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17. Akira Hanaki, Tomoko Hanaki,*1 Kazumi Ōya,*2 Atsushi Andou,*3 Tohru Hino,*1 and Sanya Akaboshi*1 : Reactivities of Radiation-protective Aminoalkylisothiuronium Salts. I. Hydrogen Ion Liberation of 2-Aminoethylisothiuronium and 3-Aminopropylisothiuronium Bromide Hydrobromide.

(*National Institute of Radiological Sciences*,*1 *Daiichi Seiyaku Co., Ltd.*,*2 *and Daiichi Pure Chemicals Co., Ltd.**3)

A considerable number of substances with -N-C-C-S-R skelton have been found to possess the protective action against an otherwise lethal dose of ionizing radiation.[1] These protective compounds fall into two sub-group by the nature of -S-R group. One is aminothiol, the prototype of which is 2-mercaptoethylamine (MEA). The other is aminothiol derivative in which thiol group is masked. A typical protector of this group is 2-aminoethylisothiuronium bromide hydrobromide (AET). AET, though stable in solid state, is unstable in neutral aqueous solution and is capable of undergoing rearrangement to mercaptoethylguanidine (MEG) having a reactive sulfhydryl group. Some of the homologous compounds not having -N-C-C-S-R skelton have been shown to possess the protective ability. 3-Aminopropylisothiuronium bromide hydrobromide (APT), a typical compound of tnis category, is transformed to 3-mercaptopropylguanidine (MPG) and possesses the protective ability.[3] These results suggest that the substances having either the reactive sulfhydryl or the sulfur-containing group capable of being transformed to reactive sulfhydryl group might be highly effective in the radiation protection.

The possible reactions of AET in aqueous solution are as follows : The first is the intramolecular transguanylation in neutral solution, the second is the cyclization, *i.e.*, thiazoline ring formation, and the third is the cleavage of the isothiuronium group (Chart 1). Doherty suggested that both the transguanylation and the cyclization proceeded through a same cyclic intermediate, and that MEG, the transguanylation

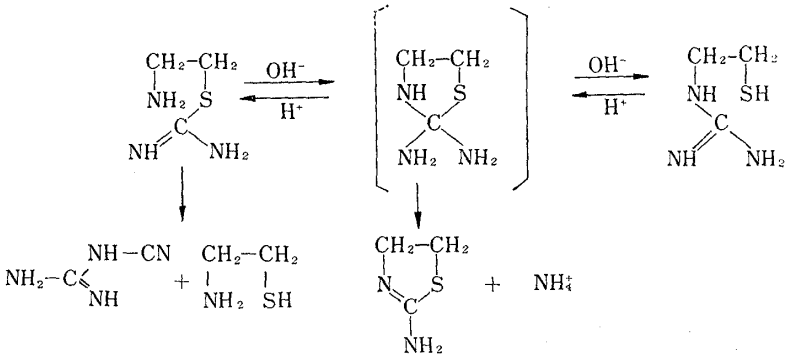

Chart 1. Possible Reactions of 2-Aminoethylisothiuronium Bromide Hydrobromide in Aqueous Solution[2]

*1 Anagawa, Chiba-shi (花木 昭, 花木トモコ, 日野 亨, 赤星三弥).
*2 Hirakawabashi, Sumida-ku, Tokyo (大屋和美).
*3 Tokai-mura, Naka-gun, Ibaragi-ken (安東 醇).

