Influence of Nucleic Acid Bases on the Light-induced Reduction of Methylene Blue: ESR and Optical Measurements

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(Z. Naturforsdi. 28b, 82—85 [1973]; received August 30/October 17, 1972)

Nucleic acid bases, electron acceptors, ESR, optical absorption, photo-induction

The effect of the nucleic acid bases adenine, cytosine, thymine, uracil, and 5-bromo-uracil on the light-induced reduction of methylene blue in dimethylsulfoxide has been investigated by means of ESR and optical absorption studies. It could be shown that the nucleic acid bases act as electron acceptors. The experimentally obtained electron affinities are of the following order: cytosine > adenine > thymine » uracil and confirm, thus, the theoretical predictions.

During the past few years it has been shown that the electron transport is a characteristic property of many biological systems. Still unsolved, however, is in many cases the question of whether a biomolecule acts as donor or as acceptor. Indications for these properties have been obtained by calculating the energy levels for most of the important biomolecules. The energy values obtained for the highest occupied (donor) and the lowest empty (acceptor) molecular orbitals of the nucleic acid bases are of special interest for the present considerations. Based on these calculations, BRILLOUIN proposed a model according to which the deoxyribonucleic acid, DNA, is supposed to be a semiconductor with the purines and pyrimidines acting as donors and acceptors, resp.

Because of the extreme importance for molecular biology, the electron affinities of the nucleic acid bases and their relative strength have to be verified. The light-induced reduction of methylene blue in dimethylsulfoxide was selected as method of choice. We have shown that the reduction of methylene blue in dimethylsulfoxide by illumination was reversed partially by the addition of Cu²⁺ depending on the concentration. In this case, the metal ion acts as electron acceptor. In the experiments reported here, Cu²⁺ was replaced by the nucleic acid bases in order to elucidate their electron attractive properties by means of electron spin resonance spectrometry and optical absorption spectrophotometry.

Materials and Methods

The nucleic acid bases adenine, cytosine, thymine, uracil, as well as the dye methylene blue (MB) and

IR-Spektrum (CCl₄):

2074, 1979 cm⁻¹ ν(C=O) Aₕ u. E.

Massenspektrum (bezogen auf ⁵⁶Fe):

346, 348 M⁺
318, 320 M⁺ - CO
290, 292 M⁺ - 2 CO
262, 264 M⁺ - 3 CO
236, 238 Br - C = CH

267 346 - ³⁹Br, 348 - ⁸⁰Br
239 318 - ³⁹Br, 320 - ⁸⁰Br
211 290 - ³⁹Br, 292 - ⁸¹Br
183 262 - ³⁹Br, 264 - ⁸¹Br

Der Deutschen Forschungsgemeinschaft und dem Verband der Chemischen Industrie danken wir für großzügige Förderung dieser Arbeit.

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dimethylsulfoxide (DMSO) were obtained from Merck, Darmstadt, and were of reagent-grade quality. 5-bromo-uracil was supplied by Koch-Light Laboratories, Colnbrook Buckinghamshire, England. Guanine could not be investigated due to its insolubility in DMSO. The substances were used without further purification. The nucleic acid base solutions were prepared fresh daily. Since the ability of the MB solution to exhibit radicals after illumination decreased for about 25% within the first few hours before reaching a stationary level, 2 days old dye stock solutions were used. All samples investigated were oxygen free.

The electron spin resonance (ESR) spectra were determined with a Varian V 4502 100-kc ESR spectrometer using a liquid sample accessory.

The optical absorption studies were carried out with a Cary 14 spectrometer using 5 mm cells. Immediately after preparing the samples the spectra were recorded in the wavelength region between 250 and 750 nm. A Hilger-Watts quartz-monochromator D 296 with a XBO-1600 W high pressure Xenon lamp as well as a 300 W slide projector lamp were used for illuminating the samples. In both cases, two additional filter were inserted into the light path in order to eliminate infrared rays (by H₂O-filter) and UV-light (by K₂Cr₂O₇ solution). Since only rel. changes were observed, no absolute flux determination has been made.

Results and Discussion

In Fig. 1 the ESR signal of a 10 mM MB-DMSO solution, illuminated for 83 min with visible light, is shown. The hf structure, which cannot be interpreted as yet, can be obtained only in oxygen-free solutions using the appropriate modulation amplitude and amplification. Usually, only the unresolved singlet is observed at \( g = 2.003 \). After illumination, the signal increased up to a saturation value which is concentration dependent.

