  Bull. Chem. Soc. Jpn., 62, 3783—3789 (1989)

Electrochemical Studies of the Reduction Mechanism of Tetrazolium Salts and Formazans

Kisaburo UMEMOTO
Department of Pure and Applied Sciences, College of Arts and Sciences,
The University of Tokyo, Komaba, Meguro-ku, Tokyo 153
(Received March 24, 1989)

The electrochemical study clarified that ditetrazolium salts are reduced not only to the half-reduced "monoformazans" but also to the fully-reduced "diformazans" at the potential where the first electron is transferred to generate the "tetrazolinyl radical". The key reaction leading to the simultaneous generation of mono- and diformazans was the disproportionation reaction of the tetrazolinyl radicals. The identification of absorption spectra of reaction intermediates was performed at several reaction stages.

Although tetrazolium salts are useful qualitative reagents for the detection of dehydrogenase[1-3] and superoxide radical,[4-7] their use as quantitative reagents has been less successful because of their complex chemistry.[1-4]

For example, Nitro Blue Tetrazolium chloride (NBT)—a tetrazolium salt most widely used for the detection of superoxide radical—is reduced to a blue colored diformazan by a four-electron reaction, and as such, NBT is taken as a four equivalent reagent in the redox reaction. However, when NBT was used as an electron acceptor in organic solvents, the final reaction product contained not only the diformazan but also the half-reduced monoformazan.[8] The reaction of ditetrazolium salts with sodium ascorbate in ethanolic solution also gave a mixture of the mono- and diformazans.[2]

Although ditetrazolium salts are known to exhibit several reaction stages,[1,2,4,10-16] the reaction paths leading to the simultaneous generation of mono- and diformazans are little known. The purpose of the present work is to clarify the reaction paths of the tetrazolium salts and their formazans in order to elucidate the problems encountered in the quantitative use of the tetrazolium salts.

Experimental

The tetrazolium salts†—2,3,5-triphenyltetrazolium chloride (TTC), Neotetrazolium chloride (NT), Blue Tetrazolium chloride (BT), and Nitro Blue Tetrazolium chloride (NBT)—were obtained from Tokyo Kasei and purified by recrystallization from ethanol-diethyl ether mixture. The formazans of TTC, NT, BT, and NBT were prepared by the method of Altman and Butcher.[2] The preparation of tetraethylammonium perchlorate (TEAP) and the purification of dimethyl sulfoxide (DMSO) were carried out by the usual methods.[8]

† NT: 3,3′-[1,1′-biphenyl]-4,4′-diylbis[2,5-diphenyl-2*H*-tetrazolium] dichloride. BT: 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2,5-diphenyl-2*H*-tetrazolium] dichloride. NBT: 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)-bis[2-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium] dichloride. The molecular structure and the abbreviation of the molecular species are given in Figs. 2, 6, 8, and 10.

The controlled potential electrolysis was carried out by using an H-type cell equipped with a sintered glass separator. The cathode compartment of the cell was made of a standard 1.00 cm spectrophotometric quartz cell, and the absorption spectra of the reaction products were measured by in-situ method.[8] The cathode was a Pt gauge and the anode was a spiral Pt wire. Potentials were all referred to an SCE. The salt bridge connected to the SCE was put in the anode compartment of the cell. Nitrogen gas was bubbled through both compartments in order to deaerate and to agitate the solution during the electrolysis. All experiments were carried out at the room temperature—ca. 20 °C.

Results and Discussion

Reduction of TTC and Its Formazan.[9] The cyclic voltammogram of TTC is given in Fig. 1a. The peak potential separation of the first redox couple (A/A′) is ca. 60 mV, indicating the electrode reaction is a reversible one-electron reaction. Coulometry at −0.8 V, corresponding to the second wave, revealed the overall electrode reaction is a two-electron reaction, and as the result of the electrolysis, a highly red colored solution of formazan anion (TF^-) was obtained as shown in Fig. 2c. From these results, the following stepwise electron transfer reactions are given.

$$TT^+ + e \rightleftarrows TT\cdot \quad (1)$$

$$TT\cdot + e \rightarrow TF^- \quad (2)$$

When the coulometry at −0.55 V, carried out for an attempt to obtain a solution of tetrazolinyl radical (TT·), was brought to completeness, two electrons, contrary to the probable one electron, were found to be transferred per molecule of TTC. The resulted solution gave no ESR signal and gave exactly the same absorption spectrum as that of TF^-. However, when the electrolysis was interrupted half-way, the ESR spectrum of 2,3,5-triphenyl tetrazolinyl radical[10,11,15] was obtained as shown in Fig. 3, irrespective of the electrolysis potential being either −0.55 V or −0.8 V. The same radical was also generated when TTC was added to a solution containing TF^- alone. These results suggest that the following disproportionation reaction of TT· is operating in the solution.

