Rendiconti
Accademia Nazionale delle Scienze detta dei XL
Memorie di Scienze Fisiche e Naturali
98° (1979-80), Vol. IV, fasc. 11, pagg. 161-168.

MAURIZIO CASARCI, PASQUALE DE SANTIS, STEFANO MOROSETTI, ANTONIO PALLESCHI, MARIA SAVINO and ANTONIO VERDINI (*)

Superstructure of DNA induced by a synthetic polypeptide (**)

SUMMARY. - The DNA-$(Lys^{33}, Leu^{67})_{[illegible]}$-$Orn_{[illegible]}$ association complex has been investigated by physico-chemical methods and X-Ray diffraction. The results indicate that DNA is supercoiled around a copolypeptide core with dimensions similar to those found in chromatin.

RIASSUNTO. - Il complesso tra DNA e il copolipeptide $(Lys^{33}, Leu^{67})_{[illegible]}$-$Orn_{[illegible]}$ è stato studiato mediante metodi chimico-fisici e diffrazione di raggi X. I risultati indicano che il DNA si avvolge, formando una superelica, intorno ad un nucleo polipeptidico con dimensioni simili a quelle trovate nella cromatina.

INTRODUCTION

DNA-basic polypeptide complexes have been widely investigated with the aim of elucidating the nature of DNA/proteins recognition [1]. Whilst DNA-poly-lysine complexes show striking similarity in their physico-chemical properties and structure with nucleoprotamines, none of the various DNA-cationic copolypeptide so far investigated exhibits structural analogy with nucleohistones.

In fact, since until 1974 [2] the key role of histone-histone interactions in determining the structure of association complexes was not clearly understood, (perhaps as a consequence of the preconceived idea that DNA has a central role in nucleating biological structures), the synthetic polypeptides adopted as histones models were generally designed taking into account only the DNA-polypeptide interactions. The Kornberg proposal [2] of the nucleo-histone structure reversed this point of view so that a proteic core, constituted by the octamer (H4, H3,

(*) Istituto di Chimica Fisica and Centro di Studio per gli Acidi Nucleici CNR, Istituto di Fisiologia Generale, Università di Roma.
(**) Presentato dal Socio G.B. MARINI-BETTÒLO il 10 ottobre 1980.

ISSN-0370-3460

H2A, $H2B)_2$, is now generally recognized to be the central block in chromatin building.

X-ray and neutron scattering investigations [3] and the recent X-ray crystal analysis at 20 Å resolution of the nuclease digestion product of chromatin [4], the nucleosome, provided a sound experimental basis for this model. The resulting picture of nucleosome corresponds to a flat solenoidal structure of diameter 100 Å, where kinking or supercoiling 1 + 3/4 turns of DNA, 140 base pairs in length, are wound around the proteic core.

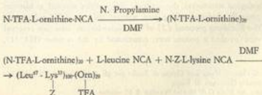
As the interpolypeptide interactions are now considered of primary importance, we have synthetized a model polypeptide, designed to mimic the fundamental feature of the histones, i.e. having a part of the aminoacid sequence highly basic and hydrophilic and a part containing a proportion of hydrophobic residues reminiscent of globular proteins. This feature is readily detected in fig. 1, where the profiles of the hydrophobicity along the primary structures of the four histones, involved in the nucleosome core, are shown. Two hydrophobicity scales are adopted. The first one results from the relative frequency of finding the different aminoacid residues in the internal regions of proteins of known structure [5]; the other derives from the thermodynamic properties of a suitable derivative of the isolated aminoacids [6].

The regions characterized by both low hydrophobicity and high basicity, are those fully available to fitting the DNA structure along the narrow groove as in nucleoprotamines [7], whereas the other regions expected to be characterized (as are the globular proteins) by secondary and tertiary structures, provide the sites of interhistones interactions.

We have designed block copolypeptides where these features are idealized. The highly basic sequence is mimicked by a polyornithine block of 20 aminoacid residues and the structured moiety, by a copolypeptide block constituted by the statistical sequence $(Lys^{33}, Leu^{67})_{100}$ which was expected to be able to provide strong interpolypeptide interactions, mimicking one tendency to association of histones.

