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In-situ FTIR study on adsorption and oxidation of native and thermally denatured calf thymus DNA at glassy carbon electrodes

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Abstract

In-situ Fourier transform infra-red (FTIR) spectra of native and thermally denatured calf thymus DNA (CT DNA) adsorbed and/or oxidized at a glassy carbon (GC) electrode surface are reported. The adsorption of native DNA occurs throughout the potential range ($-0.2 \sim 1.3$ V) studied, and the adsorbing state of DNA at electrode surface is changed from through the C=O band of bases and pyrimidine rings to through the C=O of cytosine and imidazole rings while the potential shifts negatively from 1.3 V to -0.2 V. An in-situ FTIR spectrum of native CT DNA adsorbed at GC electrode surface is similar to that of the dissolved DNA, indicating that the structure of CT DNA is not distorted while it is adsorbed at the GC electrode surface. In the potential range of $-0.2 \sim 1.30$ V, the temperature-denatured CT DNA is adsorbed at the electrode surface first, then undergoes electrochemical oxidation reaction and following that, diffuses away from the electrode surface.

Keywords: CT DNA; Thermally denatured; In-situ FTIR spectroelectrochemistry

1. Introduction

Nucleic acids are electroactive species producing reduction and oxidation signals in various polarographic and voltammetric techniques, such as: alternating current polarography, cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, etc. Electrochemical methods have been successfully applied in analysis of nucleic acids and their components. Reduction of nucleic acid at negative potentials takes place at mercury electrodes, whereas oxidation is mea-

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sured mainly at solid electrodes, such as glassy carbon (GC), graphite, gold electrodes, etc. [1–10]. The difference in reduction signals between single-strand and double-strand DNA in polarography is utilized in DNA structure studies. Recently, Wang, Tomschik et al. showed that the sensitivity of DNA and RNA analysis can be greatly increased if the constant current chronopotentiometry and voltammetry with sophisticated baseline correction is used, and these techniques are then applied in the development of the DNA hybridization sensors and ultratrace measurement of nucleic acids [11–14]. Nucleic acids have many structurally sensitive Fourier transform infra-red (FTIR) bands and these bands can follow the progress of conformational change or interactions. Combining spectroscopy and electrochemistry, the in-situ FTIR method becomes a powerful tool for study of reactions on the electrode surface since the pioneering work at the beginning of 1980s [15]. In-situ FTIR allows 'good' and 'intense' FTIR spectra to be obtained from minimal quantities of substance. In addition to molecular vibration spectroscopic parameters, in-situ FTIR can give information about the interaction of the molecule with electrode surface and is suitable for study of DNA.

Calf thymus DNA (CT DNA) is a type of nucleic acid with double strands. Native CT DNA has few of guanine and adenine residues, which cannot easily be oxidized at a GC electrode [6,9]. In this work, electrochemical adsorption and oxidation of native CT DNA and temperature-denatured CT DNA at GC electrode at room temperature were investigated by voltammetric and in-situ FTIR spectroelectrochemical techniques. The results indicate that: (i) native CT DNA cannot be oxidized but only adsorbed at the GC electrode surface, and the adsorbing state changes with adsorption potential; (ii) temperature-denatured CT DNA can be adsorbed and oxidized, and the oxidation process is completely irreversible.

2. Experimental

CT DNA (sodium salt) was obtained from Sigma Chemical Co. (USA) and used without further purification. DNA stock solution was prepared by the following procedure: DNA ($\sim$4 mg) was weighed and dissolved directly in 10 ml CH_3COONa–CH_3COOH buffer, stored at 4°C for 24 h with gentle occasional shaking to obtain a homogeneous sample. Temperature-denatured DNA was made by the following procedure: CT DNA stock solution was incubated at 100°C for 15 min, under this condition DNA bases were decomposed, then cooled rapidly to room temperature. All of the DNA stock solutions were stored at 4°C and used within 2 days.

Buffer solutions with ionic concentration of 0.1 M and 0.2 M were prepared by analytical grade reagents (CH_3COOH, CH_3COONa and NaOH) and doubly purified water from the Milli-Q system, which were used in all experiments. The pH value of each solution was measured before use.

