased spirality in the double helix. The CD spectrum of the DNA–polyhistidine complex in the neutral pH range (fig. 2, curve 2; fig. 3, curve 4) shows the same characteristics as observed in the presence of concentrated alkali salts [25–30]. There is a decrease of the positive CD maximum at 275 nm while the negative CD band is almost unaltered. Thus it seems probable that binding of protonated poly-L-histidine modifies the DNA B conformation by changing the winding angle like that observed in the transition from B to C form [32]. Whether the formation of the compact state (fig. 2, curve 3; and fig. 3, curve 2) of DNA involves C-type or pure B-type structures is uncertain at this stage.

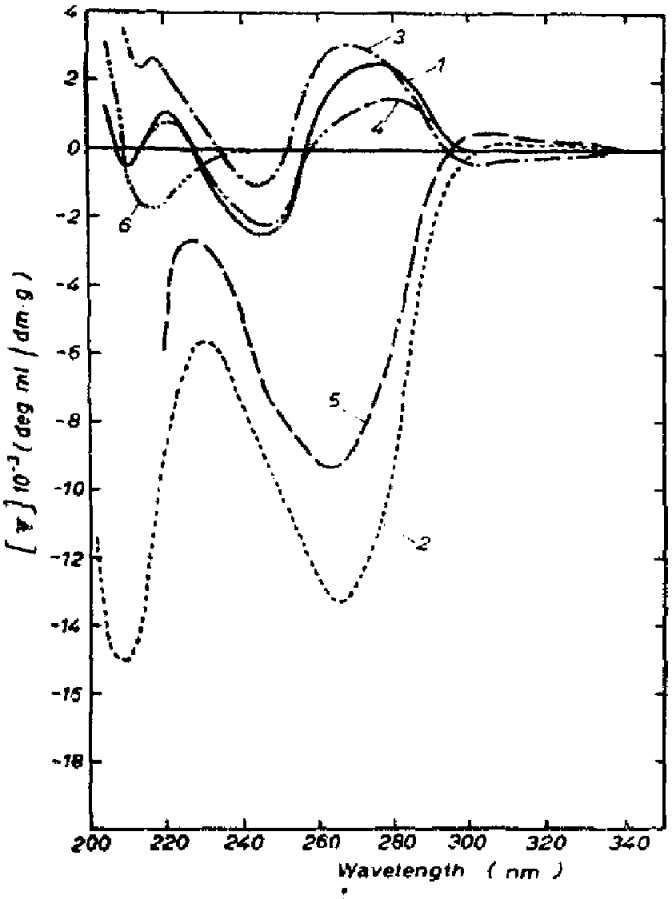

Fig. 3. CD spectra of the DNA–poly-L-histidine complex at the input ratio histidine to DNA phosphate 0.7 in 10^{-1} M Na^+. Curves indicate as follows: 1, DNA alone. Complexes: 2, after dialysis at pH 5.2; 3, titration to pH 6.5; 4, to pH 8.3; 5, second forward titration from pH 8.3 to pH 5.7.

CD spectra of a forward- and back-titration of the dialysed complex are shown in fig. 3. Upon titration from the condensed state at pH 5.2 (fig. 3, curve 2) to the region pH 6 to 6.5 (curve 3) a non-conservative CD spectrum appears as indicated by the increase of the positive CD band accompanying a blue shift and a decrease of the negative maximum when compared to the conservative spectrum of the B-DNA (curve 1). This behavior was repeatedly observed. The general shape of the non-conservative spectrum is similar to that of the A-form of DNA in ethanol [28, 33] or to double-stranded RNA [33] and to that observed for the arginine-rich histone–DNA complex [2]. Further deprotonation of

poly-L-histidine in the neutral and slightly alkaline pH region (pH 8.3) again changes the CD spectrum back to that with a conservative behavior (fig. 3, curve 4). The second forward titration from pH 8.3 to pH 5.7 demonstrates the reversibility of those conformational changes involving the formation of a condensed state (curve 5). It is most interesting that in the course of complex formation of DNA with poly-L-histidine through pH manipulation three different conformational states may be reversibly induced from B- to A- and to the condensed state containing B-type or possibly C-type structures. The conservative behavior appears when the polypeptide exists as randon coil with very few ionized imidazolic groups (pH ~ 7–8) [19–21]; the A-like structure occurs above the pK-range of poly-L-histidine (pH 6–7) and the compact form is observed at higher degree of protonation of the polypeptide where it tends to form a right-handed helical structure [22, 34]. It should be emphasized, however, that this discussion corresponds to the dissociation constant of the free polypeptide structure. The pK of poly-L-histidine may be shifted in the DNA complex due to the polyanionic nature of DNA. It is left open to conjecture whether these pH dependent binding effects and changes of the DNA structure are important in DNA–protein interactions of enzymatic reactions or in changing the conformation of DNA in chromosomal state. The behavior of our model DNA–polyhistidine complexes is certainly not directly comparable with DNA bound proteins, but it gives naval features for possible local effects of histidine adjacent to amino acids such as arginine or lysine. The importance of histidine as a trigger in the initiation of structural changes for DNA has been regarded for metaphase chromosomes [35] since the imidazolic groups of histidine of proteins show pK values between 6.4 and 7.0 [36]. Very recently a histidine switch mechanism for the specific DNA protein association from titration studies with spermine has been postulated [37]. In view of the presence of histidine in proteins associating DNA those pH-dependent conformational effects are of great biological interest. Further work on this subject is in progress.

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