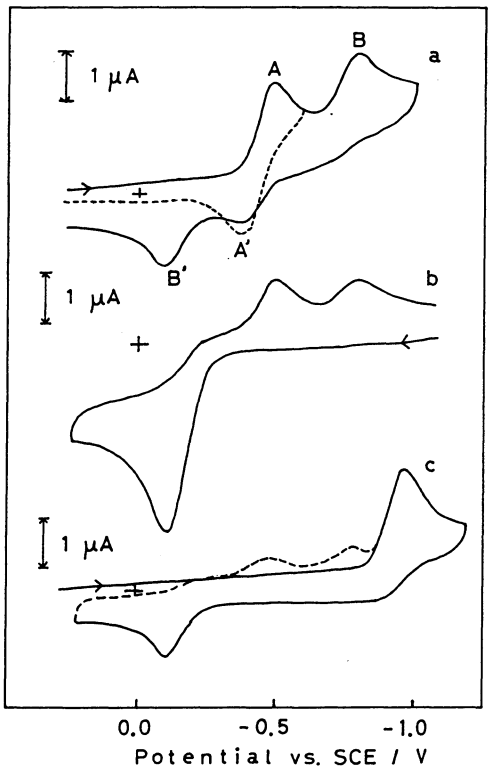


Fig. 1. Cyclic voltammograms of (a) TT^+, (b) TF^- generated by electrochemical reduction of TT^+, and (c) TF. The concentration of depolarizers is 2.5×10^{-4} mol dm^{-3} in DMSO containing 0.1 mol dm^{-3} TEAP. Scan rate: 0.05 V s^{-1}.

$$2\,TT\cdot \rightleftarrows TT^+ + TF^- \quad (3)$$

Although the monitoring of the concentration of TT· or TF^- following the controlled potential electrolysis was unsuccessful because the radical disproportionated rapidly, the disproportionation was appreciable on the cyclic voltammetry. As the half-life of the disproportionation reaction is dependent on the concentration of the radical, the cyclic voltammogram of TT^+ was affected by both the scan rate and the concentration of the depolarizer. In order to determine the rate constant of the disproportionation reaction, a series of cyclic voltammograms were taken by carrying out the potential reversal at −0.6 V, immediately after the first cathodic wave. At sufficiently high scan rate (over 0.2 V s^{-1} for a solution containing 1.0×10^{-3} M of TT^+; 1M=1 mol dm^{-3}) or at sufficiently low concentration of the depolarizer (5×10^{-4} M TT^+ at a scan rate of 0.05 V s^{-1}) only the anodic peak of TT·(A′) was observed. With the increase of the length of the time of the potential hold at −0.6 V as well as with the increase of the concentration of the depolarizer or the decrease of the scan rate, the anodic peak of TF^-(B′) increased in hight with the consumption of the anodic peak of TT·. The rate constant of the disproportionation reaction was estimated to be 2.5×10^2 mol^{-1} s^{-1} by the method of Ohmstead and Nicholson.[17]

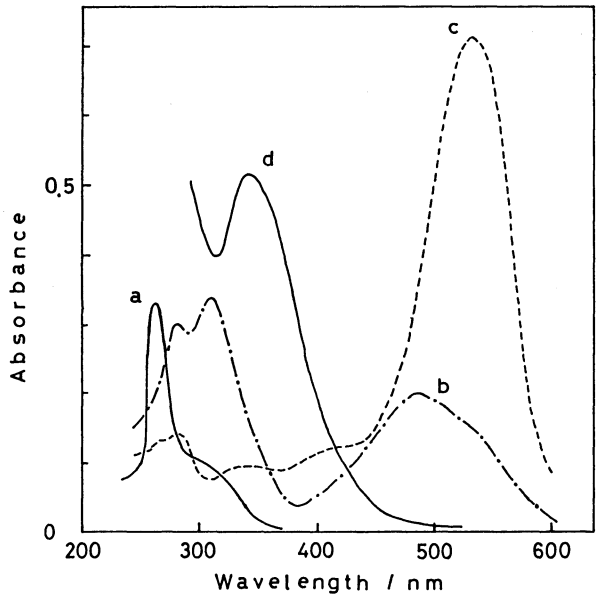
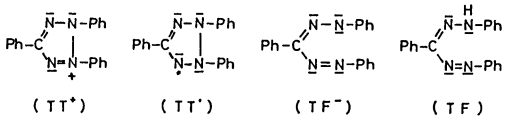


Fig. 2. Absorption spectra of (a) TT^+; 1×10^{-5} mol dm^{-3} in DMSO, (b) TF; 1×10^{-5} mol dm^{-3} in DMSO, (c) TF^- in DMSO generated by electrochemical reduction of TT^+, and (d) TT· generated by the reaction of TF^- with TT^+ in DMSO-benzene mixture (DMSO: benzene=80 : 20 in volume).



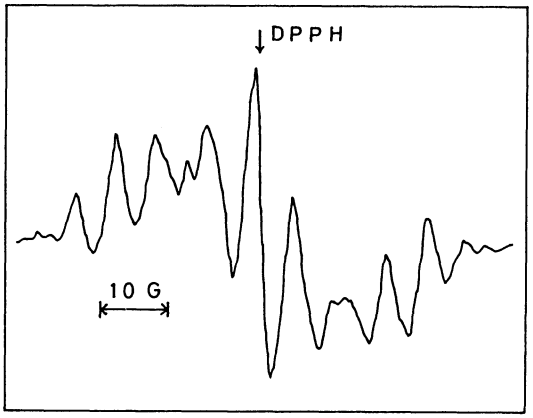


Fig. 3. ESR spectrum of TT· generated by electrochemical reduction of TT^+ in DMSO.

