direct peaks are individually resolved[10] and labeled in Figure 2A. In addition, a crosspeak arising from chemical exchange of I_zS_z two-spin order from the Tyr-35 $C^\epsilon H^1$ to the $C^\epsilon H^2$ environment is also present. The symmetric Tyr-35 crosspeak, due to exchange from $C^\epsilon H^2$ to the $C^\epsilon H^1$ environments, is partially overlapped with the Tyr-21 $C^\epsilon H$ direct peak. The same expanded region of a $^{13}C-^1H$ correlated S_z chemical exchange spectrum recorded with the pulse sequence of Figure 1B is shown in Figure 2B. In this slightly higher resolution spectrum, both the $C^\epsilon H^1$ to $C^\epsilon H^2$ and the symmetric $C^\epsilon H^2$ to $C^\epsilon H^1$ S_z exchange crosspeaks are resolved.

These results demonstrate the utility of proton-detected heteronuclear correlated chemical exchange spectroscopy for identifying slow conformational isomerization in proteins. In these experiments, the ratio of direct $^{13}C-^1H$ correlation peak volumes provides information about the equilibrium constant for the isomerization, while exchange crosspeak volumes are related to the rates of the forward and reverse exchange processes. These methods should be applicable generally to studies of slow conformational isomerization and folding/unfolding dynamic equilibria in small proteins.

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Valence Isomerization of Hexamethyl(Dewar benzene) Radical Cation. Pulse Radiolytic Investigation

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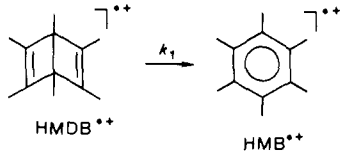
Organic radical ions are important intermediates in a wide variety of electron-transfer reactions.[1,2] Both the steady-state and time-resolved techniques have been extensively applied to probe various aspects of their chemistry. We have recently established that low-temperature pulse radiolysis can be successfully applied to the kinetic study of radical ion transformations with very low activation barriers.[3] The target of the present investigation is the hexamethyl(Dewar benzene) (HMDB)-hexamethylbenzene (HMB) system.[4] Studies by the CIDNP technique indicated the presence of two distinguishable radical cations in the system HMDB-HMB.[5] This view has not been supported by a nanosecond spectroscopic observation which failed to reveal any evidence for a radical cation other than $HMB^{\bullet+}$.[6]

The aim of this work is to present spectroscopic evidence for two different radical cations $HMDB^{\bullet+}$-$HMB^{\bullet+}$ and the activation barrier for their interconversion (reaction 1).



(1)

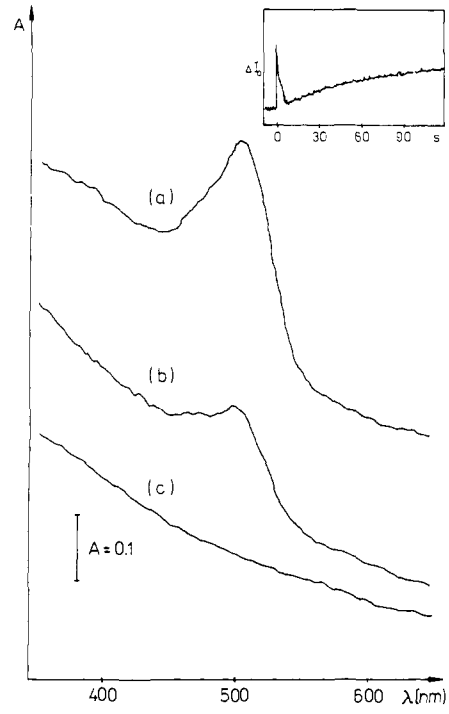


Figure 1. Absorption spectra of γ-irradiated (a) HMB (0.02 M) and (b) HMDB (0.02 M) in glassy matrices containing 3-methylpentane and 1-butyl chloride (1 M) at 77 K. The samples were 2 mm thick and received a radiation dose of 10^4 Gy. (c) Base line prior to γ-irradiation. Inset: scope trace from pulse radiolytic measurements of HMDB solution at 510 nm (T = 77 K).