Voltammetric signal was recorded with a Model 630 Electrochemical Analyzer (CH Instrument, USA) and carried out with a home-made electrochemical cell. The cell design was similar to that previously reported [16]. The working electrode was a glassy carbon disk (0.75 cm^2), the reference electrode was a saturated calomel electrode (SCE) and the counter electrode was a platinum disk (1 cm^2). The working electrode was polished to a mirror finish with 1, 0.3 and 0.05 μm α-Al_2O_3, respectively, and rinsed in an ultrasonic bath with pure water to move any adherent particles.

In-situ FTIR spectroscopy was carried out with Nicolet 520 FTIR spectrometer (USA) equipped with a home-made electrochemical cell [16] and a Model 630 Electrochemical Analyzer, a DTGs detector and Omnic E.S.P. software. The external reflection system was constructed from a variable angle specula reflectance attachment (Spectra Tech Inc.). The IR ray was adjusted so as to assure that the incidence angle at the interface of electrode/solution was 66°. The spectra were collected using a potential modulation mode to ensure optimal S/N ratios [15,16]. Three hundred interferometric scans were accumulated and averaged for a spectrum. The in-situ FTIR spectrum of each potential was collected after holding at the potential for 10 min. Spectral resolution was 2 cm^{-1}.

Circular dichroism (CD) and UV spectra were measured by a 62A DS CD spectrometer (AVIV, USA) using a 1.0-cm path length rectangular quartz cell at 25°C controlled by a thermoelectric cell holder (AVIV).

All potentials reported here were vs. the SCE.

3. Results and discussion

3.1. CD spectra of the CT DNA

CD spectra of the native and temperature-denatured CT DNA are shown in Fig. 1. The spectrum of native CT DNA (Fig. 1a) has one positive band at 276 nm due to base stacking and one negative band at 245 nm due to helicity, which is characteristic of DNA in the right-handed B form [17]. CD spectrum of the temperature-denatured CT DNA (Fig. 1b) shows that the intensity of positive band at 276 nm increases and that of negative band at 245 nm decreases. UV spectra of the native and temperature-denatured CT DNA are shown in insert in Fig. 1, the absorbance at 259 nm increases while CT DNA is denatured. The results of CD and UV spectra indicate that the structure of CT DNA is changed by temperature denaturalization.

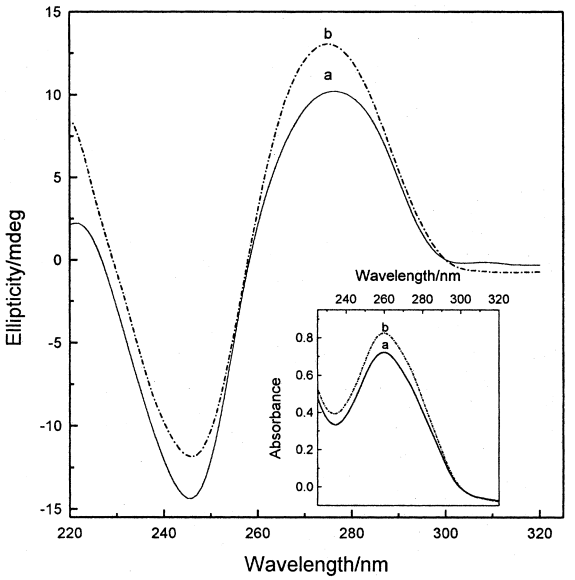

Fig. 1. CD spectra of (a) native CT DNA in (b) temperature-denatured CT DNA 0.1 M CH_3COOH–CH_3COONa (pH 6.68). Insert: UV spectra of (a) native CT DNA in (b) temperature-denatured CT DNA 0.1 M CH_3COOH–CH_3COONa (pH 6.68).

3.2. Cyclic voltammetric measurement

Shown in the insert of Fig. 2, there is no clear peak, but a thick background in cyclic voltammogram of native CT DNA in 0.2 M $NaCO_4$–$HClO_4$ buffer solution (pH 4.07), and the current becomes progressively lower with successive scans. It is identifies that the native CT DNA is difficult to be oxidized, but can be adsorbed at the electrode surface.