On the absorption spectrum, any band attributable to the radical was not observed. However, when benzene and TTC were added, the color of the solution turned from red to yellow and a new absorption band appeared at 345 nm with the consumption of the absorption band of TF^- as shown in Fig. 2d. The yellow colored solution gave a strong ESR spectrum of TT·. Thus the absorption band at 345 nm was attributed to TT·. This band was gradually substituted by that of TF^- with the increase of the content of DMSO in the DMSO-benzene mixture. The equili-

brium constant of the disproportionation reaction given by Eq. 3 was estimated to be ca. 5×10^2 in pure DMSO.

The cyclic voltammogram of formazan anion TF^-, shown in Fig. 1b, exhibited two cathodic waves at potentials quite close to those of TT^+, indicative of the regeneration of TT^+ by electrochemical oxidation of TF^-. The coulometry of TF^- at +0.2 V revealed the electrode process is a two-electron oxidation, and the original TT^+ was confirmed to be recovered in high efficiency as follows.

$$TF^- - 2e \rightarrow TT^+ \quad (4)$$

The formazan anion TF^- was stable in anhydrous DMSO, but when proton donors—water and acids, such as benzoic acid and acetic acid—were added, TF^- was protonated as follows.

$$TF^- + H^+ \rightarrow TF \quad (5)$$

The cyclic voltammogram of 1,3,5-triphenylformazan (TF) is given in Fig. 1c. The coulometry at −1.0 V revealed TF is reduced to TF^- by a one-electron reaction as follows.

$$TF + e \rightarrow TF^- + H \quad (6)$$

The reaction paths of TTC are summarized in Scheme 1.

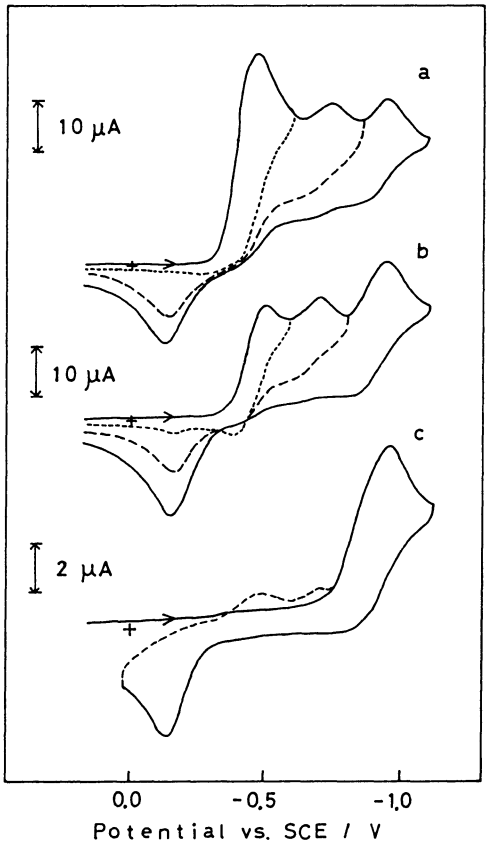
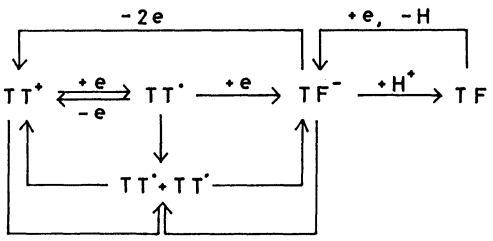


Fig. 4. Cyclic voltammograms of (a) NT^{2+} (1×10^{-3} mol dm^{-3}), (b) $NTMF^+$ (1×10^{-3} mol dm^{-3}), and (c) NTDF (saturated) in DMSO (containing 0.1 mol dm^{-3} TEAP. Scan rate: 0.05 V s^{-1}.



Scheme 1. Reaction paths of TT^+.

Reduction of NT and Its Formazans. Since NT^{2+} is a ditetrazolium ion having a molecular structure composed of two equivalent TT^+, the reaction paths clarified for TT^+ and TF provide the basis for understanding the complicated redox behaviors of NT^{2+} and its formazans. The cyclic voltammograms and absorption spectra of NT^{2+}, NT-monoformazan ($NTMF^+$), and NT-diformazan(NTDF) are given in Figs. 4—6.

