Journal of Fluorescence, Vol. 9, No. 3, 1999

Catalysis of the Back Thermal cis–trans Isomerization Reaction of Stilbazolium Betaine by Metmyoglobin

V. R. Vogel,[1,2] A. V. Pastukhov,[1] and A. I. Kotelnikov[1]

Received September 30, 1998; accepted December 11, 1998

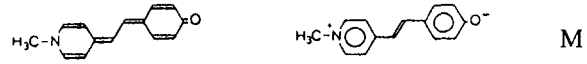
The catalytic effect of metmyoglobin on the back thermal cis → trans isomerization reaction of stilbazolium betaine M is reported. This reaction shows substantial acceleration in the presence of metmyoglobin in comparison to the same reaction without the protein or in the presence of metmyoglobin cyanide. It is suggested that the observed thermal reaction acceleration may arise from the coordination of the protonated stilbazolium betaine molecule to the sixth ligand position of the heme iron and subsequent chromophore deprotonation or from the low polarity of the heme pocket microenvironment.

KEY WORDS: Stilbazolium betaine; cis–trans isomerization; metmyoglobin; ligand.

INTRODUCTION

Whereas great attention is paid to an investigation of photophysical and photochemical properties of photochromic molecules, there is not sufficient information about back thermal stages of *cis/trans* isomerization processes. At the same time, controlling the back thermal stages of cis/trans isomerization are an actual problem in creating reversible photoregulable molecular devices. Using photochrome–protein complexes could provide perspectives for such a goal [1–4].

One of the extensively investigated compounds of this field is stilbazolium betaine M:



(**q**) (**b**) M

This compound can exist in two forms, (**q**) and (**b**). Increasing solvent polarity leads to an increasing contribution of the zwitterionic structure (**b**). The central C–C double bond of the latter provides the existence of cis and trans forms, which can be isomerized photochemically or thermally.

This paper presents the results of a study of the back dark stage of the cis/trans isomerization reaction of stilbazolium betaine M in the presence of metmyoglobin. Interaction of stilbazolium betaine with metmyoglobin heme at the sixth ligand position accelerates the back thermal isomerization. Restoration of the initial trans state of the photochrome molecule from the first prepared photostationary cis/trans state occurs much more rapidly in the presence of metmyoglobin. An analogous catalytic effect is not observed when metmyoglobin cyanide is used.

RESULTS AND DISCUSSION

4-Hydroxy-(1-methyl)stilbazolium betaine was synthesized according to a literature procedure [5]. Myoglobin from sperm whale skeletal muscle (Serva), sodium cyanide, and 0.05 *M* sodium phosphate buffer, pH 7.2, were also used. Metmyoglobin cyanide was prepared by adding sodium cyanide to the metmyoglobin solution. The final concentration of sodium cyanide in the solution was about 10^{-3} *M*. The buffered sample of the pure

[1] Department of Kinetics and Catalysis, Institute for Chemical Physics Research, Chernogolovka, Moscow Region 142432, Russia.
[2] To whom correspondence should be addressed. Fax: (096) 576-40-09.

1053-0509/99/0900-0209$16.00/0

trans form of stilbazolium betaine placed in a 1-cm quatz quvette was irradiated with UV light. A mercury high-pressure lamp (DRK-120), combined with a glass filter UFS-5 to isolate the desired mercury emission line at 366 nm, was used as a light source. The absorption spectra were recorded on a Specord M-40 spectrophotometer.

Figure 1 illustrates the absorption spectra of a solution of stilbazolium betaine (concentration, about $3*10^{-5}$ M) before irradiation (Fig. 1a) and immediately after irradiation (Fig. 1b). The solution was initially in the pure trans state. But after 5 min of irradiation with UV light at 366 nm, a new photostationary cis/trans state was reached. The optical density decrease in the solution in the pure trans form absorption maximum at 372 nm was about 35% immediately after irradiation. This was due to photochemical isomerization of the trans form to the cis form. The back thermal reaction from the photostationary cis/trans state to the entirely trans form was not observed for 12 h (Fig. 3a). If metmyoglobin (final concentration, about $5*10^{-6}$ M) was added to the trans-stilbazolium betaine solution before irradiation, then the efficiency of the forward trans → cis isomerization reaction was not affected (Fig. 2), but substantial acceleration