1) A. Hollander : "Radiation Protection and Recovery," (1960). Pergamon Press, London; S. Akaboshi: Yakugaku Zasshi, **83**, 1005 (1963).
2) J.X. Khym, *et al.* : J. Am. Chem. Soc., **79**, 5663 (1957).
3) J.X. Khym, *et al.* : *Ibid.*, **80**, 3342 (1957).

product, might be an effective form of AET in the protective action and 2-aminothiazoline, the cyclization product, has a little effect.[2] Therefore, it is of quite interest to study on the tendency to proceed of those two reactions; because the relative rate of the transguanylation to the cyclization might controll the effectiveness of a aminoalkylisothiuronium salt as the radiation-protective agent. The possibility of the cleavage of isothiuronium group can be disregarded in the present work, because it is observed only in strong alkaline medium.[2]

When alkali was added into the solution of AET or APT, was observed a rapid pH drop which continued for two or three hours. In the presence of alkali more than one equivalent with respect to the thiuronium salt, pH drop was no longer observed. This fact suggested that pH drop might be related to some reaction which is accompanied with the acid-dissociation of the thiuronium salt. However, it has yet been uncertain whether the pH drop is detected as a result of transguanylation or cyclization or other reactions. In the present paper, we attempted to investigate why AET and APT in aqueous solution liberated hydrogen ion in the presence of alkali.

Experimental

AET and APT, which were prepared by the method of Doherty and co-workers,[2,3] were dissolved in 0.1*M* NaCl just prior to use. Since both AET and APT were relatively stable in acidic media, 0.1*N* HCl, usually 0.2 equivalent with respect to aminoalkylisothiuronium salts, was added to the solution. An aliquot, usually 10 ml., of this stock solution was pippetted into 0.1*M* NaCl solution which has been kept at constant temperature. In the ordinary measurement, the temperature was maintained at 25± 0.5°. The reaction was started by adding 0.1*N* NaOH. In each case, total volume of the reaction mixture was 100 ml. and ionic strength was 0.1 with NaCl. During the measurement, the reaction mixture was agitated with a magnetic stirrer.

pH measurement was done with a Toa-Dempa Model HM-5A pH meter equipped with a HG-2005 type glass electrode. This instrument was standardized with phthalate (pH 4.01), phosphate (pH 6.86) and borate (pH 9.18) buffers before and after the measurement. pH values during the reaction were automatically recorded with a Toa-Dempa Model EPR-2T recorder.

The pH titration was semi-automatically carried out with the instrument described above. Approximately 100 ml. of the solution containing 2.00×10^{-4} moles of aminoalkylisothiuronium salts and total ionic strength of 0.1 was titrated with decarbonated 0.1*N* NaOH from a 5 ml. Metrohm piston-biuret. NaOH standard solution was added at the speed of 0.2 ml. per 30 sec., and pH values was automatically recorded. Total volume of the solution was 100 ml. at the half neutralization point. The temperature was maintained at 25° in all the determinations.

Results and Discussion

When a little amount of alkali was added to the solution containing aminoalkylisothiuronium salts, a rapid drop of pH was observed continuously. The curves given in Fig. 1 show the relation between hydrogen ion activities which were calibrated from pH values measured at constant ionic strength of 0.1 and reaction time. In the case of APT, hydrogen ion activities were linearly increased with reaction time. In the case of AET, a latent period for a few minute in the initial stage was accompanied with a period in which hydrogen ion activities were linearly increased with time. The former stage was named the initial state, and the latter the steady state.

In the presence of 0.2 equivalent sodium hydroxide, pH drops (ΔpH) for 3 hours' reaction of $5.00\times10^{-3}M$ AET and APT were about 3.0 and 2.6 respectively. After 3 hours' reaction at 25°, pH values of AET and APT solutions reached to approximately 3.8 and 5.4 respectively. Provided that the activity coefficient of hydrogen ion at ionic strength of 0.1 is unity, the liberated hydrogen ion from AET is $1.6\times10^{-4}M$ and from APT is $4.0\times10^{-6}M$. Those data showed that 1 mole AET liberated about 0.03 mole hydrogen ion in the presence of 0.2 equivalent sodium hydroxide. On the