Cyclic voltammogram of the temperature-denatured CT DNA in the same solution is shown in Fig. 2. Two oxidation peaks at 0.835 and 1.165 V corresponded to the oxidation of guanine and adenine residues are obtained, respectively [6]. Their oxidation peak currents become progressively lower with successive scans. No corresponding reduction peaks occur, which shows that oxidation of the temperature-denatured CT DNA is irreversible.

Oxidation peak potentials of the temperature-denatured CT DNA in different pH solutions at GC electrode are also studied, and the results are listed in Table 1.

Both of the two peak potentials are depended on solution pH with the relations of $Ep_1 = 1.081-0.0580$ pH for guanine residues and $Ep_2 = 1.428-0.063$ pH for adenine residues. The results are in accordance with a ratio of $2H^+/2e^-$ in the oxidative process of nucleic acid, as in the oxidation schemes for guanine and adenine previously proposed [6].

Among the nucleic acid constituents, only bases can be reduced and /or oxidized at the electrode surface. When CT DNA is incubated at 100°C, the temperature-denatured process takes place, while DNA bases decompose, resulting in regular double stranded DNA (ds DNA) macromolecules which change partly into small molecules by rupture of the hydrogen bonds and produce large quantities of single strand DNA (ss DNA). The

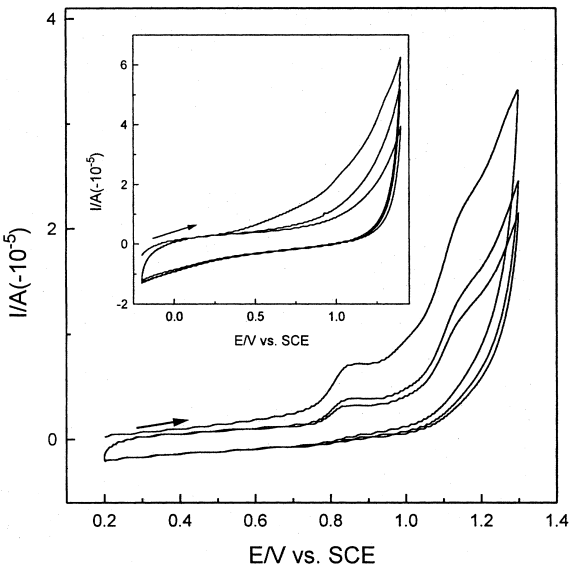

Fig. 2. Cyclic voltammograms of 0.4 mg ml^{-1} temperature-denatured CT DNA in 0.2 M CH_3COOH–CH_3COONa pH (4.07) buffer. Insert: cyclic voltammograms of 0.4 mg ml^{-1} native CT DNA in 0.2 M CH_3COOH–CH_3COONa pH (4.07) buffer. Scan rate, 5 mV s^{-1}.

double-strand structure does not resume to native CT DNA, while the temperature-denatured CT DNA solution is cooled rapidly. The secondary and tertiary structures of DNA are disturbed [9], so more fresh guanine and adenine residues are produced. Based on cyclic voltammetric experiments, it is identified that the native CT DNA has only few guanine and adenine residues, which are difficult to be oxidized, whereas the temperature-denatured CT DNA has more fresh guanine and adenine base residues that can easily to reach electrode surface, so the oxidative reaction occurs. As the adsorbed temperature-denatured DNA can form a DNA multilayer on the electrode surface [6,9] which impedes the oxidation product diffusing away from electrode surface as soon as potential scans, this leads to oxidation current decrease in successive scans.

Table 1
Oxidation peak potentials of the temperature-denatured CT DNA[a]

pH	4.07	5.52	6.68	7.50	8.10
Ep_1	0.835	0.763	0.691	0.649	0.609
Ep_2	1.165	1.09	1.01	0.952	0.920

[a] Buffer: 0.2 M CH_3COOH–CH_3COONa. Scan rate, 5 mV s^{-1}.