Experimental

The polypeptide was synthetized according to the scheme reported below; the details of the synthesis are reported elsewhere [8].



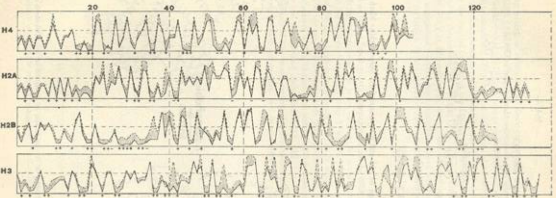
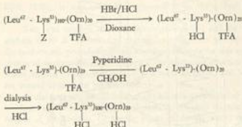


Fig. 1 — Profiles of hydrophobicity along the primary structure of histones H4, H2A, H2B and H3. ———— Gates-Fischer scale; – – – – – A. Aboderin scale. + and – indicate the basic and the acidic amino-acid residues.



In a large range of pH and temperature, the polyornithine block is random coil, whereas about 80% of the copolypeptide moiety (Leu^{33} - Lys^{67})$_{100}$ is α-helical.

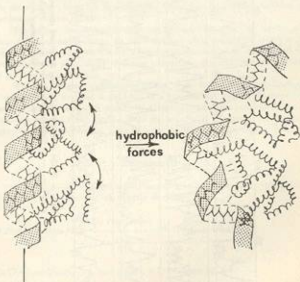


Fig. 2 — Schematic drawing of the mechanism of formation of the DNA-block copolypeptide complex.

By direct mixing of solutions the block polypeptide gives irreversible complexes with DNA [8]. The reduced viscosity decreases, increasing the input ratio r (peptide/nucleotide) to reach 20% of that of pure DNA at $r = 2.5$. Contrary to DNA-polylysine complexes, CD spectra at different r show the characteristic trend of reconstituted nucleohistones. A saturation ratio $\beta = 3.7$ was determined both by evaluating the residual fraction of free DNA, from the differential melting hyperchromism, and by determining the residual DNA in the supernatant of the centrifugation at 10,000 g at different input ratios. The titration curve of the residual DNA in the supernatant shows a clear cooperative trend which reflects a possible hydrophobic nucleation in the complex formation, according to the scheme in fig. 2.

Results and discussion

The diffraction pattern of the pellet obtained by centrifugation reveals strong reflections at 3.4 Å typical of DNA form B, and at 11.5 Å, which characterizes the pattern of the pure block copolypeptide and can be assigned to the packing of α-helices. The low-angle X-ray diffraction pattern, shown in fig. 3 (a) is characterized by reflections at 50, 30 and 23 Å reminiscent of those at 55, 37 and 27 Å of chromatin and reconstituted nucleohistones [9, 10].

The simplicity of this model system of nucleohistone and the absence of any specific interpolypeptide interactions, in principle allows the scanning of plausible

Fig. 3 (a) - Low-angle X-Ray diffraction pattern (densitometer trace) of a pellet of the DNA-block copolypeptide complex at a specimen to film distance of 20 cm.

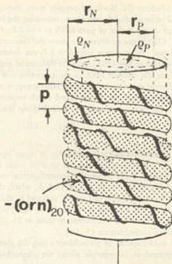


Fig. 3 (b) - Schematic drawing of the DNA-block copolypeptide structure.

structures to be restricted to those characterized by helical symmetry with DNA wound around a compact hydrophobic polypeptide core.

In a rough approximation we have assumed a tubular structure with constant density in the inner copolypeptide ρ_P, and outer DNA, ρ_N, cylindrical shells, as represented in fig. 3 (b). In this case the intensity distribution along the scattering coordinate $R = \left(\frac{2 \text{ sen } \vartheta}{\lambda}\right)$ can be approximated by:

$$I(R) = \frac{f^2(R)}{R^2} \left[r_N J_1 (2 \pi r_N R) - \frac{\rho_N - \rho_P}{\rho_N} r_P J_1 (2 \pi r_P R) \right]^2$$

where $f(R)$ is the unitary scattering atomic factor, r_P and r_N are the radii of cylindrical domains of the copolypeptide block and DNA, respectively. J_1 (   ) represents the first-order Bessel function.