The steady-state spectra of radical cations generated by γ-radiolysis of glassy solutions of HMDB and HMB at 77 K are presented in Figure 1.[7] In both cases an absorption with a maximum at 510 nm was seen, but at the same radiation dose the absorbance was higher for the HMB solution.[8] It is conceivable that the absorption at 510 nm represents the same species in both cases, i.e., $HMB^{\bullet+}$.[6] This view is compatible with a former proposition indicating a very fast interconversion process of $HMDB^{\bullet+} \rightarrow HMB^{\bullet+}$, which effectively proceeds even at 77 K.[1]

In order to verify unambiguously that the absorption at 510 nm observed for γ-irradiated HMDB solution has been correctly assigned to $HMB^{\bullet+}$ we have undertaken time-resolved studies hoping to monitor a delayed formation of $HMB^{\bullet+}$ in this case.

The transient absorption spectra of HMB at 93 K are presented in Figure 2. The spectrum determined shortly after the pulse shows a maximum at 510 nm.[9] At 2.5 s after the pulse this absorption is still present in the spectrum. The decay of this absorption presented as the oscilloscope trace is shown as inset in Figure 2. The rate constant of this decay at 93 K was found to be 0.11 s^{-1} treating the geminate recombination, responsible for the decay, as a complex first-order process (reaction 2).[10]

$$HMB^{\bullet+} \xrightarrow{k_2} HMB \qquad (2)$$

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(4) Radical cations of the solute molecules were observed in glassy solutions containing 3-methylpentane and a small addition of 1-butyl chloride acting as an electron scavenger. HMDB and HMB purchased from Aldrich were purified by standard laboratory procedures. The description of the pulse radiolysis system and other details of experimental procedure are given in ref 3.
(5) Roth, H. D.; Schilling, M. L. M.; Raghavachari, K. *J. Am. Chem. Soc.* **1984**, *106*, 253-255.
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(7) The radical cations were generated with a ^{60}Co γ-source and measured with a Beckman Acta IV spectrophotometer 5 min after the irradiation was completed. The yield of radical cations was not linear with the radiation dose for both γ-rays and electrons, showing a saturation type dependence at high doses.
(8) Following the referee's suggestion we have photolyzed (200-W high-pressure mercury lamp, 365-nm interference filter) the sample of γ-irradiated HMDB. A few minutes of irradiation caused an increase of the absorption at 510 nm to the same level as observed for the HMB solution. Analysis of γ-irradiated HMDB solution after warm up to room temperature indicated a substantial conversion of HMDB to HMB (18% at the dose of 10^4 Gy). The conversion yield did not change when the sample was photolyzed with UV light.
(9) Taking into consideration the conversion yield of HMDB to HMB, the absorption of $HMB^{\bullet+}$ (curve a in Figure 2), and assuming a quantitative neutralization of $HMB^{\bullet+}$ to HMB we have estimated extinction coefficient for $HMB^{\bullet+}$ at 510 nm (ϵ = 2300).

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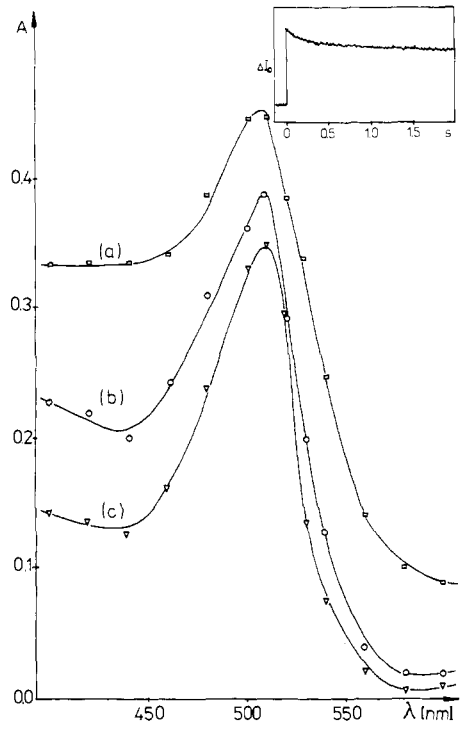

Figure 2. Transient absorption spectra of HMB solution at 93 K obtained (a) 7×10^{-4} s, (b) 0.25 s, and (c) 2.5 s after the 4 μs-electron pulse delivering a dose of 600 Gy. The sample contained HMB (0.02 M) and 1-butyl chloride (1 M) in 3-methylpentane. Inset: scope trace at 510 nm.

