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ELECTROCHEMICAL STUDY OF MONONUCLEOSOME

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Summary : The electrochemical behavior of mononucleosome has been studied in parallel with circular dichroism and trypsin degradation. In mononucleosome, DNA is never adsorbed at the electrode surface. A model of flat adsorption of the mononucleosome *via* histones is proposed.

INTRODUCTION

There is now considerable evidence that eukaryotic chromatin consists of a repeating subunit (mononucleosome) composed of the histone octamer associated with approximatively 140 base pairs of DNA. In native chromatin mononucleosomes are joined by "linker" DNA region associated with the H_1 histone (for review, see 1-3).

At the present time, a great deal of work has been devoted to the electrochemical behavior of native and denatured DNA (4-11). In this paper the influence of the mononucleosome structure on the DNA electrochemical response is reported. In parallel to circular dichroism and trypsin digestion, the behavior of mononucleosome preparation has been studied by polarographic and voltammetric methods.

METHODS

Nucleosome isolation : Chromatin extraction from chicken erythrocytes was essentially performed as described by Olins *et al.* (12). Nuclei were incubated with 35 µg/ml of micrococcal nuclease (Worthington Biochemical corp.) for 4 hours. As a substitute to sucrose gradient centrifugation, mononucleosome fraction was obtained by gel permeation on Biogel A 5m (13).

KCl soluble mononucleosomes were prepared by dialysis against 0.1 M KCl buffer (12) followed by centrifugation. One ml aliquots of the supernatant were stored frozen at -70°C.

Trypsin digestion : Mononucleosomes (OD_{260} = 4) were suspended in 0.1 M NaCl, 5 mM sodium acetate, 5 mM Tris, pH = 8. Digestion was allowed to proceed at 25°C for various times and trypsin concentrations. At selected times, aliquots

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were removed and the digestion was stopped by addition of an excess of trypsin inhibitor. We are indebted to Dr. M. Champagne for the generous gift of total histone fraction.

Isolation of mononucleosome DNA : After protein digestion by proteinase K and phenol extraction, mononucleosome DNA was precipitated by ethanol.

Gel electrophoresis : DNA was characterized with respect of molecular weight by 6 % polyacrylamide gel electrophoresis. Gels were stained with ethidium bromide (14).

Electrophoresis of histones were performed on 15 % polyacrylamide slab gels in presence of sodium dodecyl sulfate (15). Mononucleosomes were dissociated in a sample buffer containing sodium dodecyl sulfate and applied directly to the gel. The gels were stained with coomassie blue. The surface of the peaks corresponding to each histone was determined with a Joyce MK III microdensitometer. Linear variation of the peak surface with histone concentration was observed in the range of used concentration.

Circular dichroism : Circular dichroism spectra were recorded with a Roussel Jouan dichrograph III, using a 10 mm cell at room temperature. The molar extinction coefficient at 260 nm of DNA (ε_P = 6600 M^{-1} cm^{-1}) was assumed in calculation of the molecular ellipticity per mole of nucleotides.

Electrochemistry : Electrochemical measurements were carried out with a polarographic system SOLEA including a potentiostat PRT 30.01 connected with a unit PRG 3 for phase sensitive ac polarography and a PRG 4 unit allowing pulse polarography and sweep voltammetry measurements. A three electrodes system was used. The electrode potentials were referred to the saturated calomel electrode (SCE). The experiments were performed in 0.1 M NaCl with 5 mM sodium acetate and 5 mM Tris as buffer. pH was adjusted with HCl.

RESULTS

Mononucleosome characteristics

Due to the necessity of working at a relative high ionic strength with polarographic techniques, only the KCl soluble fraction of mononucleosome preparations was studied.