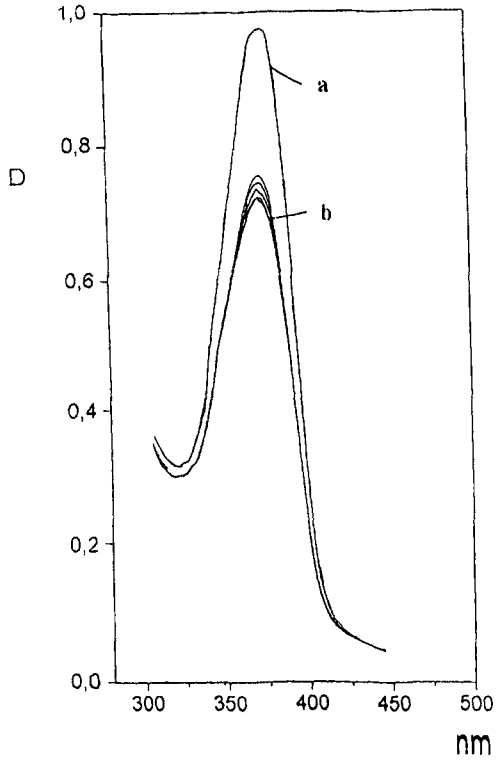


Fig. 1. Stilbazolium betaine solution absorption spectra: (a) the pure *trans* form; (b) the photostationary cis/trans state after irradiation at 366 nm.

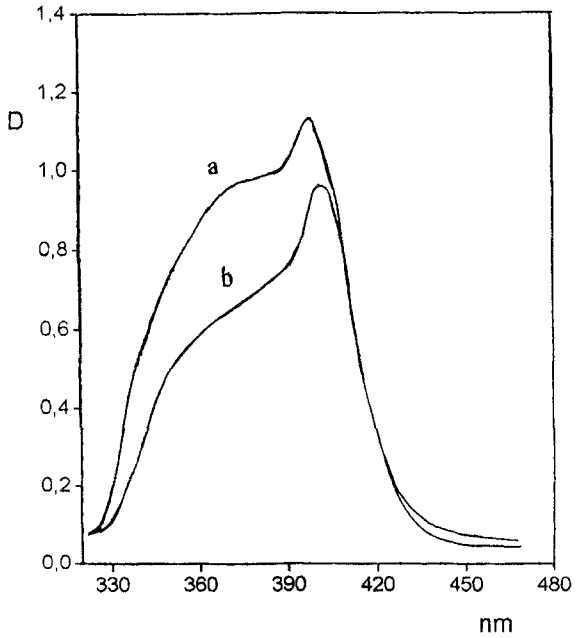


Fig. 2. Absorption spectra of a stilbazolium betaine solution in the presence of metmyoglobin, pH 7.2, 17°C: (a) before irradiation; (b) after 5 min of irradiation at 366 nm.

of the rate of the back dark stage of the cis → trans isomerization reaction was observed (Fig. 3b). At the same time, the addition of metmyoglobin cyanide affected negligibly the rate of the back cis → trans isomerization reaction (Fig. 3c). This may indicate metmyoglobin heme participation in catalysis of the thermal cis → trans isomerization reaction.

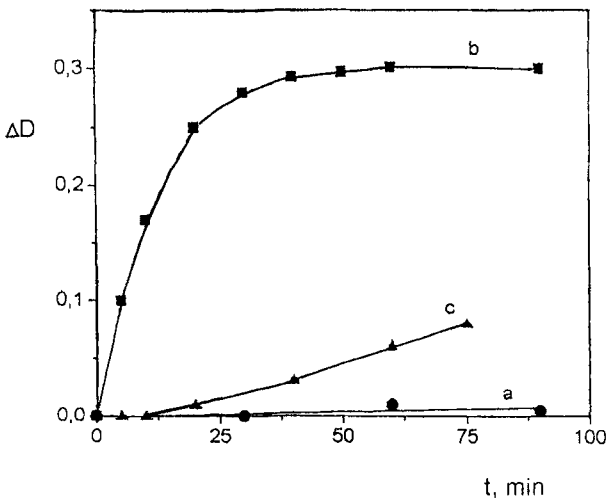


Fig. 3. Time dependence of the back thermal cis → trans isomerization reaction: (a) in a buffered solution; (b) in a buffered solution in the presence of metmyoglobin; (c) in a buffered solution in the presence of metmyoglobin cyanide.

