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ELECTROPHORETIC AND ELECTROOPTICAL STUDIES ON THE CONFORMATION AND SUSCEPTIBILITY TO PSORALEN CROSSLINKING OF MAGNETICALLY ORIENTED DNA

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ABSTRACT

Gel electrophoresis and electrooptical birefringence measurements were performed on the replicative form of bacteriophage ΦX-174 DNA subjected to orientation in a homogeneous stationary magnetic field. The conformation of this superhelical double-stranded form of DNA, and its sensitivity to intercalation and crosslinking by a psoralen derivative, were found to be unaffected by a 1 h exposure to a 2.15 Tesla field. In addition, no alteration was detected in the infectivity of the exposed ϕX-174 DNA in E. coli bacterial hosts.

INTRODUCTION

Magneto-orientation has been observed in several macromolecular and membrane systems that possess an anisotropic diamagnetic susceptibility. The most widely studied examples of this phenomenon are pigmented membrane systems, including retinal photoreceptor disk membranes (1,2) and chloroplasts (3,4), which can be oriented within seconds by a 1 Tesla (1 Tesla = 10^4 Gauss) magnetic field (5). The diamagnetic anisotropy exhibited by these systems has been attributed to the planar chlorophyll ring in the case of chloroplasts (4,5), and to the planar peptide bonds (7,8) and aromatic amino acid residues (9) of rhodopsin molecules in the disk membranes of retinal rods. Another biologically important macromolecule that has been demonstrated by optical birefringence measurements to orient in a strong stationary magnetic field is DNA (10,11). The magnetically-induced orientation of DNA is attributable to the diamagnetic anisotropy of its constituent planar nucleotide bases (10).

Studies of magneto-orientation phenomena have been focused primarily on its use as a method for analyzing the structural arrangement of membrane-bound photopigments such as chlorophyll and rhodopsin (4-6,9), and as a technique for determining the extent of local order within long flexible polymers such as DNA (10) and fibrin (12). From the viewpoint of assessing potential magnetic field effects on biomolecular systems with a high degree of diamagnetic anisotropy, it is also of interest to study magneto-orientation effects on the conformation of anisotropic macromolecules, including the evaluation of irreversible structural changes and their functional consequences. In the studies reported here, two independent physicochemical techniques were used to determine

whether exposure to a 2 T field alters the superhelical density of covalently-closed, double-stranded DNA from the bacteriophage ϕX-174. In addition, the occurrence of irreversible alterations in the biological activity of phage DNA subjected to magnetic orientation was tested by means of transfection assays in bacterial hosts.

MATERIALS AND METHODS

Bacteriophage DNA and psoralen

The double-stranded replicative form (RF I) of bacteriophage ϕX-174 covalently-closed DNA (MW = 3.6×10^6) was either supplied by Dr. William Taylor, Pennsylvania State University, or purchased from Bethesda Research Laboratories (Bethesda, Maryland). The DNA stock solutions were stored at -60 °C in 10 mM Tris, 1 mM Na_2EDTA. The psoralen derivative 4'-aminomethyltrioxalen (AMT) was purchased from Calbiochem-Behring (La Jolla, California). The concentrations of DNA and AMT in aqueous solution were determined spectrophotometrically from their absorbance at 260 and 250 nm, respectively.

Magnetic field exposure

Prior to exposure, DNA stock solutions were dialyzed overnight at 4 °C in a 0.5 mM Tris, 0.1 mM $MgCl_2$ solution (pH 7.9). A 0.6 ml sample of DNA (~20 μg/ml) was placed in a 1 cm square glass cuvette that was immersed in an ice bath contained within a cylindrical lucite vessel. The sample was exposed for 1 h to a 2.15 T stationary magnetic field produced by an electromagnet. The magnetic induction at the sample location was measured with a transverse Hall

effect probe attached to a Bell Model 620 gaussmeter (F.W. Bell Co., Orlando, Florida). The applied magnetic field was homogeneous to within 0.5% over the entire sample volume. Each control DNA sample was placed in the magnet gap in a glass cuvette immersed in an ice bath, and sham-exposed for 1 h. Following the magnetic field exposure (or sham exposure), a 10 µl aliquot of each DNA solution was removed for analysis by gel electrophoresis and the remainder of the solution was used for electrooptic birefringence measurements.

In experiments involving the crosslinking of DNA by AMT during the course of the magnetic field exposure, 0.6 ml samples with equimolar DNA (~20 µg/ml) but with varying concentrations of AMT were prepared to give graded AMT to DNA concentrations up to a maximum level of 460 moles AMT per mole DNA. During the second 0.5 h segment of the 1 h exposure (or sham-exposure) interval, the solution was irradiated with 360 nm light at an incident power density of 50 mW/cm^2. This procedure converted DNA-AMT monoadducts to diadducts that covalently crosslink the DNA (13).

