Moscow University
Physics Bulletin
Vol. 51, No. 3, pp. 38–41, 1996

Vestnik Moskovskogo
Universiteta. Fizika
UDC 535.3:577.32

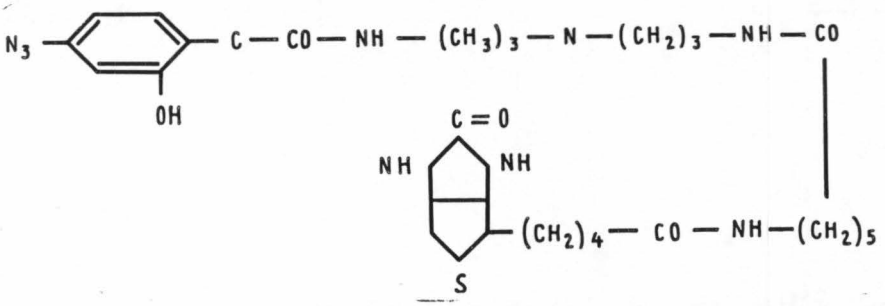
ELECTRON ABSORPTION SPECTRA AND PHOTOCHEMICAL PROPERTIES OF A NEW COMPOUND, PHOTOBIOTIN

K. G. Blinova, V. I. Yuzhakov, S. N. Shcherbo, and M. F. Turchinskii

Electron absorption spectra of a new first synthesized compound, photobiotin, have been investigated in relation to the pH value and to an exposure dose of an aqueous solution of this compound. Optimal conditions of labeling nucleic acids with the new photobiotin have been determined. The proposed method of labeling nucleic acids proved to be very sensitive. The spectral and photochemical characteristics of the new photobiotin are compared with the characteristics of biotins investigated earlier.

A method of non-radioactive labeling of nucleic acids, developed recently on the basis of biotinylation of nucleic acids, has the advantages of being highly sensitive, simple, and quick [1–3]. The development of this method calls for the synthesis of novel compounds, photobiotins. This is a general name of biotin derivatives incorporating photoactive groups whose chemical nature can be different. To use photobiotins effectively for labeling nucleic acids, one should know their spectral and photochemical properties. An important parameter in DNA modification is the exposure time in the presence of a photoactive reagent.

This work is devoted to spectroscopic investigation of a new photobiotin (PB) which was synthesized by one of the authors on the basis of di-(3-aminopropyl)-methylamine of ε-amidocaproylbiotin and azidosalicylic acid for the purpose of optimizing the method of DNA modification with photobiotin to prepare DNA-probes employed in the non-radioisotopic hybridization analysis of nucleic acids. The structural formula of the PB molecule is presented below:



We have investigated the electron absorption spectra of an aqueous solution of PB, their dependence on the acidity of the medium, and on the radiant exposure of the PB solution to different wavelengths. Such data are required for choosing optimal conditions of labeling nucleic acids in carrying out the photochemical reaction. The absorption spectra were recorded with a Specord-M40 spectrophotometer (FRG) over the range from 210 to 450 nm. The degree of acidity of solutions was determined with the help of a pH-304 pH meter. The solutions were exposed in the cell compartment of the spectrofluorimeter. The radiation intensity was about 1 W/m^2, a one-centimeter cell was used. A hybridization analysis of preliminarily denaturated thymus DNA applied to a nylon filter [4, 5] was carried out to check the degree of photobiotin incorporation.

The obtained spectral and photochemical characteristics of PB were compared with similar character-

istics of the earlier investigated photobiotin PB1 produced by Clontech Co. (USA) and of the polymeric photoactive biotin derivative (PB2) [3], which have already been used as compounds for non-radioisotopic labeling of nucleic acids in the hybridization analysis, particularly, in diagnostics of herpes simplex of type 2 and of influenza A [6].

RESULTS AND DISCUSSION

The absorption spectrum of a neutral (pH 7) aqueous solution of PB (Fig. 1) contains three peaks at $\lambda = 273$, 304, and 323 nm, the ratio of their intensities being 1 : 0.5 : 0.25. In the absorption spectrum of the aqueous solution of the polymeric biotin derivative PB2 having a hydronitrophenyl photoactivated group common with PB, the absorption peaks are observed approximately at the same wavelengths, $\lambda = 271$ and 305 nm.

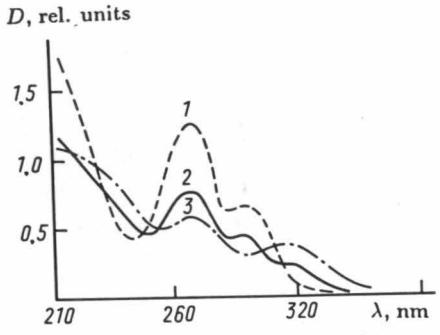


Fig. 1

Absorption spectra of aqueous PB solutions at $C = 10$ mol/l and pH 4 (*1*), 7 (*2*), and 10 (*3*).