3.3. In-situ FTIR spectroscopy

FTIR spectroscopy is extensively used in the study of nucleic acid conformation and the mechanism of interaction with other compounds and substrate surface [18–26]. Nucleic acids show many structurally sensitive FTIR bands and these bands can follow the progress of conformational change or interactions. The main vibration bands from the PO_2^- group, the ribose moiety and the bases occur at 900 ~ 1800 cm^{-1} region [18–26]. A series in-situ reflection-absorption difference FTIR spectra of the native and temperature-denatured CT DNA at GC electrode surface are shown in Figs. 3–6. The potential (E_1) for each of the spectra is marked in the curve. The reference potential (E_2) of these spectra is set by experimental condition. In such spectra upwards point-

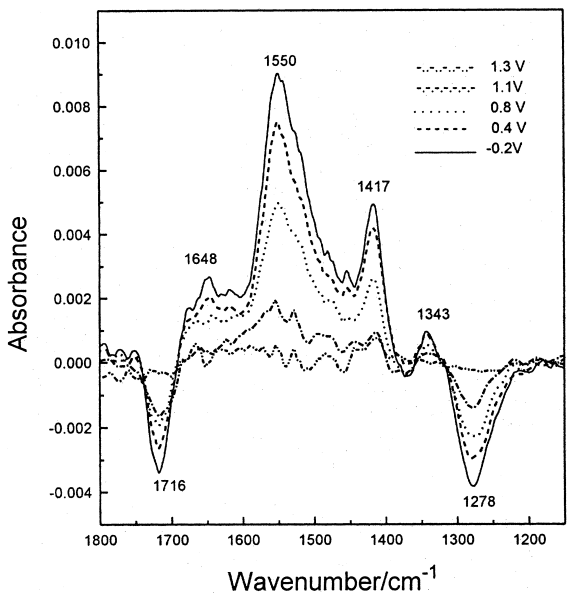

Fig. 3. In-situ FTIR spectra of 0.42 mg ml^{-1} temperature-denatured CT DNA in 0.1 M CH_3COOH–CH_3COONa solution (pH 6.68). Reference potential: 1.3 V.

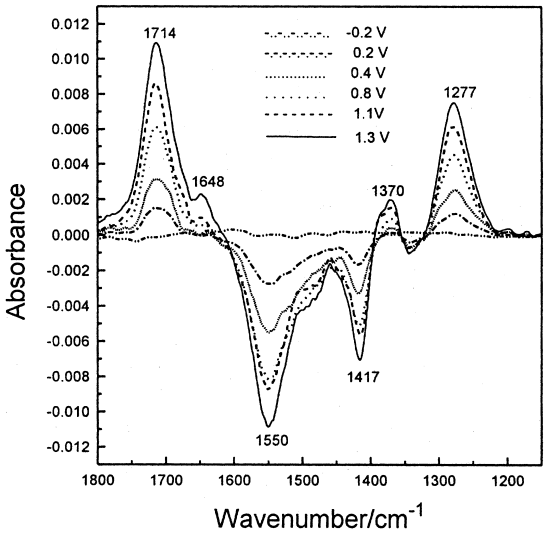

Fig. 4. In-situ FTIR spectra of 0.42 mg ml^{-1} temperature-denatured CT DNA in 0.1 M CH_3COOH–CH_3COONa solution (pH 6.68). Reference potential: −0.2 V.

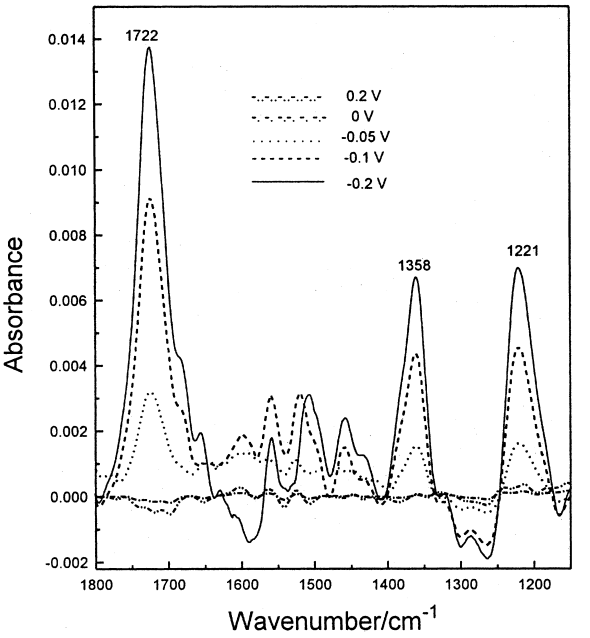

Fig. 5. In-situ FTIR spectra of 0.42 mg ml^{-1} temperature-denatured CT DNA in 0.1 M CH_3COOH–CH_3COONa solution (pH 6.68). Reference potential: 0.0 V.

ing (+) bands correspond to IR absorption by the species in excess at E_1, while downwards pointing (−) bands correspond to IR absorption by the species in reduce at E_1.