The four main histones were present in the KCl soluble fraction in a relative molar ratio of 1 ± 0.15 (Fig. 1), H_1 and H_5 were absent (12). In agreement with other reports (16-18), the mononucleosome has a maximum ellipticity at 284 nm $(\theta)_{284}$ = 2100 ± 100 deg cm^2 $dmol^{-1}$ (Fig. 2). There is a shoulder at 275 nm $(\theta)_{275}$ = 1300 ± 200 and a negative band at 295 nm $(\theta)_{295}$ = - 200 ± 100. As already shown (19) no change in ellipticity was observed between pH = 5.5 and 8.5.

ac Polarography of mononucleosome

ac polarography was the more efficient electrochemical method for the study of mononucleosomes. Mononucleosomes were adsorbed at mercury drop elec-

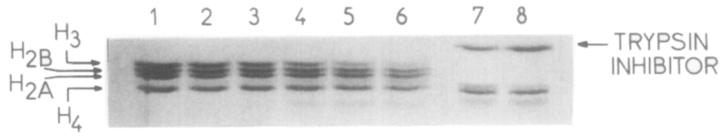


Fig. 1 - Gel electrophoresis of histones from mononucleosome.
1 Mononucleosome histones.
2-8 Trypsin hydrolyzed mononucleosome histones versus time
5 ng/ml, 2 : 10 min., 3 : 30 min., 4 : 90 min., 5 : 150 min., 6 : 190 min.
1 µg/ml, 7 : 30 min., 8 : 60 min.

trode. This was proved by the decrease of the out of phase component of the current as compared to the supporting electrolyte alone (Fig. 3). The polarogram of the mononucleosome solution merges with the supporting electrolyte near - 1.6 V. Whatever the pH (between 5.6 and 8.5) no peaks were visible on the curves. Peaks were also absent on the polarograms recording the in phase component of the current (Fig. 4A). Test experiments with total chromatin and polynucleosomes gave identical results. Peaks were only observed in medium where the dissociation of mononucleosomes could occurred (Fig. 4 B) (15,20).

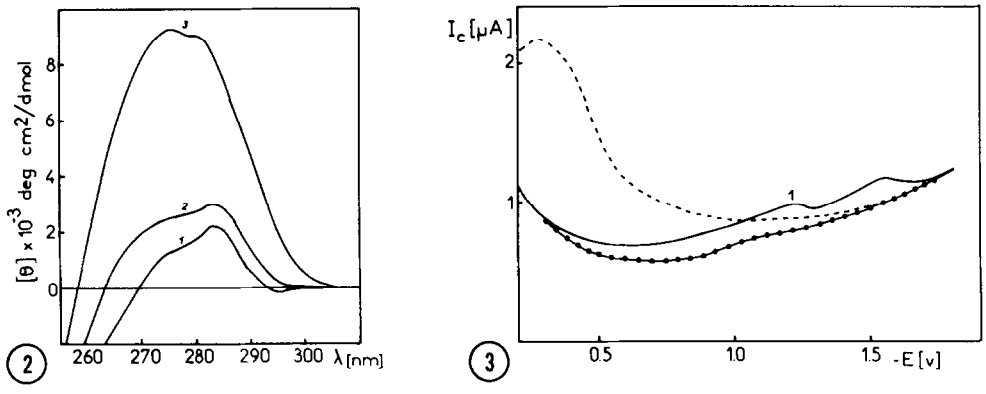
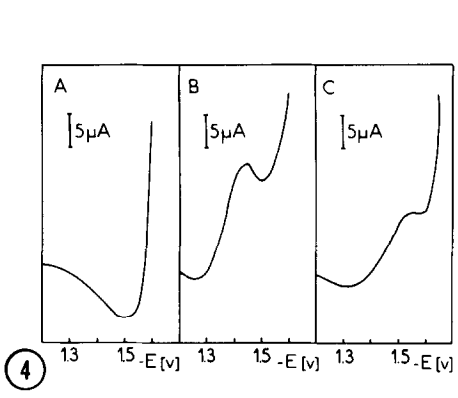


Fig. 2 - Circular dichroism spectra. NaCl 0.1 M, sodium acetate 5 mM, Tris 5 mM, pH : 8.
1 Mononucleosome
2 Trypsin digested mononucleosome, 1 µg/ml trypsin, 60 min. (see Fig. 1)
3 Mononucleosome DNA.