In the PB1 spectrum, where the photoactivated group is the azidonitrophenyl group, the absorption peaks correspond to $\lambda = 262$ and 472 nm. Therefore, the absorption at $\lambda = 260$–270 nm is common to biotin derivatives, while the photoactivated group, different in different derivatives, is responsible for the peak at $\lambda = 472$ nm in PB1 and for the peaks at $\lambda = 304$–305 nm in PB and PB2. All the three photobiotins intensively absorb light in the wavelength range of $\lambda < 200$ nm, but the nature of this band is still unclear.

The acidulation of the PB solution to the pH 3 does not bring about any noticeable changes in the form of the absorption spectrum (Fig. 1), but the intensity of peaks increases and they shift to the short-wave region (the maximum at $\lambda = 273$ nm shifts down to 271 nm). On the alkalization of the initial PB solution from the pH 7 to the pH 10 the peak intensity decreases at $\lambda = 273$ nm, the absorption band with peak disappears completely at $\lambda = 304$ nm and two new absorption bands appear at $\lambda = 238$ and 334 nm. The spectrum of an alkaline solution of PB2 (pH 10) looks similar: three peaks are observed at $\lambda = 240$, 270, and 330 nm. The absorption spectrum of an acidulated solution of PB2 (pH 2) differs little from the spectrum of the neutral solution.

When the PB solution is exposed to ultraviolet light with wavelengths of 250–350 nm, the time of complete irreversible decomposition is 10–15 min, this being almost half the decomposition time of PB2 (about 20 min) and one-fourth of the decomposition time of PB1 (35–40 min). A reduction of the PB decomposition time can be used to accelerate the process of labeling the nucleic acids using the new photobiotin in hybridization analysis of DNA and RNA. In the course of the exposure of the PB aqueous solution the intensity of peaks at $\lambda = 273$ and 304 nm gradually decreases. The absorption spectra, depending on the exposure time, are characterized by an isobestic point at $\lambda = 288$ nm (Fig. 2). A similar pattern is observed when exposing the PB2 solution. Both peaks in the spectrum ($\lambda = 271$ and 305 nm) decrease simultaneously. The PB2 absorption spectra, depending on the exposure time, are characterized by three isobestic points: at $\lambda = 230$, 240, and 320 nm. The presence of isobestic points suggests the presence of two types of the absorbing centers in the PB and PB2 aqueous solutions: the initial molecule and the product of its photodecomposition. During the exposure of the PB1 aqueous solution, the spectral behavior becomes more complicated. In the absorption spectrum the intensity of the bands with peaks at $\lambda = 262$ and 472 nm

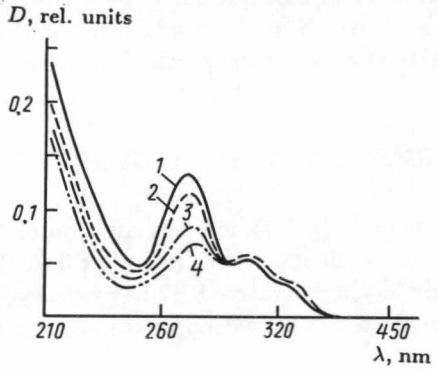


Fig. 2

Absorption spectra of aqueous PB solutions at $C = 1$ mol/l and pH 7 before (*1*) and after the exposure to UV light for 2 (*2*) 6 (*3*), and 10 min (*4*).

also drops almost simultaneously; and in addition, absorption bands of the photoproduct appear at $\lambda = 284$ and 514 nm. There also appears an absorption band with peak at $\lambda = 353$ nm.

The exposure of PB to radiation with a wavelength shorter than 230 nm does not lead to substantial changes in the absorption spectrum. A similar behavior is observed on exposure of PB1 to light with $\lambda > 320$ nm [3]. For PB2 no data of such a kind can be found in the literature.

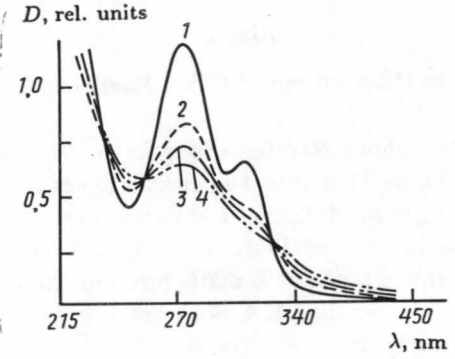


Fig. 3

Absorption spectra of PB solution at $C = 10$ mol/l and pH 4 before (*1*) and after the exposure for 5 (*2*), 10 (*3*) and 20 min (*4*).

