Role of structure-proteins in the porphyrin–DNA interaction

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ABSTRACT

We studied the complexation of meso-tetrakis(4-N-methylpyridyl)porphyrin (TMPyP) with HeLa nucleosomes and compared it to our earlier results on T7 phage nucleoprotein complex (NP) and isolated DNA. To identify binding modes and relative concentrations of the bound TMPyP forms, the porphyrin absorption spectra were analyzed at various base pair/porphyrin ratios. Spectral decomposition and circular dichroism measurements proved that the two main binding modes of TMPyP, i.e., external binding and intercalation occur also in the nucleosomes. The DNA superstructure maintained by the proteins decreases its accessibility for TMPyP similarly in both nucleoproteins. A difference is observed between the partitioning of the two binding modes: in the case of nucleosome the ratio of intercalation to groove-binding is changed from 60/40 to 40/60 as determined for T7 NP and for isolated DNA-s. Using UV and CD melting studies, we revealed that TMPyP destabilizes the DNA–protein interaction in the nucleosomes but not in the T7 phage. Lastly, photoinduced reaction of bound TMPyP caused alterations in DNA structures and DNA–protein interactions within both nucleoprotein complexes; the nucleosomes were found to be more sensitive to the photoreaction.

1. Introduction

Cationic porphyrins are of great interest as they are inhibitors of human telomerases [1,2], components of artificial receptors [3], delivery vehicles [4] and agents used in photochemotherapy [5]. Most of these activities are facilitated by their binding to macromolecules, specifically to nucleic acids.

The binding of cationic porphyrin derivatives to nucleic acids has been the subject of extensive research since Fiel and colleagues discovered that such compounds can form various complexes with DNA [6]. It has been shown that the binding can be either intercalative or external, in the minor groove (in some special cases with self-stacking) [7–11] depending on the DNA base composition, the presence and type of the central metal ion in porphyrin, as well as the peripheral substituents. Some peptides also have been reported to be bound by cationic porphyrins [12]. One such peptide with the amino acid sequence His-Ala-Ser-Tyr-Ser was selectively screened from a phage library and bound to meso-tetrakis(4-N-methylpyridyl)-porphyrin (TMPyP). The proposed binding was due to stacking from two aromatic amino acids. Nevertheless, there are very few data about the cationic porphyrin binding to nucleoprotein complexes [13,14]. Also very little is known how the presence of proteins, e.g., viral capsid proteins or histones, influences the porphyrin–DNA interaction.

Recently, we investigated the complex formation between the TMPyP and free or encapsidated DNA of T7 bacteriophage [13]. Our results suggested that TMPyP binds to DNA regardless of whether the polynucleotide is part of a nucleoprotein complex (NP) or not. We found similar binding forms, i.e., intercalation and outside, non-intercalative binding both in isolated and encapsidated DNA.

In our previous studies, we selected T7 as nucleoprotein complex for two reasons. (1) Samples of both T7 phage and its isolated DNA can be prepared in optical grade purity and in sufficiently high concentration [15]. In this way, two different conformations of the same natural polynucleotide could be investigated. (2) Phage nucleoprotein has been considered as a model for compact packing of DNA within chromosomes [16]. As such, T7 was used in several studies where the genotoxic effect of various physical and chemical agents was tested [17,18]. However, in spite of significant similarities in the compactness of the genetic material in the viruses and within the chromosome, the DNA–protein contacts of viruses differ from that of chromatin.

The fundamental packaging unit of the chromatin is the nucleosome [19] consisting of the core particle and linker DNA between successive nucleosome cores. The core particle is one of the most conserved structures containing two copies of each histone protein

(H2A, H2B, H3 and H4) and cca 146 base pairs of DNA wrapped around the histone octamer [20,21]. The length of the linker DNA varies between 20 and 60 base pairs, dependent upon organism. The solution-structure of this linker DNA is not specifically known, but seems to be more similar to the canonical B form DNA than to the distorted DNA wrapped around the histone octamer. Different linker histones, but primarily H1, stabilize this structure through further contacts between the core nucleosome and the linker DNA. The nucleosome represents the first order of DNA packaging in the nucleus, and as such, is the principal structure that determines DNA accessibility to the binding agents.

The aim of this work was a qualitative and quantitative comparison of TMPyP binding (1) to T7 phage nucleoprotein and nucleosome; and (2) to nucleosome and isolated nucleosomal DNA.

Quantitative characterization of TMPyP binding modes was performed by the decomposition of the Soret band within the porphyrin absorption spectra [13,14]. CD and absorption spectroscopy were used for the identification of the various binding forms of TMPyP. Structural consequences of porphyrin binding on NP complexes were followed by both CD and absorption melting measurements.

Thermal denaturation of nucleoproteins is one of the most frequently used physical methods for studying the stability changes of a nucleoprotein complex [22–25] dependent on the ionic strength and composition of the buffer solution. The melting parameters of T7 nucleoprotein have been previously determined [22,23] under experimental conditions similar to ours, however, no such data were published for HeLa nucleosomes. There have been so far very few examples using in parallel applications of absorption and CD melting analyses.