Agarose gel electrophoresis

Vertical 1.4% (w/v) agarose gels with 40 mM sodium acetate, 1 mM Na_2EDTA electrophoresis buffer (pH 7.9) were run for 16 h in an applied electric field of 4 V/cm. Each well contained 10 µl of a DNA test sample (~0.2 µg DNA) combined with 3 µl of a 25% (v/v) aqueous glycerol solution. The gels were stained for 1 hr with 0.5 µg/ml ethidium bromide dissolved in the electrophoresis buffer solution. They were then illuminated with a UV light source, and the ethidium bromide fluorescence was filtered to remove wavelengths below 560 nm. The gels were photographed and traced with an RFT densitometer (Transidyne,

Ann Arbor, Michigan). Areas under the peaks of the densitometer traces were calculated by using an on-line PDP-8 computer program via an interactive terminal (14).

Electrooptical birefringence

DNA samples were subjected to 200 μs, 9.25 kV/cm square-wave pulses in a computerized electrooptic (EO) spectrometer described elsewhere (15). Birefringence induced in the DNA solution by the applied electric field pulse was measured as a light signal on an EMI 98568 phototube after passage through an optical system containing nearly-crossed Nicol prisms and a quarter-wave plate. The transient signal was captured in a Biomaton 805 recorder and read into a PDP 11/10 on-line computer equipped with an AED 2500 flexible disk storage system. Computer programs were developed to analyze EO signal amplitudes and to fit the decay portion of each curve to a sum of three exponentials by means of a nonlinear least-squares algorithm. In equation form, the amplitude S(t) was represented as

$$S(t) = \sum_{i=1}^{3} a_i \, e^{-t/\tau_i}$$

where each coefficient, a_i, is the fraction of the molecular population with the relaxation time τ_i. The signal to noise ratio in the EO signal amplitude limits the fit of the decay curve to three exponential terms. The three individual relaxation times represent a parametric fit of the decay portion of the EO signal, and they cannot be correlated directly with specific macromolecular motions during the relaxation process. Nevertheless, changes in the values of τ_i and a_i provide a qualitative picture of the changes in macromolecular size and flexibility that result from a structural perturbation.

The three relaxation times obtained for each fit were used to compute the mean relaxation time, $\bar{\tau}$, as the weighted sum of the three τ_i values:

$$\bar{\tau} = \left(\sum_{i=1}^{3} a_i \tau_i\right) / \left(\sum_{i=1}^{3} a_i\right)$$

Bioassays for DNA infectivity

Transfection assays were carried out with ϕX-174 DNA samples to which no AMT was added. Exposed and control samples of DNA were tested for infectivity proficiency in Escherichia coli AB 1157 by methods described elsewhere (16).

RESULTS AND DISCUSSION

Electrophoretic measurements

Electrophorograms and densitometer traces are shown in Figure 1, panel A, for control ϕX-179 DNA and for DNA that was exposed to a stationary 2.15 T magnetic field for 1 h. It is evident for both samples that a major fraction (~85%) of the DNA is in the RF I form, which has approximately 20 negative superhelical turns (17). The remainder of the DNA molecules in the control and magnetic field treated samples are predominantly in the open circular RF II form, which contains one or more single-strand breaks and has a significantly lower mobility than the RF I species. The minor peaks with an intermediate mobility between the maximally supercoiled RF I species and the open circular RF II species represent DNA molecules with decreasing superhelical densities. From a quantitative comparison of the areas under the peaks in the exposed and control DNA electrophorograms, no evidence was obtained for a

magnetic field effect on the relative proportions of any of the conformationally-distinct DNA species.

In Figure 1, panel B, electrophorograms are shown for ΦX-174 DNA samples that were crosslinked with AMT (368 moles AMT per mole DNA) by applying 360 nm irradiation during the final 0.5 h segment of a 1 h exposure (or sham exposure) to a 2.15 T magnetic field. It is clear from the densitometer traces for both the control and exposed samples that crosslinking by AMT at this high concentration produced single-strand breaks and a resultant conversion of the ΦX-174 DNA predominantly to an RF II form. At much lower AMT concentrations, the conversion of RF I to RF II is less and crosslinking results primarily in a decrease in the number of superhelical turns, probably due to partial unwinding of the DNA double helix. Control experiments demonstrated that the addition of AMT alone, or the application of 360 nm light in the absence of AMT, does not cause changes in superhelical RF I DNA. In replicate experiments, no difference between the electrophorograms for control and magnetic field treated DNA samples was observed with concentrations of 0, 92, 184, 276, 368 or 460 moles AMT per mole DNA.

