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SPECIFIC INTERACTIONS OF 4′-6-DIAMIDINO-2-PHENYLINDOLE WITH NUCLEOSOME

C. Bumma, S. Cacchione, R. Caneva, M. Savino

Dpt Genet. Biol. Mol. – CS Acidi Nucleici CNR,
Universita' Roma I

In a research program on the interactions between biologically active molecules and nucleosome, after the study of a typical intercalator such as actinomycin D (ACTD) [1], we are now analysing the interaction of 4′-6-Diamidino-2-phenylindole (DAPI) with nucleosome and nucleosomal DNA, by topological analysis and spectroscopic measurements. DAPI forms fluorescent complexes with natural and synthetic double-stranded deoxyribonucleic acids containing AT, AU and IC clusters [2] and with double-stranded RNAs containing AU clusters [3], while no fluorescent complexes have been found for the interaction of DAPI with single stranded ribonucleic acids. Because of these properties DAPI is a useful tool in various biochemical and cytochemical investigations. Recent researches have evidenced the biological importance of DAPI: it induces undercondensation of certain specific human chromosome sites and causes breaks in other chromosome sites with the characteristics of common fragile sites [4], and it inhibits the initiation of in vitro trascription through its effect on open complex formation and stability [5]. DAPI binds to DNA in two different modes [2, 6]: 1) a strong binding, specific for AT, AU and IC clusters, which gives rise to a large increase in fluorescence, and 2) a weaker one, non specific to nucleotide sequence, which only slightly increases the fluorescence of DAPI and can be attributed mainly to electrostatic interactions.

Two models have been proposed for the stronger type of interaction, one based on intercalation [2], the other on external binding in the narrow groove [6]. The outside binding model is now generally accepted and it is confirmed by our results. Notwithstanding the hypochromism and the red-shift observed in the spectra of DAPI in the presence of DNA and nucleosome (fig.1), that could indicate an intercalation, our results of topo-

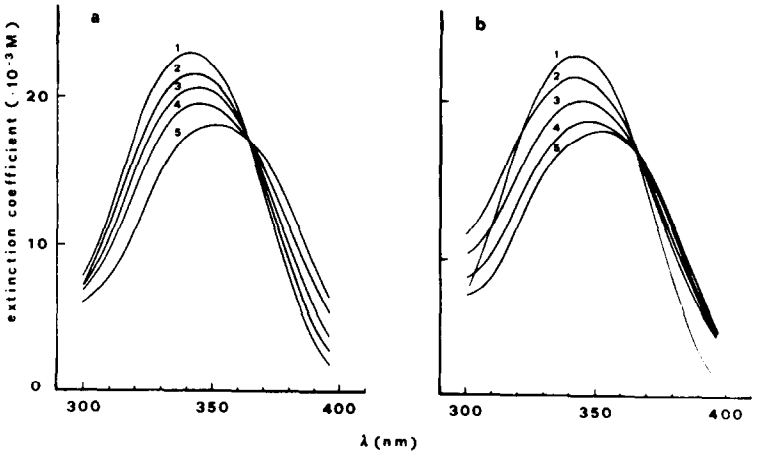

Fig. 1. Absorption spectra of DAPI at various drug/phosphate ratios. a) DAPI/DNA: [D]/[P] from 0 to 0.11; b) DAPI/nucleosome: [D]/[P] from 0 to 0.18.

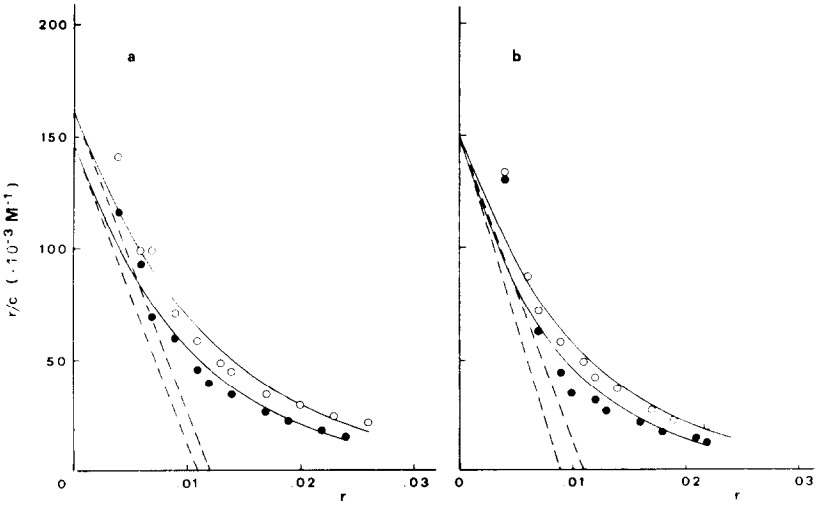

Fig. 2. Isotherms for the binding of DAPI to DNA (o) or to 175 bp nucleosomes (o), shown as Scatchard plots. a) 10 mM Tris-HCl pH 7.4; b) 10 mM Tris-HCl pH 7.4 + 0.1 M NaCl; r, molar ratio of bound drug to total DNA bp; m, concentration of free drug. Dashed lines are the tangents to y-intercepts of semilogarithmic fits.

logical analysis of complexes between DAPI and pBR322 supercoiled DNA at different ratios suggest that the intercalative model can be excluded. In fact, the DNA is fully relaxed upon topoisomerase I treatment of the complexes (not shown).

The strong fluorescence increase of DAPI, caused by the binding with DNA and nucleosome, has been exploited for the measurement of the association constant and the number of binding sites for the interactions of DAPI with nucleosome and nucleosomal DNA. Data obtained with spectrofluorimetric measurements at two different ionic strenght, organized in the form of Scatchard plots (fig.2), indicate a strong similarity for the binding of DAPI to DNA and nucleosome: the association constants are almost equal (about 1.3-1.6 x 10^7 M^{-1}) and the number of binding sites found for the interaction between DAPI and nucleosome is slightly smaller than the number found for DNA (by about 15%). On the contrary, for the interaction of nucleosome with an intercalator such as ACTD, the number of binding sites is strongly decreased, compared with that found for the interaction between ACTD and DNA [1].

These findings are in agreement with results on the accessibility of bases in nucleosomes previously reported [7]: guanine residues in the large groove and, less certainly, adenine residues in the narrow groove are in the nucleosome as accessible to dimethylsulfate as they are in naked DNA. Our results confirm that nucleosomal DNA is accessible in the narrow groove to relatively small molecules: this indicates that, in the nucleosome, histones do not engage in extensive contacts with nucleotide bases of DNA, suggesting an organization of the nucleosome which assures the maximum of DNA readability compatible with its compact structure.

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