Addition of adenine to an MB-DMSO solution did not exhibit any ESR signal at all. Upon illumination, however, adenine caused a reduction in spin concentration. The extent of this reduction is concentration dependent. The effect of different adenine concentrations of up to 10.4 mM on the light-induced spin concentration is shown in Fig. 2a.

Cytosine behaves very similar to adenine but its efficiency in reducing the spin concentration is more pronounced. The influence of adenine and cytosine, resp., on the rel. spin concentration of a nucleic acid base-MB-DMSO solution, illuminated for 20 min, is shown in Fig. 2b. As can be seen by this comparison, cytosine is about 10 times more effective than adenine.

Comparable concentrations of uracil, 5-bromo-uracil, and thymine had no effect at all. A 10% reduction in spin concentration was observed when 30 mM thymine were used. In the case of uracil, a 10% decrease was obtained with a 100 mM concentration.

The effect of illumination on the absorption spectrum of MB in a DMSO solution is shown in Fig. 3. The decrease of the two bands at 296 and 670 nm occurs exponentially with the dose applied; a new band appears at 261 nm. Addition of adenine, cyto-

Fig. 1. ESR signal of 10 mM methylene blue in dimethylsulfoxide illuminated for 83 min with visible light.

Fig. 2 Effect of adenine (2a) and cytosine (2b) on the light-induced methylene blue radical.
Fig. 3. Effect of illumination on the absorption spectrum of methylene blue in dimethylsulfoxide.

Fig. 4. Modification of the absorption spectrum of an illuminated methylene blue-dimethylsulfoxide system by adenine (4a) or cytosine (4b).

adenine or cytosine are present. In the case of adenine, a new band appears at about 279 nm which is the more pronounced the larger the adenine concentration (s. Fig. 4a) or the larger the radiation dose.

In the presence of cytosine, a new band appears at 283 nm being also dependent on the concentration (s. Fig. 4b) and the dose as well. The light-induced decrease of the bands at 296 and 670 nm is not influenced by either adenine or cytosine; the band at 261 nm doesn't seem to be as expressed as without addition of the two nucleo-bases.

The results obtained by optical absorption studies agree with the ESR data: only cytosine and adenine affect, to any larger extent, the light-induced changes in a MB-DMSO system. Since none of the nucleic acid bases investigated modifies the unilluminated MB spectrum it seems to be obvious that there is no interaction between them and MB in DMSO. These findings are in agreement with nuclear magnetic resonance studies and calculations according to which nucleic acid bases interact much stronger with DMSO than with MB and that there is no interaction, in the ground state, between them and MB.

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As we have shown, an electron is transferred from DMSO to MB upon illumination only, that is the redox potential of the ground state of the dye molecule is lower than that of DMSO. The formation of a charge-transfer complex resulting in the absorption band at 261 nm can be described by a model proposed by Weller. A subsequent electron transfer from MB to adenine or cytosine occurs when these substances are present. This is expressed by the reduction of spin concentration in the ESR data as well as by the formation of new optical bands located at \( \lambda > 261 \) nm. The appearance of the "cytosine"-band at \( \lambda \) slightly higher than the "adenine"-band exhibits a greater electron affinity, \( E_A \), of cytosine according to Mulliken's charge-transfer theory. This result is in agreement with Pullman's calculations (s. Table I). According to this table, oxidized MB has the smallest \(-k\) value and, thus, the greatest \( E_A \) compared with the other substances listed. After the electron transfer from DMSO to MB, the \(-k\) value of the lowest empty molecular orbital (\(-1.0\)) is considerably larger than the \(-k\) values for cytosine or adenine. In this case, these two nucleic acid bases act as electron acceptors with the reduced MB as donor. Moreover, the greater efficiency of cytosine is also predicted by the theory. The rel. inefficiency of uracil and thymine can also be explained by the \(-k\) values: their values differ only slightly from the value of reduced MB. A significant effect should be observed at larger concentrations only. The experimental results obtained confirm this prediction.

Furthermore, polarographic investigations have shown that only adenine and cytosine are reducable while uracil and thymine cannot be reduced. Again, only adenine and cytosine should be effective.

From the data obtained one might conclude that, using the MB-DMSO system, the nucleic acid bases act as electron acceptors and that their rel. strength in electron affinity follows, in agreement with the theory, the order: cytosine \( > \) adenine \( > \) thymine \( \approx \) uracil.

The excellent technical assistance of Miss A. Dimmerling is greatfully acknowledged. This work was supported in part by Fraunhofer - Gesellschaft.