Electrooptical studies

EO measurements of relaxation times and population parameters were made for ΦX-174 DNA samples that were exposed to a 2.15 T stationary magnetic field for 1 h, and for control samples (Figure 2). Experiments were conducted either with native DNA, or with DNA in which AMT crosslinks were introduced during the magnetic field exposure. Control experiments demonstrated that AMT concentrations up to 460 moles per mole DNA (the maximum level used in the EO

studies) had no effect on the τ_i values when crosslinking was not introduced by UV irradiation.

The decay of the EO signal amplitude for native ϕX-174 DNA can be represented as a sum of three exponentials with characteristic relaxation times of 12, 84 and 640 μs. The shortest relaxation time, τ_1, had an identical value of 12 μs for the native and the crosslinked DNA samples. Based on studies in our laboratory with DNA fragments, τ_1 can be attributed to internal molecular motion. The two longer relaxation times, τ_2 (= 84 μs) and τ_3 (= 640 μs), which are associated with molecular rotation, are sensitive to AMT crosslinking, as evidenced by a ~30% increase in τ_2 and τ_3 for AMT concentrations in the range 370 to 460 moles per mole DNA. The population parameters a_2 and a_3 also changed as a function of AMT concentration, with a_2 decreasing and a_3 increasing. From an intercomparison of the EO and gel electrophoresis measurements, this shift in population parameters can be attributed to a transition of the DNA from the RF I to the RF II form as a result of AMT crosslinking. The mean relaxation time $\bar{\tau}$, which represents a weighted sum of the three τ_i values, steadily increased from a value of 130 μs at zero AMT concentration to 260 μs at an AMT concentration of 460 moles per mole of DNA. This increase in $\bar{\tau}$ is attributable both to an increase in τ_2 and τ_3, and to an increase in the population of molecules with a characteristic relaxation time τ_3. The data presented in Figure 2 clearly demonstrate that the individual relaxation times τ_i, and the mean value $\bar{\tau}$, were not affected by exposure to a 2.15 T field, or by application of the field during the UV-induced AMT crosslinking procedure.

DNA infectivity assays

The results of ϕX-174 DNA transfection assays with E. coli hosts did not demonstrate any effect of a 1 h exposure to a 2.15 T field (Table I). In three replicate experiments, no statistically significant difference was observed in the number of plaque-forming units in control and exposed DNA samples. This result is in agreement with the lack of a stationary magnetic field effect on the infectious properties of purified tobacco mosaic virus DNA in solution as reported by Maj and Dutczak (18).

Summary and conclusions

The experimental results reported here demonstrate that the torsional stress experienced by ϕX-174 DNA during magnetic orientation does not lead to irreversible alterations in molecular structure or function. The accessibility of pyrimidine residues to binding and crosslinking by a psoralen derivative was also found to be unaltered by magneto-orientation, thereby indicating that this process does not affect internucleotide spatial relationships within the DNA double helix. These findings suggest that the genetic consequences of exposure to large stationary magnetic fields should be minimal, and this expectation is supported by the results of several recent studies with plant (19), insect (20) and animal (21) systems.

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TABLE I

INFECTIVITY OF ΦX-174 DNA IN E. COLI[a]

Experiment number	Experimental conditions	Average plaques per 100 mm dish ± 1S.D.	Plaque-forming units per ml of DNA sample
1	Control	42 ± 3	5.0×10^5
	2.15 T, 1 h	41 ± 3	4.9×10^5
2	Control	78 ± 6	4.7×10^5
	2.15 T, 1 h	77 ± 6	4.6×10^5
3	Control	106 ± 7	1.6×10^7
	2.15 T, 1 h	110 ± 6	1.7×10^7

(a) The plaque-forming units (PFU) per ml of DNA solution are defined as the product of the average number of infectivity centers (plaques) per dish and the dilution factor for the DNA sample prior to addition to the dishes. The DNA stock solutions used in experiments 1 and 2 had been stored at - 60 °C for several months, whereas the solution used in experiment 3 was from a new DNA stock. It is apparent from the PFU assay that the infectivity of the latter DNA stock was approximately 35 times greater than that of the DNA stocks used in experiments 1 and 2.