When an acidulated PB solution (pH 4) is exposed to ultraviolet light, the peaks at $\lambda = 271$ and 305 nm disappear simultaneously (Fig. 3). The absorption spectra of this solution, depending on the exposure time, have two isobestic points, at $\lambda = 258$ and 334 nm. The time of complete decomposition, as compared with the neutral solution, increases to 20–25 min.

In the absorption spectra of the alkalized PB solution, depending on the exposure time, a simultaneous drop of the peaks at $\lambda = 238$, 273, and 334 nm is observed.

The time of complete decomposition of PB in the alkaline solution corresponds to the time of decomposition in the neutral solution and amounts to 10–15 min. Similarly, the exposure of the initially acidulated PB2 solution (pH 2) does not lead to essential changes in the absorption spectra as compared with the neutral solution, however, the time of complete decomposition decreases approximately by one half as compared with the neutral solution rather than increases as in the case of PB.

Similarly to the case of PB, the intensities of the bands with peaks at $\lambda = 240$, 270, and 330 nm in the absorption spectrum of the alkaline solution of PB2 decrease on exposure, with the short-wave peak

disappearing more rapidly than the two other peaks. The time of complete decomposition of PB2 in this case does not change either [3].

On acidulation of the initial PBS solution (pH 2), the absorption peaks at $\lambda = 284$ and 514 nm are absent; at the same time, an absorption band at $\lambda = 340$ nm, due to a photoproduct, is observed. We note that the time of PB1 decomposition at the above-stated pH reduction is also 40 min. Similar spectral changes occur on exposure of an alkaline (pH 10) solution of PB1 in water [3].

The presence of an azide group in the photobiotin molecules brings about a high photochemical activity of these compounds. When photobiotin solutions are exposed to ultraviolet light, a very active nitrene radical is formed, which can participate in different chemical reactions. In particular, it can add to molecules of nucleic acids along the C–H, O–H, and N–H bonds. In the equimolar ratio 5 to 10 photobiotin molecules are incorporated per 1000 DNA bases [7]. The exact point of addition of photobiotins to DNA and RNA is so far unknown.

The probe for carrying out hybridization analysis of DNA was prepared by modifying thymus DNA with photobiotin. The degree of biotin incorporation and the modification conditions were checked by applying the DNA probe to a nylon filter. To identify labeled DNA, a conjugate of colloidal carbon with avidin was used. 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, and 10^{-13} g of biotinylated DNA was applied to a nylon support. Then the filter with applied DNA-probe was placed into a solution containing NaCl salt which increases the ionic strength and thus stabilizes the DNA structure, a detergent Triton X100 which precludes binding of the probe with the support, and chaotropic reagents which bind water. After treating the filter with the avidin–carbon conjugate, the parts of the filter to which from 10^{-11} to 10^{-10} g DNA had been applied became stained.

This means that the sensitivity of our system is about 10^{-10} g. The sensitivity of the method can be improved substantially by using highly active enzymes: peroxidase, alkali phosphatase, and their chromogenic substrates. Increasing the development time under these conditions, one can increase the sensitivity to units of a picogram [5].

CONCLUSIONS

In our work we have investigated the dynamics of the electronic absorption spectra of aqueous solutions of a new photobiotin under changes of their pH and exposure dose. The applicability of this compound for labeling nucleic acids is elucidated. The optical properties of the new photobiotin are compared with the properties of photobiotins studied earlier.

The use of the new photobiotin makes it possible to reduce by half the time of labeling nucleic acids as compared with PB2 and almost a fourfold reduction as compared with PB1. Labeling will be more effective, when conducted in a neutral or alkalized medium when exposed to light with wavelengths ranging from 250 to 350 nm. The sensitivity of the method is the same for all three photobiotins and reaches a few units of a picogram of biotinylated nucleic acid.

REFERENCES

1. R. M. Buckland, *Nature*, vol. 320, p. 557, 1986.
2. D. J. Brigati, D. Myerson, J. J. Leary, *et al.*, *Virology*, vol. 126, p. 32, 1983.
3. S. N. Shcherbo, S. V. Patsaeva, V. I. Yuzhakov, and M. F. Turchinskii, *Biofizika*, vol. 37, no. 1, p. 34, 1992.
4. T. Maniatis, E. Fritsch, and J. Sambrook, *Molecular Cloning. A Laboratory Manual*, (Russian translation) Cold Spring Harbor Laboratory, New York, 1982.
5. A. T. Al'zhanova and V. M. Generalova, *Molekulyarnaya Biologiya*, vol. 22, no. 3, p. 780, 1988.
6. L. V. Uryvaev, E. A. Rusavskaya, N. M. Sinagatullina, *et al.*, *Voprosy Virusologii*, no. 6, p. 464, 1990.
7. A. C. Forster, J. L. McInnes, D. C. Skingle, and R. H. Symons, *Nucleic Acid Res.*, vol. 13, no. 3, p. 745, 1985.

26 June 1995

Department of General Physics