As $NTMF^+$ has a molecular structure composed of one teterazolium ring and one formazan moiety, the cyclic voltammogram of $NTMF^+$ revealed the characteristics of both structures. By referring to the cyclic voltammograms of TT^+ and TF, the first and the

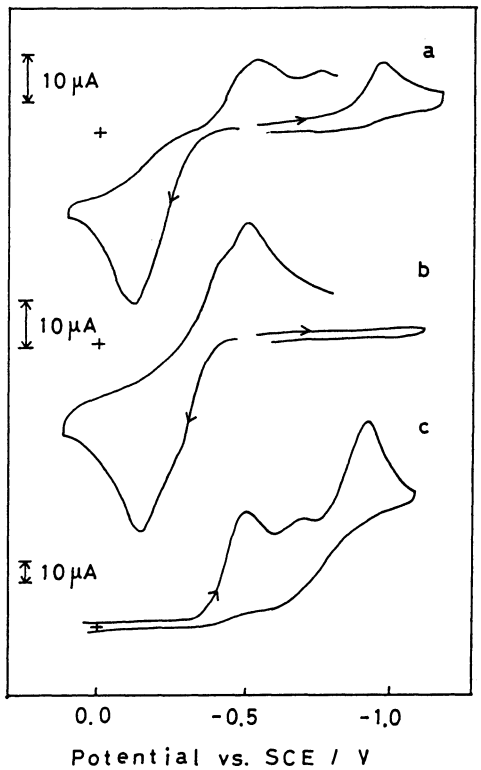


Fig. 5. Cyclic voltammograms of (a) the solution obtained by the exhaustive electrolysis of NT^{2+} at −0.55 V, (b) $NTDF^{2-}$ generated by the reaction of NTDF with tetraethylammonium hydroxide (TEAOH), (c) NT^{2+} in the presence of equimolar amount of acetic acid. The concentration of depolarizers is 1×10^{-3} mol dm^{-3} in DMSO containing 0.1 mol dm^{-3} TEAP. Scan rate: 0.05 V s^{-1}.

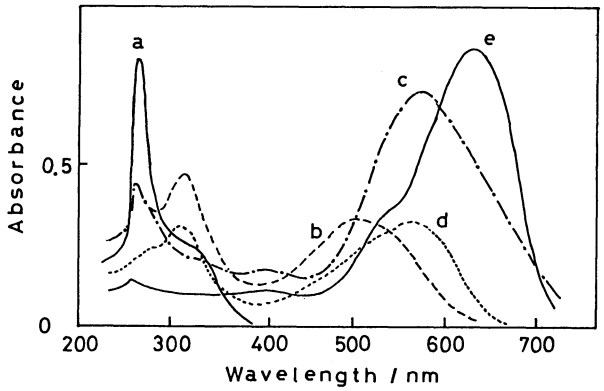
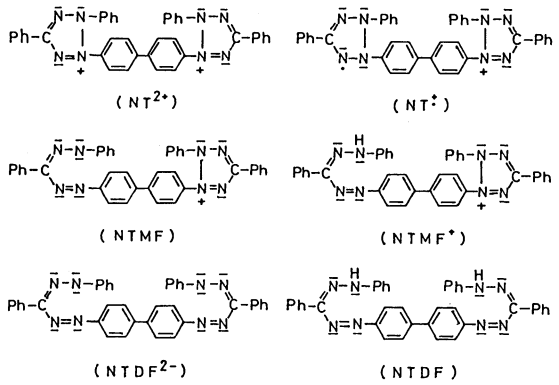


Fig. 6. Absorption spectra of (a) NT^{2+}, (b) $NTMF^+$, (c) NTMF, (d) NTDF, and (e) $NTDF^{2-}$. The concentration is 1.5×10^{-5} mol dm^{-3} in DMSO. The range of absorbance of (d) and (e) is 1/2.



second cathodic waves were attributed to the reduction of the tetrazolium ring. The third wave agreed with the reduction wave of TF.

NTDF exhibited only one cathodic wave at a potential quite close to that of TF, suggesting that there is no or little difference in the potential required for the reduction of any formazan structure in TF, $NTMF^+$, or NTDF. In the presence of base, NTDF dissociated to generate $NTDF^{2-}$, and the cathodic wave of NTDF disappeared as shown in Fig. 5b.

By analogy with $NTMF^+$ the first and the second wave of NT^{2+} were attributed to the reduction of the tetrazolium ring. It is seen that there is no or little difference in the reduction potentials of the mono- and ditetrazolium ions. The observation of the third wave of NT^{2+} indicates the formazan anion of NT is more easily protonated than TF^-. In the presence of proton donor, the formazan peak of NT^{2+} increased in height and the anodic peak of formazan anion disappeared by the protonation reaction as shown in Fig. 5c.

When the coulometry of $NTMF^+$ at -0.55 V, corresponding to the first wave, was completed, overall two electrons were transferred per molecule of $NTMF^+$ as in the case of TT^+. The resulted solution was deep purplish colored. By addition of acids, the solution gave an absorption spectrum of NTDF (λ_{max}=558 nm) as shown in Fig. 6d. These results indicate the reaction paths given by Eqs. 1—5 are exactly working in the reduction of the one-sided tetrazolium moiety of $NTMF^+$ as follows.

$$NTMF^+ + e \rightleftarrows NTMF\cdot \quad (7)$$

$$2\,NTMF\cdot \rightleftarrows NTMF^+ + NTDF^- \quad (8)$$

$$NTDF^- + H^+ \rightarrow NTDF \quad (9)$$

Due to the disproportionation reaction of the tetrazolinyl radical (Eq. 8), the anodic peaks of the cyclic voltammogram of $NTMF^+$ were affected by both the scan rate and the concentration of the depolarizer as in the case of TT^+. The rate constant of the disproportionation reaction was estimated to be 2×10^3 mol^{-1} s^{-1}.