2. Materials and methods

2.1. Porphyrin

Meso-tetrakis(4-N-methylpyridyl)porphyrin (TMPyP) was purchased from Porphyrin products (Logan, UT). Porphyrins were stored at 4 °C in powder form or as a stock solution in distilled water. Before the experiments the porphyrin stock solutions were diluted into a buffer solution composed of 20 mM Tris–HCl and 50 mM NaCl (pH = 7.4). The concentrations of porphyrin was determined from its optical density using molar extinction coefficients of $3.17 \times 10^5 \, M^{-1} \, cm^{-1}$ at $\lambda = 422$ nm obtained from the initial slope of Beer–Lambert plots in our laboratory.

2.2. T7 Bacteriophage

T7 (ATCC 11303-B7) was grown on *Escherichia coli* (ATCC 11303) host cells. The cultivation and purification were carried out according to the method of Strauss and Sinsheimer [26]. The phage suspension was concentrated on a CsCl gradient and dialyzed against buffer solution composed of 20 mM Tris–HCl and 50 mM NaCl (pH = 7.4). The concentration of T7 bacteriophage was determined from its optical density using a molar absorptivity of $\varepsilon_{260} = 7.3 \times 10^3 \, (Ml_{nucleotide \, bases} \, cm^{-1})$ in phosphate buffer [27].

2.3. Preparation of nucleosomes from HeLa cells

HeLa cells were maintained in suspension culture in RPMI 1640 medium (Invitrogen, Germany) without phenol red supplemented with 10% foetal calf serum. Isolation of nuclei and fractionation of chromatin into mono and oligonucleosomes was performed as previously described [28]. Fractions containing primarily mononucleosomes were dialysed overnight against a buffer containing 20 mM Tris, 50 mM NaCl (pH = 7.4), and concentrated in Centricon cartridges (Centricon, MA). SDS and native polyacrylamide gel analy-

sis of whole sample or isolated DNA were used to determine whether mononucleosomes are truncated to core particles, which contain DNA fragments, no longer than 150 base pairs and the four core histones in equimolar proportion. Linker histones or non-histone proteins were not detectable within the samples. Supposing a 1:1 GC:AT ratio in nucleosomes, the base pair concentration in nucleosome solutions was approximated by the molar absorptivity of T7 NP. This can lead to a small inaccuracy, but due to the sequence heterogeneity of nucleosome, determination of a precise molar absorptivity is not possible.

2.4. Preparation of DNA from nucleoprotein complexes

DNA was prepared from nucleoprotein complexes by incubating with 0.5% SDS for 30 min at 65 °C; followed by precipitation with 1 M KCl on ice for 10 min. The precipitate was centrifuged twice for 10 min in an Eppendorf microcentrifuge at 13,000 rpm. The DNA was precipitated with ethanol from the supernatant. The pellet was washed with 70% ethanol, and resuspended in buffer solution 20 mM Tris–HCl, 50 mM NaCl (pH = 7.4). The amount of DNA was determined spectrophotometrically. The quality of the DNA was checked by gel electrophoresis and by its absorption spectrum.

2.5. Absorption spectroscopy

Ground-state absorption spectra were recorded with 1 nm steps and 2 nm bandwidth using a Cary 4E (Varian, Mulgrave, Australia) spectrophotometer.

2.6. Decomposition of absorption spectra

Absorption spectra of TMPyP bound to DNA or NP were analyzed with the method described before [13]. Briefly, the spectral decomposition was performed for absorption spectra [$A(\lambda)$, absorbance versus wavelength] of the series of TMPyP–DNA and TMPyP–NP solutions with various base pair/porphyrin molar ratios (r). All of the spectra were analyzed in the 390–480 nm wavelength range.

For fitting we used the Gaussian multipeak fit routine from the Microcal Origin software. The error of the fit was determined as

$$\chi^2 = \frac{\sum_{\lambda=390}^{480} [A(\lambda)_{measured} - A(\lambda)_{calculated}]^2}{\sum_{\lambda=390}^{480} A(\lambda)_{measured}} \quad (1)$$

For further analysis of absorption spectra we made the following assumptions:

(1) All of the measured spectra [$A(\lambda)$] can be considered as a sum of three component spectra belonging to three possible porphyrin states, namely, to free [$A_F(\lambda)$], to externally bound [$A_E(\lambda)$] and to intercalated [$A_I(\lambda)$] porphyrins:

$$A(\lambda) = A_F(\lambda) + A_E(\lambda) + A_I(\lambda) \quad (2)$$

(2) The spectrum of each state [$A_X(\lambda)$] can be fitted as a sum of two Gaussians, a band [$A_x(\lambda)$] and its shoulder [$A'_x(\lambda)$]:

$$A_X(\lambda) = \frac{A_x}{w_x \sqrt{\pi/2}} \exp\left(\frac{-2(\lambda - \lambda_x)^2}{w_x^2}\right)$$
$$+ \frac{A'_x}{w'_x \sqrt{\pi/2}} \exp\left(\frac{-2(\lambda - \lambda'_x)^2}{w'^2_x}\right) \quad (3)$$

A_x, and A'_x are the total areas under the curves; λ_x, and λ'_x are the centers of the peaks; w_x, and W'_x are the full widths for the band and for the shoulder, respectively.