The time-resolved spectra for the HMDB system are very much different. The spectrum determined after the pulse has practically no absorption in the observation range (Figure 3). However, the $HMB^{\bullet+}$ absorption at 510 nm appeared to grow with time, and 0.25 s after the pulse a spectrum with a maximum at 510 nm was clearly seen.[11] The evident growth of this absorption was noticed both at 77 and 93 K (insets in Figures 1 and 3). The delayed formation of a signal at 510 nm can be assigned to the unimolecular valence isomerization of $HMDB^{\bullet+}$ (reaction 1). This picture is also consistent with the steady-state measurements. Ignoring the decay of $HMB^{\bullet+}$ ($k_2 \ll k_1$) one can calculate the rate constant k_1. At 77 K this assumption is even unnecessary since k_2 is practically zero. The calculated values of k_1 are 1.71 and 0.015 s^{-1} at temperatures of 93 and 77 K, respectively. Activation parameters associated with the isomerization process were calculated to be $E_A = 17.6$ kJ/mol and $A = 1.3 \times 10^{10}$ s^{-1}. We believe that these values are related to the intrinsic process of valence isomerization, and they are not associated with softening of the matrix, which controls the decay of $HMB^{\bullet+}$.[3] The processes concerning dissipation of the excess energy in rigid matrices are faster and do not coincide with our observation.[13-15] This lends support to a view that the reaction studied involves vibrationally relaxed radical ions.

Our efforts to monitor directly the absorption of $HMDB^{\bullet+}$ have not been successful. If the absorption of $HMDB^{\bullet+}$ lies below 350 nm the detection is difficult or even impossible since that range is obscure by the strong absorption from the radicals. In the region of 350-700 nm the absorption of $HMDB^{\bullet+}$ might escape from the detection only when it is very weak, i.e., $\epsilon < 100$. We have not searched for $HMDB^{\bullet+}$ in the region of $\lambda > 700$ nm.

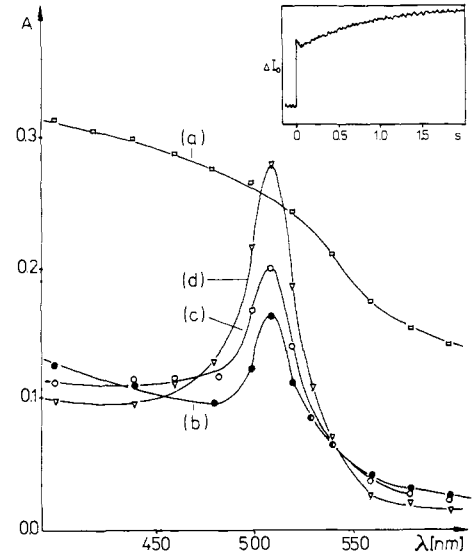

Figure 3. Transient absorption spectra of HMDB solution at 93 K obtained (a) 7×10^{-4} s, (b) 0.25 s, (c) 0.5 s, and (d) 2.5 s after the 4 μs-electron pulse delivering a dose of 600 Gy. The sample contained HMDB (0.02 M) and 1-butyl chloride (1 M) in 3-methylpentane. Inset: scope trace at 510 nm.

Acknowledgment. We acknowledge a cooperation of the staff of the accelerator laboratory. This work was supported by the research program CPBP 01.19.

Registry No. $HMDB^{\bullet+}$, 85293-78-3; $HMB^{\bullet+}$, 34473-51-3; HMDB, 7641-77-2; HMB, 87-85-4.

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Phospholipids Chiral at Phosphorus. 18. Stereochemistry of Phosphatidylinositide-Specific Phospholipase C[1]

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Phosphatidylinositides-specific phospholipase C (PI-PLC), a key enzyme in the metabolism of phosphatidylinositides, catalyzes the formation of three second messengers: diacylglycerol, inositol 1,4,5-trisphosphate, and inositol 1,2-cyclic 4,5-trisphosphate.[2-4] Despite its biological significance and its mechanistic uniqueness in producing both cyclic and open inositol phosphates simultaneously, little mechanistic information about this enzyme has been available. We report the stereochemical mechanism of PI-PLC from *Bacillus cereus*.

Scheme I outlines the synthesis of R_p and S_p isomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphoinositol (DPPsI). The starting material **1** (DL) was synthesized from *myo*-inositol as described by Garegg et al.[5] Resolution of D and L enantiomers was achieved by derivatization with (-)-camphanic acid chloride

(1) Supported by research Grant GM 30327 from NIH. Paper 17: Sarvis, H. E.; Loffredo, W.; Dluhy, R. A.; Hernqvist, L.; Wisner, D.; Tsai, M.-D. *Biochemistry* **1988**, *27*, 4625-4631.
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