When the constant potential electrolysis of NT^{2+} at -0.55 V was interrupted at the early stage of the electrolysis, the absorption spectrum of NTMF (λ_{max}=570 nm) and an ESR spectrum quite similar to that of TT· were obtained. These species turned to $NTMF^+$ (λ_{max}=500 nm) by addition of acid. These experimental results suggest the reaction paths leading to $NTMF^+$ from NT^{2+} must be the disproportionation reaction of the one-sided tetrazolinyl radical as follows.

$$NT^{2+} + e \rightleftarrows NT^{\cdot+} \quad (10)$$

$$2\,NT^{\cdot+} \rightleftarrows NT^{2+} + NTMF \quad (11)$$

$$NTMF + H^+ \rightarrow NTMF^+ \quad (12)$$

The rate constant of the disproportionation reaction was estimated to be 3×10^3 mol^{-1} s^{-1} by the cyclic voltammetry. As NT^{2+} is a ditetrazolium ion, the generation of a biradical seems to be possible. However, the ESR measurement showed the presence of only one radical species which did not show any line broadening characteristic of the biradical.[15] Such a biradical may be unstable, if existent, since following electron transfer reaction is probable as $NT^{\cdot+}$ must be more stable than NT:.

$$NT^{2+} + 2e \rightarrow NT: \xrightarrow{NT^{2+}} 2\,NT^{\cdot+} \quad (13)$$

When the coulometry of NT^{2+} at the first wave was brought to completeness, the overall reaction was found to be a four-electron reaction. The reaction products were $NTDF^{2-}$ (λ_{max}=626 nm) and its formazans as shown in Fig. 5a. By addition of acid, NTDF (λ=558 nm) was obtained. The generation of diformazan at the first wave suggests the reduction of the residual one-sided tetrazolium ring of NTMF must follow the reactions shown by Eqs. 10 and 11 as follows.

$$NTMF + e \rightleftarrows NTMF^{\cdot-} \quad (14)$$

$$2\,NTMF^{\cdot-} \rightleftarrows NTMF + NTDF^{2-} \quad (15)$$

$$NTDF^{2-} + 2\,H^+ \rightarrow NTDF \quad (16)$$

When NT^{2+} was added into the solution of $NTDF^{2-}$, NTMF was generated. Accordingly, the following disproportionation reaction is also working in the solution.

$$2\,NTMF \rightleftarrows NT^{2+} + NTDF^{2-} \quad (17)$$

As the reduction potential of the one-sided tetrazolium ring of NTMF is almost the same with that of NT^{2+}, the reactions shown by Eqs. 14—17 will occur in parallel with those given by Eqs. 10—13. As the disproportionation reaction given by Eq. 17 is advantageous for the generation of NTMF at high concentration of NT^{2+}, the preferencial generation of monoformazan was observed at the early stage of electrolysis, but the simultaneous generation of both formazans became remarkable on proceeding the electrolysis.

$NTMF^+$ and NTDF gave $NTDF^{2-}$ by the constant potential electrolysis at −1.0 V.

$$NTMF^+ + 3e - H \rightarrow NTDF^{2-} \quad (18)$$

$$NTDF + 2e - 2H \rightarrow NTDF^{2-} \quad (19)$$

As shown above, the complicated reactions of NT^{2+} is considered to be composed of the combination of the fundamental reactions characteristic of the single tetrazolium ion.

Reduction of BT and Its Formazans. BT^{2+} has a molecular structure in which two methoxy groups are introduced into the biphenyl structure of NT^{2+}. The present study was focused on how the fundamental reactions of NT^{2+} are modified by the introduction of methoxy group.

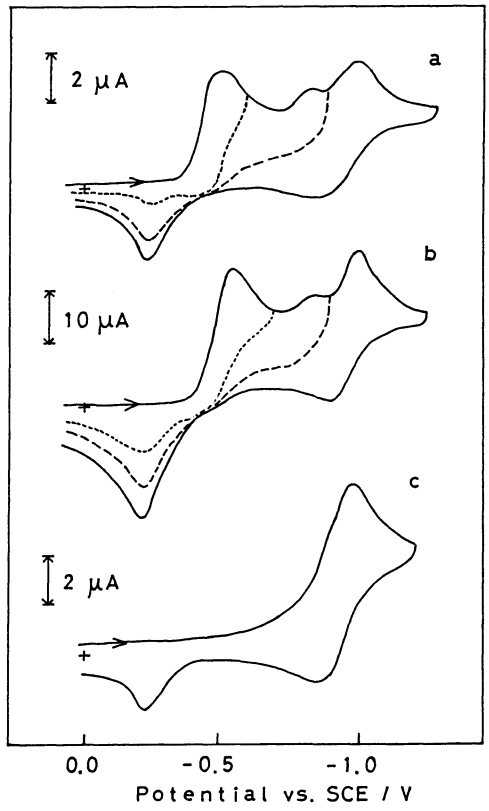


Fig. 7. Cyclic voltammograms of (a) BT^{2+} (saturated -2×10^{-4} mol dm^{-3}), (b) $BTMF^+$ (1×10^{-3} mol dm^{-3}), and (c) BTDF (saturated) in DMSO containing 0.1 mol dm^{-3} TEAP. Scan rate: 0.05 V s^{-1}.