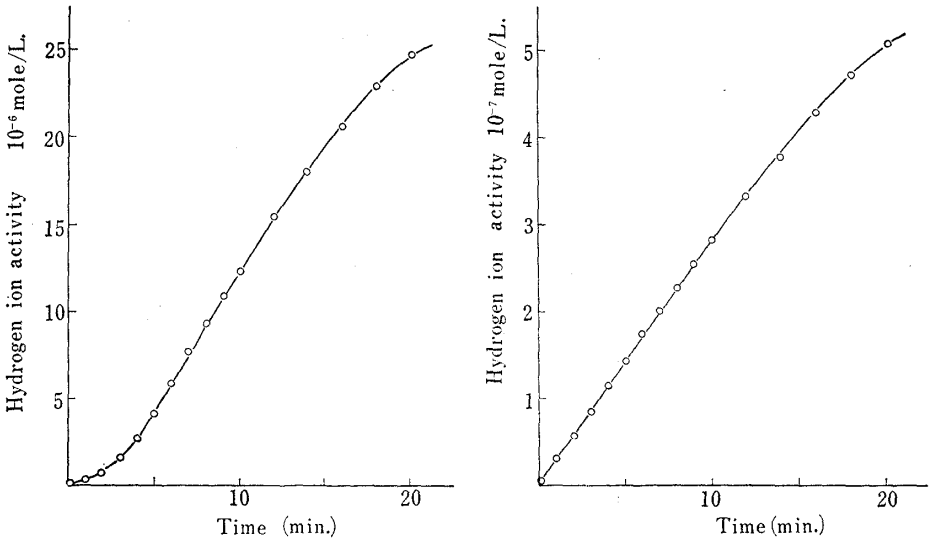

Fig. 1. Hydrogen Ion Liberation Curves of 2-Aminoethylisothiuronium Bromide Hydrobromide (left) and 3-Aminopropylisothiuronium Bromide Hydrobromide (right)

$2\times10^{-3}M$ AET or APT containing 0.4 equivalent NaOH was stirred at 30°.

other hand, 1 mole APT liberated hydrogen ion less than 0.001 mole in the same condition. In the presence of 0.6 equivalent alkali, pH drops for 3 hours were approximately 3.4 for AET and 2.6 for APT. In Table I were presented pH drops for 10 minute of AET and APT in the presence of varying amounts of alkali. Though ΔpH

Table I. pH Drops of 2-Aminoethylisothiuronium Bromide Hydrobromide and 3-Aminopropylisothiuronium Bromide Hydrobromide solution in the Presence of Varying Amounts of Alkali

AET			APT		
Alkali equivalent	Initial pH	ΔpH for 10 min.	Alkali equivalent	Initial pH	ΔpH for 10 min.
0.2	6.95	−1.82	0.2	7.99	−1.19
0.4	7.36	−1.89	0.4	8.38	−1.46
0.6	7.66	−1.70	0.6	8.65	−1.60
0.8	7.97	−0.92	0.8	8.88	−1.69
			1.0	9.06	−1.48

Concentration of AET and APT: $2.00\times10^{-3}M$
Temperature: 25° Ionic Strength: 0.1 (NaCl)

appeared to be resemble in each case, the pH values after 10 minute were quite different; the pH values of AET solution were lower than that of APT. It means that AET may be less stable than APT and liberate more hydrogen ion. Thus, it appeared that the pH drop, that is the increment of hydrogen ion concentration, might be related to the nature of aminoalkylisothiuronium salts as well as the amounts of alkali presented. In Fig. 2 was presented the relationship between the rate of hydrogen ion liberation, $\Delta a_{H^+}/\Delta t$, and the amount of alkali added; Δa_{H^+} represented the increment of hydrogen ion activity. In each case, the rate appeared to be decreased with the increasing amounts of alkali added. In the case of AET, the curve showed concave forms, and in the presence of alkali more than 0.8 equivalent, there observed no hydrogen ion liberation. On the other hand, the curve for APT showed a convex form in the presence of alkali less than 1.1 equivalents. Aminoalkylisothiuronium