FIGURE LEGENDS

Figure 1 Agarose gel electrophoresis profiles are shown for control ΦX-174 DNA samples, and for samples exposed to a 2.15 T magnetic field for 1 h. For both gels, electrophoretic migration of the DNA molecules was from left to right. The gel profiles in panel A show the dominant RF I peak of native superhelical ΦX-174 DNA, and a minor peak associated with a small population of open circular RF II molecules. In the ΦX-174 DNA gel electrophoresis profiles shown in panel B, both the control and exposed samples were crosslinked with AMT (368 moles AMT per mole DNA), which at this high concentration converts a major fraction of the DNA to an RF II form.

Figure 2 The relaxation times (τ_i) and population parameters (a_i x 100%) are shown as a function of the molar AMT/DNA ratio for both control and exposed (2.15 T, 1 h) ΦX-174 DNA samples. The data for control and exposed samples are represented by circles and crosses, respectively. In addition, the mean relaxation time $\bar{\tau}$, calculated as a weighted sum of the three τ_i values, is shown as a function of the molar AMT/DNA ratio. Each τ_i represents the average value obtained in three independent measurements. For all of the plotted data points, the standard deviation was ≤5% of the mean value.

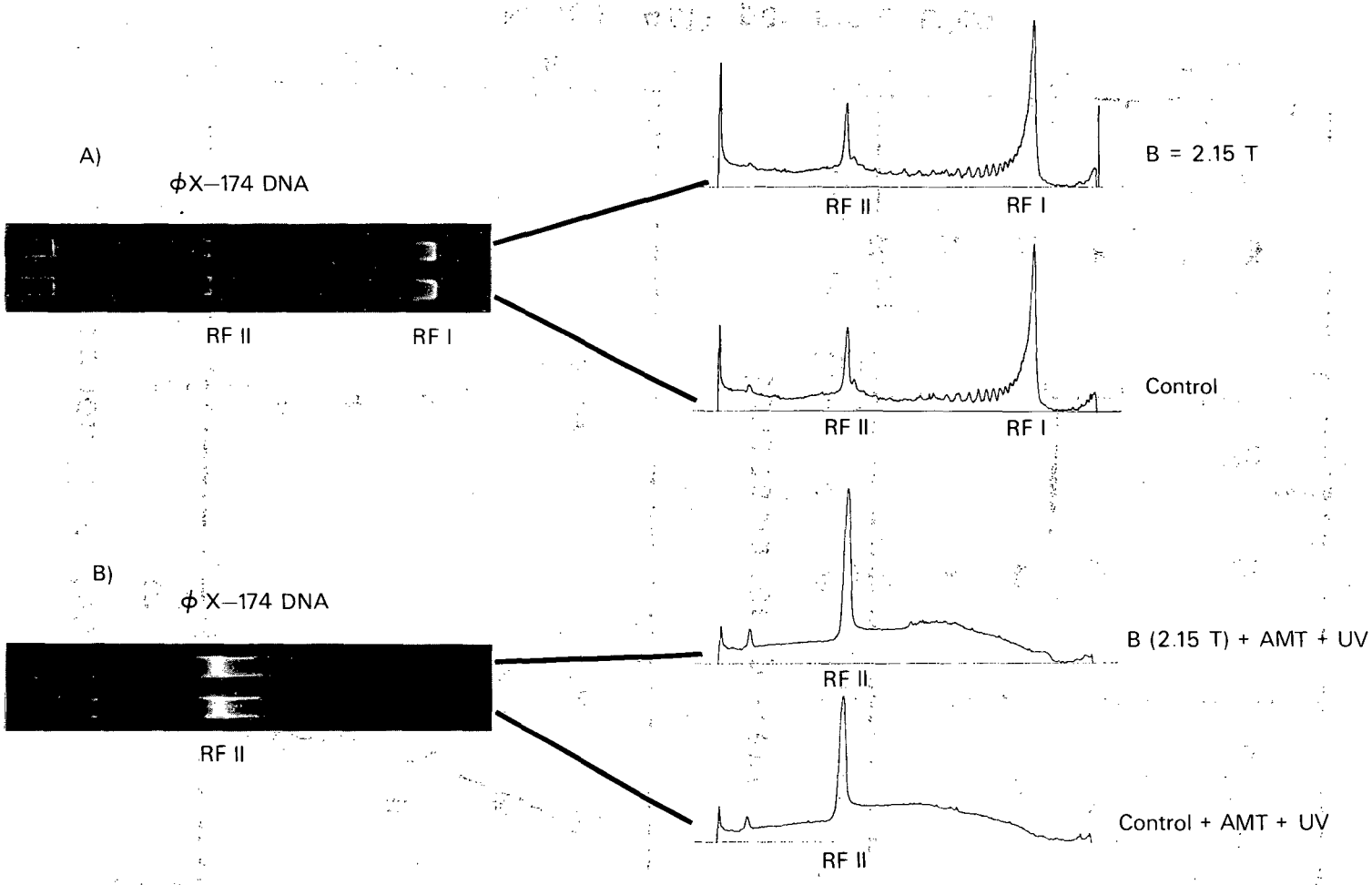
A)
ϕX–174 DNA
RF II
RF I
RF II
RF I
B = 2.15 T
RF II
RF I
Control
B)
ϕX–174 DNA
RF II
RF II
B (2.15 T) + AMT + UV
RF II
Control + AMT + UV
XBB 802-14840