The cyclic voltammograms and the absorption spectra of BT^{2+} and its reaction products are given in Figs. 7 and 8. By referring to the results obtained for NT^{2+}, the first two cathodic waves on the cyclic voltammograms of BT^{2+} and $BTMF^+$ were attributed to the reduction of the tetrazolium ring and the third wave to the reduction of the formazan moiety. The anodic wave was attributed to the oxidation of the formazan anion.

The constant potential electrolysis revealed BT^{2+} is reduced to $BTMF^+$ (λ_{max}=540 nm) by a two-electron reaction and to BTDF (λ_{max}=608 nm) by a four-electron reaction at the first cathodic wave. BTDF was reduced to $BTDF^{2-}$ (λ_{max}=640 nm) at −1.0 V, and $BTDF^{2-}$ reacted with BT^{2+} to give BTMF by a disproportionation reaction analogous to Eq. 17.

From these results, it is likely that the reaction paths of BT^{2+} are exactly analogous to those of NT^{2+} given by Eqs. 7—19, indicative of no effect of the methoxy group.

Reduction of NBT and Its Formazans. The cyclic voltammograms and the absorption spectra of NBT^{2+} and its reduction products are given in Figs. 9 and 10.

As $NBTMF^+$ has one tetrazolium structure and one formazan structure, the cyclic voltammogram of $NBTMF^+$ shown in Fig. 9b is well understood by

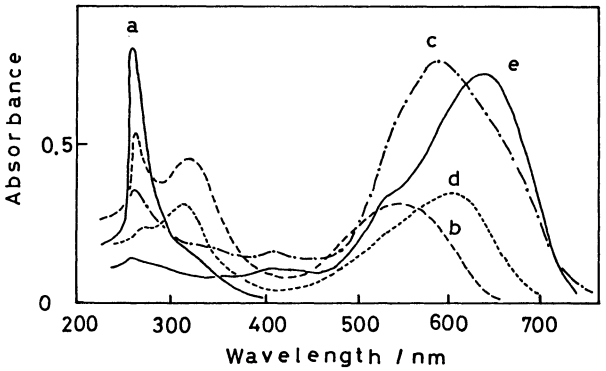
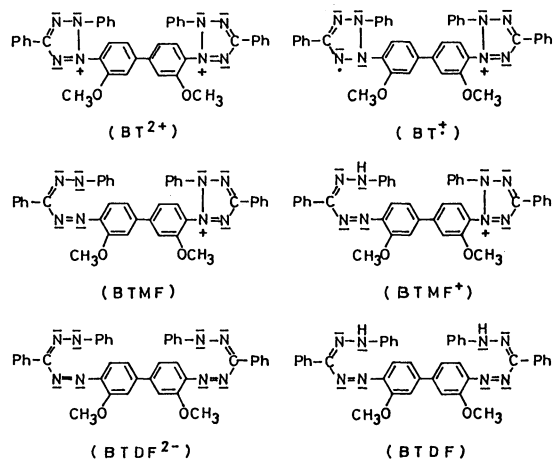


Fig. 8. Absorption spectra of (a) BT^{2+}, (b) $BTMF^+$, (c) BTMF, (d) BTDF, and (e) $BTDF^{2-}$. The concentration is 1.5×10^{-5} mol dm^{-3} in DMSO. The range of absorbance of (d) and (e) is 1/2.



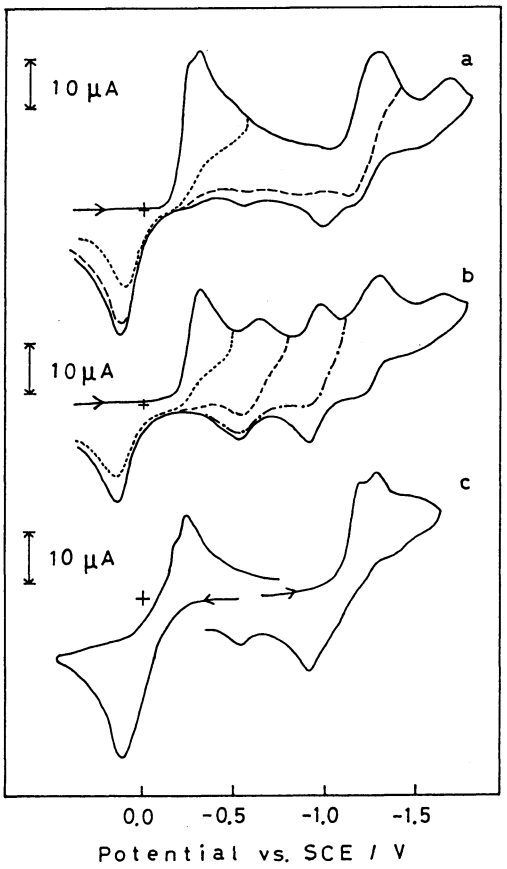


Fig. 9. Cyclic voltammograms of (a) NBT^{2+}, (b) $NBTMF^{+}$, and (c) $NBTDF^{2-}$ generated by the reaction of NBTDF with TEAOH. The concentration of depolarizers is 1×10^{-3} mol dm^{-3} in DMSO containing 0.1 mol dm^{-3} TEAP. Scan rate: 0.05 V s^{-1}.