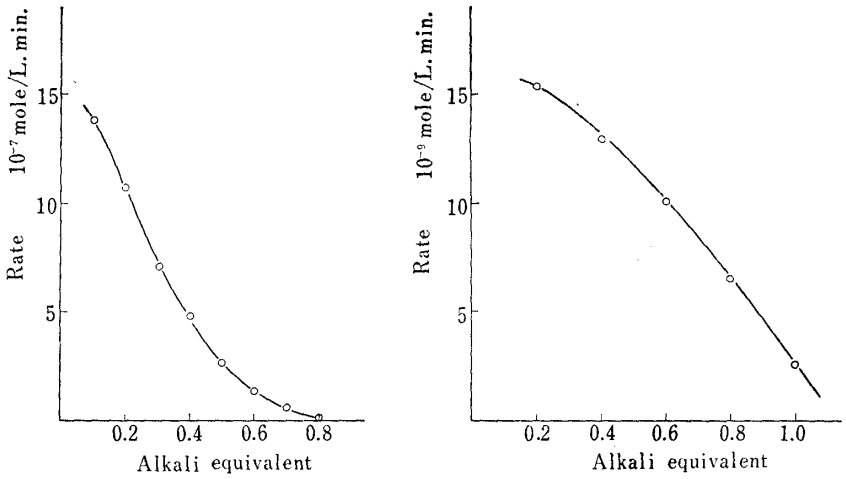

Fig. 2. The Rate of Hydrogen Ion Liberation from 2-Aminoethylisothiuronium Bromide Hydrobromide (left) and 3-Aminopropylisothiuronium Bromide Hydrobromide (right) in the Presence of Varying Amounts of Alkali

Concentration of AET and APT; $2.00\times10^{-3}M$.
The reaction was carried out at 25° under mechanical stirring.

salts have two ionizing groups; one is amino group, and the other is isothiuronium group. As to pKa values, the former might have lower value. Since the first ionizing constants of AET and APT due to amino group were approximately 7.5 and 8.5,*4 respectively, and the second constants, which are due to isothiuronium group and probably independent on the structure of the acids were estimated at least 10.[4] pKa values of those two groups, amino and isothiuronium groups, appeared to be adequately separated. These informations supported that one equivalent alkali added first might be consumed almost with the neutralization of amino, precisely ammonium group, and that hydrogen ion liberation might be connected with the dissociation of amino group.

The titration curves of aminoalkylisothiuronium salt appeared to be irreversible at one glance. If the titration was stopped at the half neutralization point and then, after excess hydrochloric acid was added, the solution was re-titrated with alkali, it was found that only half equivalent of initially presented aminoalkylisothiuronium salt could be detected titrimetrically and another half was converted to an different compound, the amount of which corresponded nearly to that of alkali first added. The typical curves were presented in Fig. 3. This finding suggested that the conjugate base of aminoalkylisothiuronium salt was unstable and instantly transformed to another different compound, which was unable to associated with hydrogen ion. The probable reaction was schematically shown as follows;

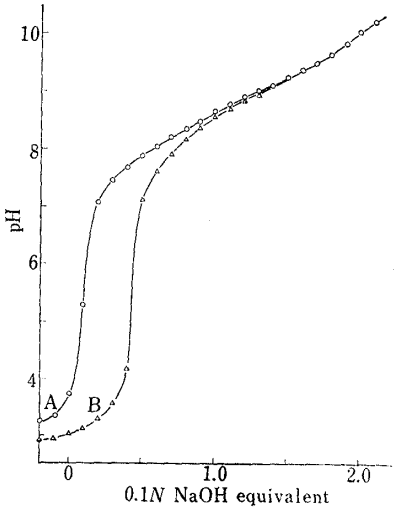

Fig. 3. Titration Curves of 3-Aminopropylisothiuronium Bromide Hydrobromide

Curve A (○——○):
$2.00\times10^{-3}M$ APT solution was titrated with 0.1*N* NaOH at the speed of 0.2 ml./30 sec.(ionic strength 0.1)

Curve B (△——△):
The APT solution which was half-neutralized was stood for 10 min., and then, after excess HCl was added, titrated with 0.1*N* NaOH. The temperature was maintained at 25°

*4 They are approximate values estimated from the pH values at the half neutralization point.