Fig. 3 - Out of phase component of the ac polarographic current. Superimposed frequency 78 Hz, current amplitude 10 mVpp, drop time : 7.6 sec., NaCl 0.1 M, pH : 8
——— mononucleosome DNA OD_{260} = 4
●—● mononucleosome OD_{260} = 4
---- supporting electrolyte.



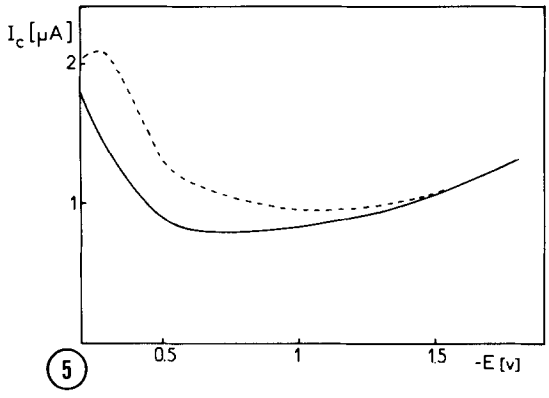


Fig. 4 - In phase component of the ac polarographic current. Superimposed frequency 78 Hz, current amplitude 10 mVpp, drop time : 3 sec., pH : 8.
A : mononucleosome NaCl 0.1 M
B : mononucleosome NaCl 2 M
C : mononucleosome DNA NaCl 0.1 M.

Fig. 5 - Out of phase component of the ac polarographic current. Superimposed frequency 78 Hz, current amplitude 10 mVpp, drop time : 7.6 sec. NaCl 0.1 M, pH = 8.
——— total histone fraction 100 μg/ml
---- supporting electrolyte.

Peaks similar to those already observed with higher molecular weight DNAs (4) were obtained with mononucleosome DNA (Fig. 3 and 4C) but peak 1 was much smaller. The second peak recorded on the in phase (Fig. 4 C) and out of phase (Fig. 3) polarograms was different in shape and potential from the characteristic one of native or denatured DNA (4). This behavior might be related to the fast opening of this very low molecular weight DNA at the electrode surface (unpublished results).

In the same experimental conditions, histones alone were adsorbed in a similar range of potential (Fig. 5).

Trypsin digestion of mononucleosome

Histone degradation was followed by electrophoresis and circular dichroism. With low concentration of trypsin (5 ng/ml) histones were slowly hydrolyzed (Fig. 1). Relative variation of histone bands as a function of time was plotted in parallel with the variation of the molecular ellipticity at 284 nm (Fig. 6).

A linear decrease of H_3 is observed, the other histones being less rapidly hydrolyzed. Similar results has already been observed (21-23). The

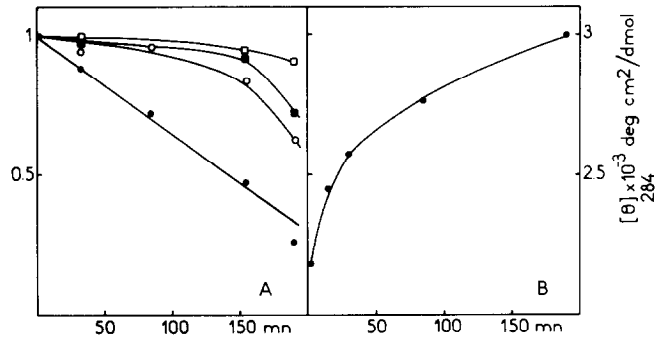


Fig. 6 - A : Relative variation of histone versus time of hydrolysis by trypsin (5 ng/ml). ●—● H_3, ○—○ H_{2A}, ■—■ H_{2B}, □—□ H_4
B : Variation of the molar ellipticity at 284 nm versus time of hydrolysis by trypsin (5 ng/ml).

hydrolysis of histones induced a molecular ellipticity increase which rapidly reached a plateau at 3000 ± 200 deg cm^2 $dmol^{-1}$. At high trypsin concentration, total blotting out of the histone bands can be obtained (Fig. 1) but the molecular ellipticity is no more modified (Fig. 1 and Fig. 2).