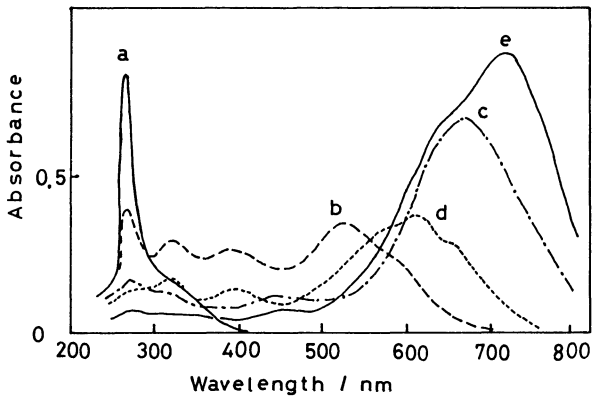
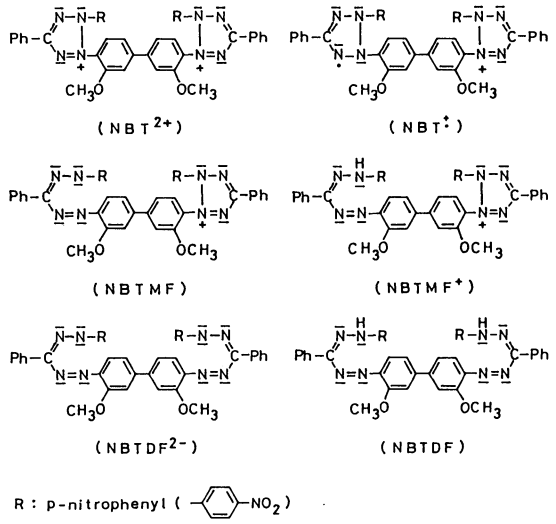


Fig. 10. Absorption spectra of (a) NBT^{2+}, (b) $NBTMF^{+}$, (c) NBTMF, (d) NBTDF, and (e) $NBTDF^{2-}$. The concentration is 1.5×10^{-5} mol dm^{-3} in DMSO. The range of absorbance of (d) and (e) is 1/2.



analogy with that of $NTMF^{+}$. The first and the second cathodic waves were characterized to the reduction of the tetrazolium ring, the third wave to the reduction of the formazan structure and the rests to the reduction of the nitro group, since nitrobenzene derivatives gave reduction waves around these potentials.

The coulometry at the first wave revealed that $NBTMF^{+}$ was reduced to $NBTDF^{-}$ (λ_{max}=709 nm) by a two-electron reaction. The reaction paths leading to $NBTDF^{-}$ from $NBTMF^{+}$ are considered to be similar to those of $NTMF^{+}$ shown by Eqs. 7—9. The rate constant of the disproportionation reaction of the tetrazolinyl radical was estimated to be 5×10^{3} mol^{-1} s^{-1} by cyclic voltammetry. The reduction of $NBTMF^{+}$ at the third wave gave $NBTDF^{2-}$ (λ_{max}=724 nm). $NBTDF^{-}$ and $NBTDF^{2-}$ thus generated gave NBTDF (λ_{max}=600 nm) by addition of acid. NBTDF is insoluble not only in DMSO but also in usual organic solvent, such as ethanol, benzene, ether, acetonitrile, and *N,N*-dimethylformamide. However, the DMSO solution of NBTDF appeared to be homogeneous immediately after being shaken with the ultrasonic vibrator, and gave a reproducible absorption spectrum as shown in Fig. 10d. Although the reduction potential of NBTDF could not be determined, the electrolysis of NBTDF was performed at −1.0 V, a potential now specified to be characteristic of the reduction of the formazan structure. Nitrogen purged through the solution for the deaeration secured the continuous contact of NBTDF particle with the Pt mesh electrode. The current density was low, but as the result of the electrolysis, a sky blue solution of $NBTDF^{2-}$ was obtained. $NBTDF^{2-}$ gave a cyclic votammogram as shown in Fig. 9c. The reduction waves at ca. −1.2 V are due to the nitro group. By the electrolysis at +0.2 V, $NBTDF^{2-}$ was oxidized to NBT^{2+}.

The cyclic voltammogram of NBT^{2+} is given in Fig. 9a. By analogy with $NBTMF^{+}$, the first cathodic wave was attributed to the reduction of the tetrazolium ring and the rests to the reduction of the nitro group. The small shoulder at the first wave may discriminate the small difference in the potential of the generation of mono- or ditetrazolinyl radical, but the waves corresponding to the second and the third waves exhibited for NT^{2+} and BT^{2+} were not observed.