4) M. Charton: J. Org. Chem., **30**, 969 (1965).

$$RH_2^{++} \rightleftarrows RH^+ + H^+ \quad (1)$$

$$RH^+ \longrightarrow R'H^+ \quad (2)$$

$$R'H^+ + H^+ \longrightarrow \text{No change} \quad (3)$$

where RH_2^{++}, RH^+, and $R'H^+$ represented aminoalkylisothiuronium salt, its conjugate base and the reaction product, respectively. Since $R'H^+$ has the ability to reduce 2,4-dichlorophenol indophenol and ferric ion, and also the characteristic absorption band due to RS^- structure,[5] which appears at the vicinity of 230 mμ in alkaline solution, it is probably 2-mercaptoalkylguanidine.*5 Provided that reaction 1 is reversible and reaction 2 is irreversible, the over-all reaction seems to be irreversible; and liberation of hydrogen ion is attributed to the disturbance of equilibrium of reaction 1.

In the absence of alkali, there observed no hydrogen ion liberation in both AET and APT, so that aminoalkylisothiuronium salt appeared to be stable in aqueous solution. Certainly APT was stable in such a condition, while AET was unstable and cyclized to 2-AT during the storage, though a pH drop of the solution was scarcely observed.*5 The experimental results above mentioned support that hydrogen ion liberation from AET or APT is related to the transguanylation which is accompanied with the acid dissociation of aminoalkylisothiuronium salt.

The rate of hydrogen ion liberation was depend on the reaction temperature, as well as the amount of alkali added. In Fig. 4 was presented the curves shown the relation between the rate of hydrogen ion liberation and reaction temperature. Though we have not yet determined the kinetic data of the reaction, such as rate constant, activation energy and frequency factor, it seems probable to compare the reactivity of AET to that of APT and estimate the relative value of kinetic data; because the mechanism of transguanylation of both AET and APT may be resemble. If the logarithmic value of the apparent rate ($\Delta a_{H^+}/\Delta t$) is plotted against the reciprocal

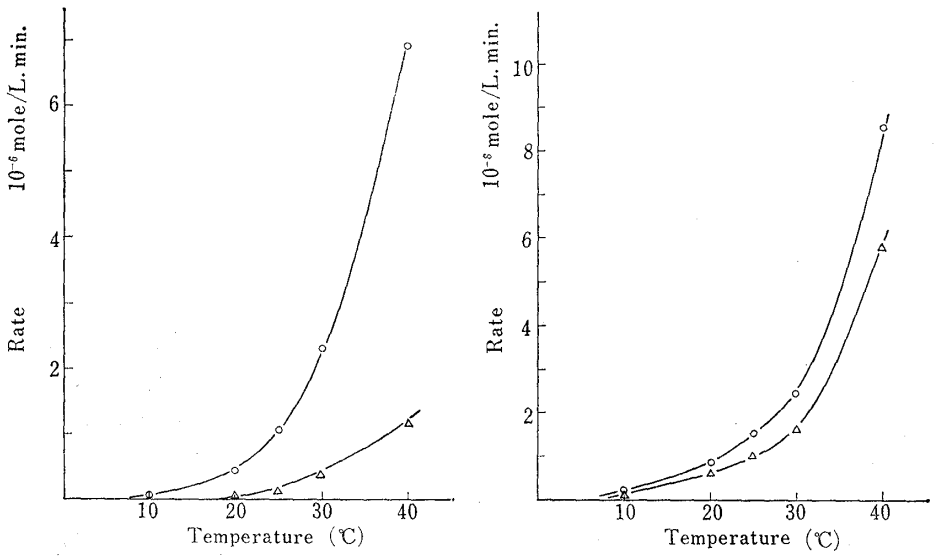

Fig. 4. Effect of Temperature on the Rate of Hydrogen Ion Liberation from 3-Aminoethylisothiuronium Bromide Hydrobromide (left) and 3-Aminopropylisothiuronium Bromide Hydrobromide (right)

