Application of Oscillographic Polarography in Photochemistry (II) Nucleic Acids

D. KALÁB

Research Department of Bioveta, n. p., Ivanovice na Hané

The heat denaturation of bacteriophage T 2 DNA before and after the UV irradiation (2.537 Å) has been followed oscillopolarographically. The changes found were discussed and the results obtained were compared with the published data, obtained by some other methods.

The influence of ultraviolet light (UV) and of some photodynamically active organic dyes on the nucleic acids has been studied by numerous investigators at the present time [1—5].

Studies were performed at the physical, chemical and biological levels. The purpose of the following communication is to present data concerning the application of alternating current oscillographic polarography in the investigation of the effects of UV irradiation on the deoxyribonucleic acid (DNA) isolated from a bacteriophage T 2.

Experimental

Materials and Methods

For this study the bacteriophage T 2 DNA was prepared in a solution of 0.15 M sodium chloride and 0.015 M sodium citrate at a concentration of 100 μg DNA/ml.

The bacteriophage T 2 DNA solution was irradiated at a distance of 5 cm using a Philips TUV, 30 W, low pressure mercury lamp with the emission maximum at the wave lenght of 2.537 Å.

The Polaroscope P 524 (Křižík, Praha) was used with a mercury dropping electrode [6].

For the oscillopolarographic analyses 0.2 ml of the DNA solution were always added to 0.8 ml of 1 M ammonium formate electrolyte in the usual polarographic cell and the size of anodic incision of DNA was registred photographically [7, 8].

Results

The heat denaturation of the bacteriophage T 2 DNA solution has been followed quantitatively using the A. C. current oscillographic polarography. The depth of anodic incision of DNA increasing with the rising temperature was measured oscillopolarographically in the ammonium formate medium (Fig. 1, curve *1*).

The DNA denaturation curve expressed a course of the relative depths D_r of the anodic incisions of DNA solutions as a function of temperature. The relative depth of

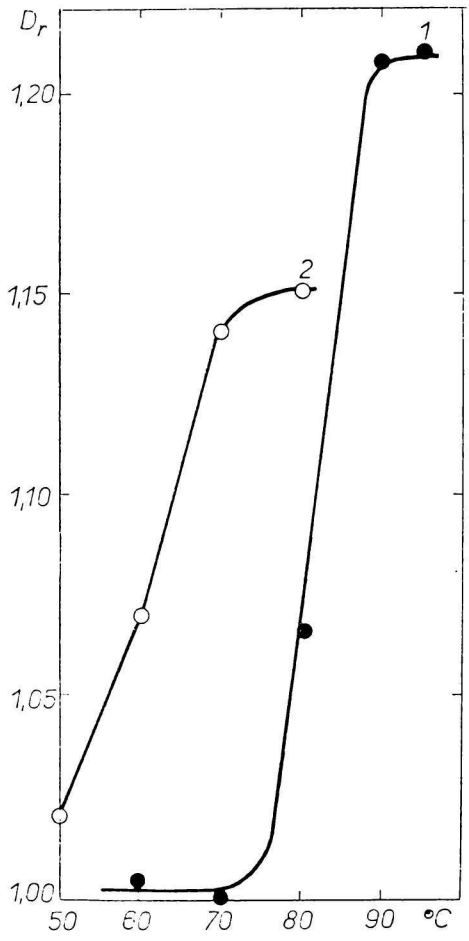

Fig. 1. Heat denaturation curves of the bacteriophage T 2 DNA, derived from the oscillopolarographic measurements. *1.* DNA before the UV irradiation; *2.* DNA after 180 minutes of the UV irradiation.

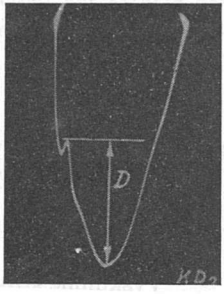

Fig. 2. Anodic part of the $dE/dt = f(E)$ curve of the bacteriophage T 2 DNA in 1 M ammonium formate.

incision D_r means the proportion of the depths of incisions at the definite temperature t °C (Fig. 2) and at the temperature of 25 °C:

$$D_r = \frac{D(t\ °C)}{D(25\ °C)}.$$

The course of heat denaturation of the bacteriophage T 2 DNA after 180 minutes of UV irradiation was followed oscillopolarographically in the same way (Fig. 1, curve *2*).