FIGURE 1

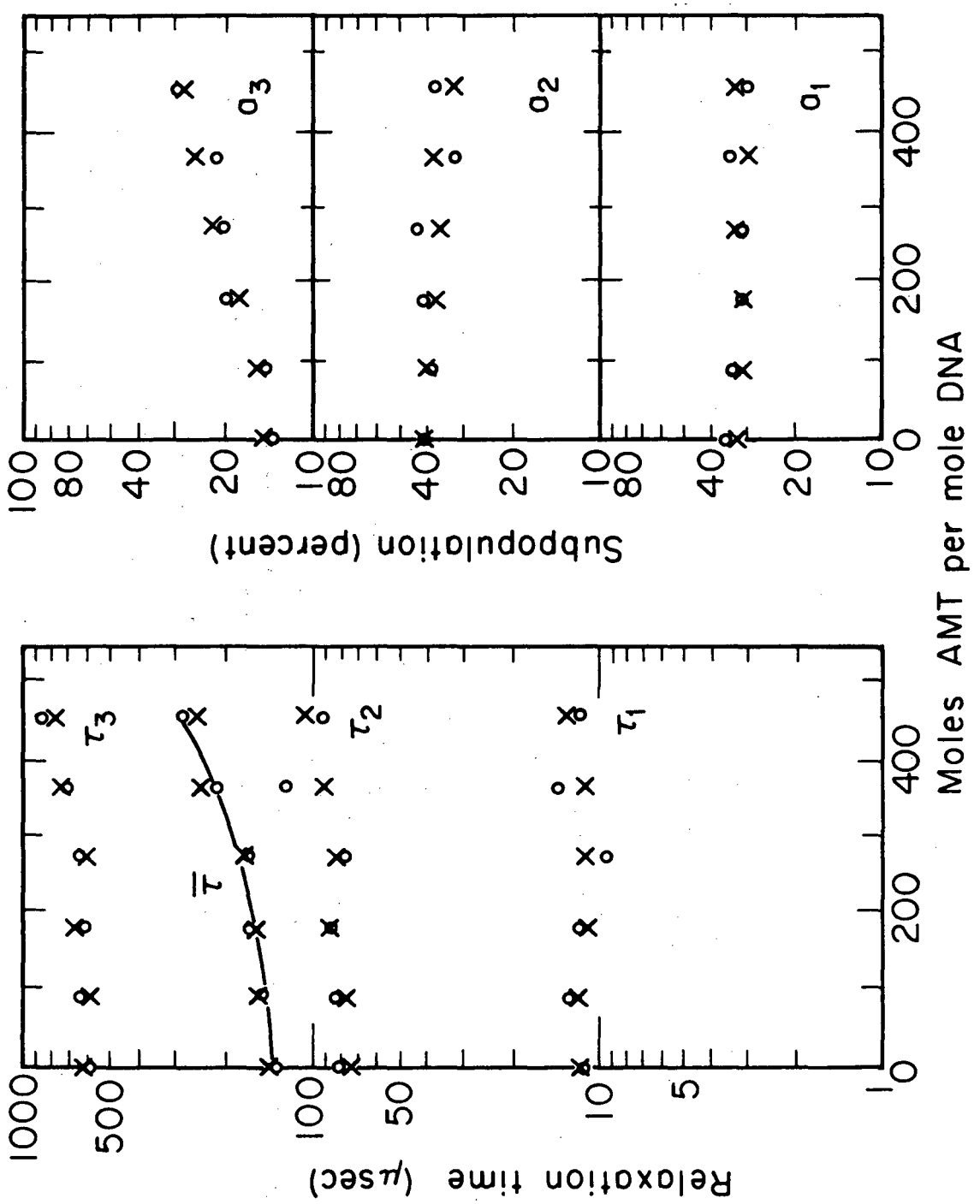
Relaxation time (μsec)
1000
500
100
50
10
5
1
0
200
400
Moles AMT per mole DNA
τ3
τ2
τ1
τ̄
Subpopulation (percent)
100
80
40
20
10
a3
a2
a1
XBL8011-3843

FIGURE 2

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