At the present time we can offer two possible explanations for the observed processes. The first is that the cis and trans forms of stilbazolium betaine have different capabilities for associating with heme at the sixth ligand position. In the case of metalloporphyrins it has been established that stilbazoles bond to the heme metal by nitrogen. Since the volume of the myoglobin heme pocket is restricted enough, it is possible that the cis compound has some advantage, due to its more compact geometry, over the trans compound in associating with myoglobin heme. Furthermore, as Steiner *et al.* [5] established, the protonated form of stilbazolium betaine is stable with respect to thermal isomerization both in the cis form and in the trans form, whereas the deprotonated cis form may be isomerized thermally (at 23°C, $\tau_{1/2}$ = 260 min). They also found that the pK of stilbazolium betaine is 8.54 at 25°C. So at pH 7.2 the protonated form predominates and there is equilibrium in the stilbazolium betaine solution after irradiation:

$$\begin{array}{ccc} {}^{trans}M + H^+ & \Leftrightarrow & {}^{trans}MH^+ \\ \Uparrow & \Updownarrow h\nu & \\ {}^{cis}M + H^+ & \Leftrightarrow & {}^{cis}MH^+ \end{array}$$

It is thus possible to suggest that coordination of the protonated cis form by the heme iron leads to its deprotonation and the subsequent thermal cis → trans isomerization.

Another way to explain our finding is based on the polarity dependence of the thermal isomerization of stilbazoles. As Abdel-Halim *et al.* [6] reported, the rate constant of the thermal cis → trans isomerization of stilbazolium betaine at room temperature is increased by about seven orders of magnitude when going from water to acetone. This corresponds to the decrease in Dimroth's solvent polarity parameter, E_T, from 63.1 to 42.2, respectively [7]. Since the heme pocket provides a hydrophobic, low-polar microenvironment, we suppose that under these conditons the predominant form of coordinated stilbazolium betaine will be the **q**-type resonance structure with a C–C single bond. Subsequent ligand exchange will produce the thermodynamically more favorable trans configuration.

Taking into account the possible pH and polarity dependences of stilbazolium betaine, it is interesting to prepare a photoregulable cis/trans isomerization cycle of stilbazolium betaine in the presence of metmyoglobin. The detailed study of the catalytic mechanism of the thermal cis → trans isomerization reaction of stilbazoles by metmyoglobin and other hemeproteins will be continued.

ACKNOWLEDGMENTS

The authors thank Dr. V. Maier (Institue of Organic Chemistry, Tubingen) for performing the stilbazolium betaine synthesis. This work was supported by Russian Foundation for Basic Research Grant 97-0448646.

REFERENCES

1. U. Pfeifer, H. Fukumura, H. Misawa, N. Kitamura, and H. Masuhara (1992) *J. Am. Chem. Soc.* **114**, 4417–4418.
2. P. R. Westmark, J. P. Kelly, and B. D. Smith (1993) *J. Am. Chem. Soc.* **115**, 3416–3419.
3. Y. Iseki, E. Watanabe, A. Mori, and Sh. Inoue (1993) *J. Am. Chem. Soc.* **115**, 7313–7317.
4. I. Willner (1997) *Acc. Chem. Res.* **30**, 347–356.
5. U. Steiner, M. H. Abdel-Kader, P. Fisher, and H. E. A. Kramer (1978) *J. Am. Chem. Soc.* **100**, 3190–3197.
6. S. T. Abdel-Halim, M. H. Abdel-Kader, and U. E. Steiner (1988) *J. Phys. Chem.* **92**, 4324–4328.
7. C. Reichardt (1988) *Solvents and Solvent Effects in Organic Chemistry*, VCH, Weinheim.