Taking into account the experimental value of $\beta = 3.7$ and a density of 1.25 gr/cm³ of the copolypeptide core, the structure which emerges corresponds to a compact solenoidal DNA with an external enveloping surface of ~ 90 Å of diameter and a pitch of ~ 29 Å. This value is not far from the X-ray equatorial spacings found in fibers of DNA nucleoprotamines and DNA polylysine complexes at high relative humidity, revealing a tendency of the DNA superspirals to close packing.

Table 1 summarizes the structural parameters of the model system as compared with the corresponding values derived, for the nucleosome core, by X-ray single crystal analysis [4].

TABLE 1

	Diameter	Pitch	Base pairs/turn	β	Protein molecules/turn
nucleosome core	100 Å	28 Å	80	3.5	4.6
model system	90Å	29 Å	68	3.7	4.2

On the basis of the results of this paper the conclusion can be drawn that the mutual hydrophobic interactions between the copolypeptide blocks anchored to DNA, stabilize a cylindrical micellar structure, whose dimensions are dictated by the dimensions of the polypeptide hydrophobic core. It is, however, possible that DNA itself has the property of stabilizing a solenoidal superstructure, as already suggested [11, 12]. A systematic analysis of model systems by varying the dimensions of hydrophobic core would be necessary to assess this point. On the basis of this model, the inability of binary DNA association complexes with single histone fractions to assume a chromatin-like structure, may be ascribed to the reduced tendency of histones to self-association as compared with the block copolypeptide; this is, however, very high in the ternary complex DNA-(H4, H3)₂ which exhibits practically the same structure as chromatin [13].

In spite of the rigid conservativity of the histone structures that can be, however, required for other subtle and essential biological controls as specific regulatory processes, the gross superstructure induced in DNA apperars to be correlated to the disproportion in the distribution of basic and hydrophobic residues along the primary structure and the tendency to self associate of the globular structure of histones.

The recent evidence that a similar DNA superstructure can be induced by a basic detergent [14], further supports this conclusion.

References

[1] *Chromatin and Chromosome structure* (Li, H.S. and Eckhardt, R.A., eds) Academic Press, 1977, and references therein quoted.

[2] Kornberg R.D. (1974) - « Science », *184*, 868-871.

[3] Pardon J.F., Worcester D.L., Wooley J.C., Catter R.I., Lilley D.M.J. and Richards B.M. (1977) - « Nucleic Acids Res. », *4*, 3199-3214.

[4] Finch J.T., Lutter L.C., Rhodes D., Brown R.S., Rushton B., Levitt M. and Klug A. (1977) - « Nature », *269*, 29-36.

[5] Gates R.E. and Fisher H.F. (1971) - « Proc. Nat. Acad. Sci. USA », *68*, 2928-2931.

[6] Aboderin Akintola A. (1971) - « Int. J. Biochem. », *2*, 537-544.

[7] De Santis P., Forni E. and Rizzo R. (1974) - « Biopolymers », *13*, 313-326.

[8] Costantino P., De Santis P., Rizzo R., Savino M. and Verdini A.S. (1979) - « Biopolymers », *18*, 9-24.

[9] Sperling M. and Klug A. (1977) - « J. Mol. Biol. », *112*, 253-263.

[10] Boseley P.G., Bradbury E.M., Butler-Browne G.S., Carpenter B.G. and Stephens R.M. (1976) - « Eur. J. Biochem. », *62*, 21-31.

[11] Crick F.H.C. and Klug A. (1975) - « Nature », *255*, 530-535.

[12] Sobell H.Y., Tsai C.C., Gilbert S.G., Jain S.C. and Sakore T.D. (1976) - « Proc. Nat. Acad. Sci. USA », *73*, 3068-3072.

[13] Bradbury E.M., Moss T., Hayashi H., Hjelm R.P., Snan P., Stephens J.M., Baldwin J.P. and Crane-Robinson C. (1978) - « Cold Spring Harbor Symp. Quant. Biol. », *42*, 287.

[14] Verkin B.I., Osica V.D., Sukharevsky B.Ya. and Fedgin L.A. (1978) - « Studia Biophysica », *67*, 35-36.

Direttore respon.: Prof. P. Di Mattei - Autorizz. Trib. di Roma n. 7269 del 28-12-1959 - Tip. della Pace, Roma