The in-situ FTIR spectra of native CT DNA varying with potential E_1 in the range of 1800 ~ 1100 cm^{-1} are shown in Fig. 3. E_2 is set at 1.3 V. Two downwards pointing (−) bands at 1716 (strong) and 1278 (strong) cm^{-1} and four upwards pointing (+) bands at 1648 (medium), 1550 (very strong), 1417 (strong) and 1343 (medium) cm^{-1}, which can be clearly discerned while the potential shifting negatively from 1.30 to −0.2 V.

As discussed in previous literatures [18–26], assignment of these bands are complicated by several factors including coupling between vibration modes and the interaction of DNA with water. On the basis of comparison of several previous report the assignment of four strong bands are shown in Table 2. The band at 1716 (−) cm^{-1} has been assigned to the in-plane stretching vibration mode of C=O of nucleic acid bases, and is very similar with the spectra in literature for aqueous solution of DNA. The band is strong and sharp, indicating that DNA is not

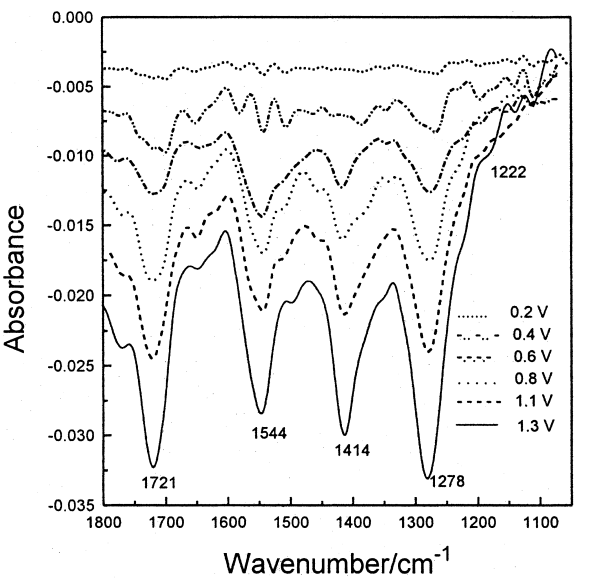

Fig. 6. In-situ FTIR spectra of 0.42 mg ml^{-1} temperature-denatured CT DNA in 0.1 M CH_3COOH–CH_3COONa solution (pH 6.68). Reference potential: 0.0 V.

significantly denatured and remains mainly under the B-form while CT DNA is adsorbed at electrode surface [20,25,26]. The band at 1550 (+) cm^{-1} has been assigned to the in-plane ring vibration mode of C–C and C=N, and the bending vibration mode of C–H and N–H. The band at 1417 (+) cm^{-1} has been assigned to the in-plane imidazole ring vibration, the band at 1278 (−) cm^{-1} has been assigned to the pyrimidine (mainly thymine) ring vibration. The results of in-situ FTIR show that the intensities of C=O and pyrimidine ring decrease, and the intensities of C–H, N–H, C=N and imidazole ring increase at the electrode surface while the potential shifts negatively.

The in-situ reflection-absorption difference spectra in the range of 1800 ~ 1100 cm^{-1} at the DNA/GC electrode varying with potential E_1 are shown in Fig. 4. E_2 is set at −0.2 V. Obviously, all the spectral changes except 1648 cm^{-1} are just in reverse to that of Fig. 3. The band at 1648 cm^{-1} has been assigned to the in-plane stretching vibration mode of C=O of the cytosine [20], indicating that cytosine is adsorbed at the electrode surface throughout the potential range (−0.2 ~ 1.3 V) studied. The results of in-situ FTIR show that intensities of C=O and pyrimidine ring increase and intensities of C–H, N–H, C=N and imidazole ring decrease at the electrode surface while the potential shifts positively.

Above all, the results of in-situ FTIR indicate that the adsorbing state of native DNA at electrode surface is changed with electrode potential. The DNA is adsorbed at the electrode surface through C=O of bases and pyrimidine ring at high potential, and turns to through C=N, C–H and N–H of imidazole ring and C=O of cytosine adsorption at low potential.