Whatever the level of histone degradation, no peak is recorded in ac polarography or with other polarographic techniques.

DISCUSSION

According to the generally accepted model (21-24), the mononucleosome is a flat particle of about 110x110x60 Å. The DNA of 140 base pairs forms nearly two loops on the outside of the histone octamer. The exact position of the histones are not yet established. However it is known that large parts of them are accessible as shown by extensive trypsin degradation without dramatic change in the structure. This trypsin degradation of histones induces the lost of the NH_2-terminal part and the hydrolysis of many peptidic bonds in the internal part of the protein (25-27).

The purpose of this work was to study the influence of the histones on the electrochemical response of the DNA. It has been shown that with intact or hydrolyzed mononucleosomes, DNA does not result in the appearance of an electrochemical signal. In order to explain these experimental results, three assumptions can be put forward.

1) The presence of histones stabilizes DNA as shown by the increase of the melting temperature (1-3). On the other hand, the base adsorptions at the electrode surface can only occur after the opening of the double helix (5-7). The increased stability of DNA in the mononucleosome cannot explain our results since very stable DNAs (like G-C rich DNAs at high ionic strength) are easily opened at the electrode and an electrochemical signal appears.

2) At the interface the mononucleosome structure is destroyed, histones and DNA are allowed to be adsorbed at the surface but histones are more strongly adsorbed and prevent DNA adsorption. In medium conditions where DNA and histones are already dissociated (2 M NaCl), this competition is effective but the peaks of DNA are still observed. Therefore one can assume that the association between DNA and histones is preserved at electrode surface

3) The mononucleosome, the structure of which is largely preserved, is adsorbed with an orientation which prevents DNA adsorption. It should be possible to assume that only the NH_2-terminal part of the histones is at the periphery of the particule and prevents DNA adsorption. The absence of variation in electrochemical signal with trypsin degraded mononucleosomes rules out this interpretation.

The experimental results can be explained assuming the mononucleosome adsorption at the electrode by its flat surface (Fig. 7). In this scheme,

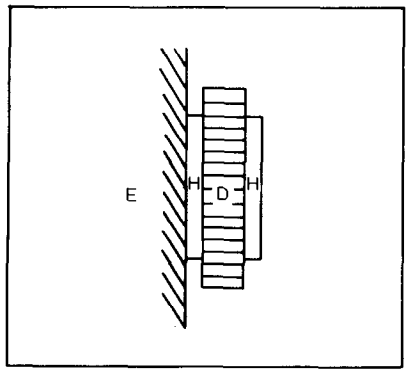


Fig. 7 - Schema of adsorption of mononucleosome.
E : electrode ; H : histone octamer ; D : DNA loops.

histones are adsorbed at the electrode surface and the double helix of the DNA is outside of the double layer. It is admitted that histone octamer has a global diameter larger than the DNA loops. If no strong modification of structure is induced by adsorption (this is likely due to the high stability of the octamer), the DNA cannot come into contact with the electrode. In our experimental medium, the double layer of the electrode has a thickness of about 10 Å, therefore one can assume that the surface of the histone octamer exceed the DNA loops of more than this distance. Trypsic degradation of histones does not modify the relative position of histones and DNA in the mononucleosome.

CONCLUSION

DNA in mononucleosome is not accessible at the electrode surface in spite of the fact that the particle is adsorbed. This result can be explained by the preferential adsorption of the histones which are accessible in the flat part of the mononucleosome and without strong modification of the structure. In agreement with the results of trypsin digestion, this electrochemical study confirms the very stable structure of mononucleosome. It also gives some information on the relative position of DNA and histones in mononucleosome particle.

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