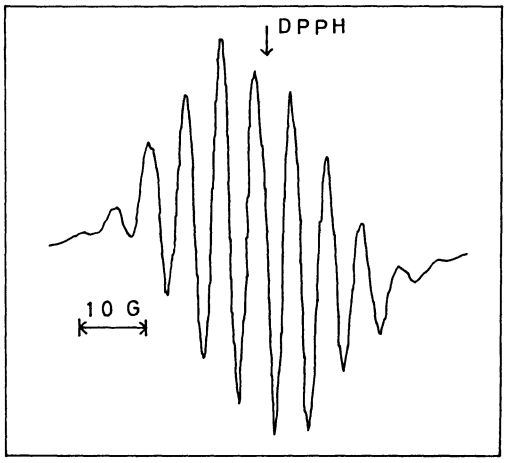


Fig. 11. ESR spectrum of the tetrazolinyl radical generated by electrochemical reduction of NBT^{2+}.

At the early stage of the constant potential electrolysis at −0.4 V, NBT^{2+} gave NBTMF (λ_{max}=680 nm) and a tetrazolinyl radical NBT^{+} which gave an ESR spectrum as shown in Fig. 11. The ESR spectrum with nine line hyperfine structure is characteristic of the tetrazolinyl monoradical, and the biradical of NBT was not evidenced as in the case of NT. The rate constant of the disproportionation reaction of the tetrazolinyl radical was evaluated to be 5×10^3 mol^{-1} s^{-1} by the cyclic voltammetry. By addition of acids, both the ESR spectrum and the absorption spectrum of NBTMF disappeared and the absorption spectrum of $NBTMF^+$ (λ_{max}=530 nm) was obtained.

When the constant potential electrolysis at the first wave was completed, NBT^{2+} was reduced to $NBTDF^{2-}$ by a four-electron reaction. $NBTDF^{2-}$ was stable in anhydrous DMSO and accordingly the cyclic voltammogram of NBT^{2+} did not exhibit any formazan wave. When NBT was added into the solution of $NBTDF^{2-}$, NBTMF was generated by a disproportionation reaction analogous to that shown by Eq. 17.

The reaction scheme shown for NBT^{2+} is exactly understood by reaction paths given by Eqs. 10—19, suggesting no affect of both nitro- and methoxy groups on the fundamental reactions characteristic of the tetrazolium structure.

Summary

Ditetrazolium salts were first reduced to half-reduced monoformazans and successively to fully-reduced diformazans. In both reaction stages, the key reaction leading to the generation of each formazan was the disproportionation reaction of the tetrazolinyl radical generated by one-electron reduction of the tetrazolium structure. As there was almost no difference in the potential required for the generation of tetrazolinyl radical from mono- or ditetrazolium ion, the simultaneous generation of mono- and diformazans appears to be a general feature of the reduction of any ditetrazolium salts. It was only at the early stage of the electrolysis that the half-reduced monoformazan was preferencially generated. In the analytical use of ditetrazolium salts, it was only when the reductant was very weak[2] or the tetrazolium salts existed in vast molar excess over the reductant[4] that the preferential generation of monoformazan secured the quantitative use of the ditetrazolium salts. Otherwise, the separated amount of mono- and diformazan should be determined[3] for the successful application of the tetrazolium method.

The author thanks to Dr. Ryo Hirasawa for his kind permission of using the ESR spectrometer.

References

1) A. W. Nineham, *Chem. Rev.*, **55**, 355 (1955).
2) F. P. Altman and G. R. Butcher, *Histochemie*, **37**, 333 (1973); F. P. Altman, *ibid.*, **38**, 155 (1974).
3) R. G. Butcher and F. P. Altman, *Histochemie*, **37**, 351 (1973).
4) B. H. J. Bielski, G. G. Shiue, and S. Bajuk, *J. Phys. Chem.*, **84**, 830 (1980).
5) I. Saito, T. Matsuura, and K. Inoue, *J. Am. Chem. Soc.*, **105**, 3200 (1983).
6) E. Lee-Ruff, *Chem. Soc. Rev.*, **6**, 195 (1977).
7) F. W. Beyer Jr. and I. Fridovich, *Anal. Biochem.*, **161**, 559 (1987).
8) K. Umemoto and N. Okamura, *Bull. Chem. Soc. Jpn.*, **59**, 3047 (1986).
9) K. Umemoto, *Bull. Chem. Soc. Jpn.*, **58**, 2051 (1985).
10) Y. Deguchi and H. Takagi, *Tetrahedron Lett.*, **1967**, 3179.
11) O. W. Maender and G. C. Russell, *J. Org. Chem.*, **31**, 442 (1966); F. A. Neugebauer and C. A. Russell, *ibid.*, **33**, 2744 (1968).
12) F. A. Neugebauer, *Tetrahedron*, **26**, 4843 (1970).
13) K. M. Dyumaev, G. V. Formin, and Z. K. Kriminskaya, *Khim. Vys. Energ.*, **16**, 558 (1982).
14) Z. K. Kriminskaya, K. M. Dyumaev, L. S. Vischipanova, Yu. V. Ivanov, and A. K. Pikaev, *Khim. Vys. Energ.*, **18**, 40 (1984).
15) N. Azuma, K. Mukai, and K. Ishizu, *Bull. Chem. Soc. Jpn.*, **43**, 3960 (1970).
16) I. Tabakovic, M. Trkovnik, and Z. Gruic, *J. Chem. Soc., Perkin Trans. 2*, **1979**, 166.
17) M. L. Ohmstead and R. S. Nicholson, *Anal. Chem.*, **41**, 862 (1969).