CT DNA adsorbed at GC electrode surface is also studied at another pH (4 ~ 8) solutions, the adsorbed behavior of native CT DNA is similar to that of the adsorbed DNA at pH 6.68.

The in-situ reflection-absorption difference spectra of temperature-denatured DNA/GC electrode in the range of 1800 ~ 1100 cm^{-1} varying with potential E_1 from −0.2 to 1.3 V are shown in Figs. 5 and 6. E_2 is set at 0 V. Two potential regions can be distinguished from these spectra. Shown in Fig. 5, in region I (−0.2 ~ 0.2 V), temperature-denatured DNA is not oxidized but adsorbed at electrode surface. Shown in Fig. 6, in region II (0.2 ~ 1.3 V), oxidation of the temperature-denatured DNA takes place. In region I, three IR bands extending upward (+) at 1358, 1221, 1722 cm^{-1} increase with the potential shifting negatively from 0 to −0.2 V (Fig. 5). The band at 1722 (+) cm^{-1} has also been assigned to the in-plane stretching vibration mode of C=O of the nucleic bases. For native B-form DNA, the band of C=O is at 1716 cm^{-1} [20,25,26], but the spectra of temperature-denatured DNA, the band shifts to high wavenumber. This difference is due to the double strands of DNA being decomposed to form a single strand. The structure of CT DNA is disturbed, and C=O interacts with the electrode surface resulting in the band shift. The bands at 1358 (+) and 1221 (+) cm^{-1} are assigned to the stretching vibration mode of imidazole ring [24], and the antisymmetric stretching vibration mode of PO_2^- [21–24]. The spectra changes in region I show that more temperature-denatured DNAs through C=O of bases, imida-

Table 2
Assignment of the four strong IR bands by in-situ FTIR spectra

Band position (cm^{-1})	Assignment	Reference
1716	$\upsilon_{C=O}$ (T, G)	[18,20,25,26]
1550	δ_{C-H}, δ_{N-H}, in-plane ring vibration mode of C–C and C=N, (A, C)	[24,27]
1417	In-plane imidazole ring vibration (A, C, G)	[20,24]
1278	Pyrimidine ring vibration (T, C)	[20,24]

zole ring and PO_2^- to form a thin adsorbing-layer at the electrode surface while E_1 shifts negatively from 0.2 to −0.2 V, but the adsorbing state is not similar to that of native CT DNA.

In region II, three IR bands extending downward (−) at 1721, 1544, 1414, 1278 and 1222 (shoulder) are clearly discerned while the potential is holding at 1.3 V (Fig. 6). The band at 1721 (−) cm^{-1} has been assigned to the in-plane stretching vibration mode of C=O of nucleic acid bases; the band at 1544 (−) cm^{-1} assigned to the stretching vibration mode of C–N and C–C, the band at 1414 (−) cm^{-1} assigned to the imidazole ring vibration, and C–H and N–H bending vibration; the band at 1278 (−) cm^{-1} assigned to the pyrimidine ring vibration, and 1222 (−) cm^{-1} assigned to the antisymmetric stretching vibration mode of PO_2^- [21–24]. All of these bands extend downward showing that the intensities of these vibrations are decreased at the electrode surface with potential E_1 shifting positively. The spectral changes in region II show that the thin adsorbing-layer is damaged and temperature-denatured CT DNA diffuses from the electrode surface while oxidation takes place.

4. Conclusion

The adsorption and oxidation mechanism of DNA is interpreted at the molecular level using in-situ FITR spectroelectrochemical method. Comparing the in-situ FTIR spectroelectrochemical and electrochemical behaviors of native with thermally denatured CT DNA, shows that the structure of DNA plays an important role in the adsorption and oxidation of DNA. Native CT DNA can be adsorbed at the electrode surface and its adsorbing state changes with the adsorbing potential. The temperature-denatured DNA can be adsorbed and easily oxidized at the electrode surface. The oxidation of the temperature-denatured DNA is irreversible based on the corresponding reduction peaks being absent at negative scans. DNA is desorbed from the electrode surface while the oxidation of bases takes place.

Acknowledgements

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