The denaturation curves of the bacteriophage T 2 DNA before and after the UV irradiation were guite different (Fig. 1). The melting temperature of the original bacteriophage T 2 DNA amounting to 82.5 °C was shifted after the above UV irradiation to 61 °C.

Similarly the relative depth of anodic incision of UV irradiated bacteriophage T 2 DNA after the additional heat denaturation was distinctly lower (D_r 1.15) as compared with the original sample (D_r 1.21) (Fig. 1).

At the laboratory temperature however the ratio of the depths of anodic incisions of the bacteriophage T 2 DNA before and after the UV irradiation reached only a value of 1.02.

Discussion

Similar changes in the course of the heat denaturation curves after the UV irradiation have been found spectrophotometrically in a number of bacterial DNAs by J. Marmur and co-workers [2].

After an intensive UV irradiation of D. pneumoniae DNA their melting temperature T_m was lowered from 86 °C down to 69 °C. Simultaneously a distinct drop of relative absorbance at 2600 Å of UV irradiated D. pneumoniae DNA was observed after an additional heat denaturation [2].

The above data yield further possibilities for the physicochemical assay of nucleic acids based on the oscillopolarographic estimations.

I should like express my thanks to Dr. J. Koudelka from the Biophysical Institute of the Czechoslovak Academy of Sciences in Brno, for the kind providing of the bacteriophage T 2 DNA sample.

VYUŽITIE OSCILOGRAFICKEJ POLAROGRAFIE VO FOTOCHÉMII (II) KYSELINY NUKLEOVÉ

D. Kaláb

Výskumné stredisko Bioveta, n. p., Ivanovice na Hané

Oscilopolarograficky sa sledoval priebeh termickej denaturácie DNK z bakteriofágu T 2 pred ožiarením a po ožiarení ultrafialovým žiarením o vlnovej dĺžke 2537 Å. Oscilopolarografické výsledky sa porovnávali s výsledkami dosiahnutými inými metódami opísanými v literatúre.

ПРИМЕНЕНИЕ ОСЦИЛЛОГРАФИЧЕСКОЙ ПОЛЯРОГРАФИИ В ФОТОХИМИИ (II) НУКЛЕИНОВЫЕ КИСЛОТЫ

Д. Калаб

Исследовательский центр нац. предпр. Биовета, Ивановице на Гане

Осциллополярографически исследовался ход термического денатурирования ДНК бактериофага Т 2 перед и после облучения ультрафиолетовым светом (2537 Å). Найденные изменения обсуждаются и результаты сопоставляются с опубликованными данными, полученными с использованием других методов.

Preložil I. Smoleň

REFERENCES

1. Chargaff E., Davidson J. N., *The Nucleic Acids*, Vol. III. New York 1960.
2. Marmur J., Anderson W. F., Matthews L., Berns K., Gajewska E., Lane D., Doty P., *J. Cellular Compar. Physiol.* ***58***, 33 (1961).
3. Grossman L., Stollar D., Herrington K., *J. chim. phys. et phys. chim. biol.* ***58***, 1078 (1961).
4. Coahran D. R., Buzzell A., Lauffer M. A., *Biochim. Biophys. Acta* ***55***, 755 (1962).
5. Simon M. I., Van Vunakis H., *J. Mol. Biol.* ***4***, 488 (1962).
6. Heyrovský J., Kalvoda R., *Oszillographische Polarographie mit Wechselstrom*, 75. Akademie-Verlag, Berlin 1960.
7. Paleček E., *Nature* ***188***, 656 (1960).
8. Paleček E., *Biochim. Biophys. Acta* ***51***, 1 (1961).

Received September 18, 1963