Concentration of AET and APT; $2.00 \times 10^{-2} M$
○—○ in the presence of 0.2 equivalent NaOH
△—△ in the presence of 0.6 equivalent NaOH

*5 Unpublished work.

5) L. H. Noda, S. A. Kuby, and H. A. Lardy : J. Am. Chem. Soc., **75**, 913 (1953).

of absolute temperature, an Arrhenius-like plot can be drawn. As the reaction mechanism might be resemble, the ratio of the slops presents the relative value of the activation energy. With the same assumption, the ratio of the frequency factor may be estimated. From Fig. 5 was understood that the ratio of the activation energy,

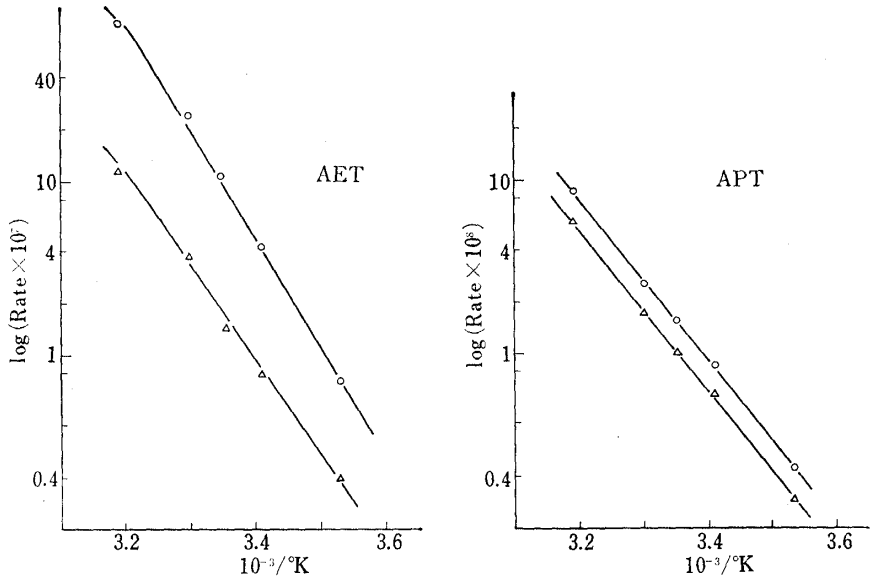

Fig. 5. Relation between the Rate of Hydrogen Ion Liberation and Temperature

Concentration of AET and APT; $2.00\times10^{-3}M$
○—○ in the presence of 0.2 equivalent NaOH
△—△ in the presence of 0.6 equivalent NaOH

AET to APT, was approximately 1.35 in the presence of 0.2 equivalent alkali and 1.20 in the presence of 0.6 equivalent alkali. These values mean that the activation energy of hydrogen ion liberation from AET is 20 or 30 per cent larger than that from APT, in spite of the high reactivity of AET. On the other hand, the frequency factor of hydrogen ion liberation may be more than 40 or 50 times larger in AET. This difference might be attributed to the probability that the reaction intermediate is produced. The intermediate from AET might have five-membered ring, and that from APT six-membered ring. Thus, it is suggested that the frequency factor has main effect on the rate of hydrogen ion liberation from aminoalkylisothiuronium salts.

The authors wish to thank Misses Tanaami and Yamada for preparing AET, APT and other related compounds.

Summary

Hydrogen ion liberation from AET and APT was investigated. In the presence of alkali less than approximately one equivalent with respect to aminoalkylisothiuronium salt, hydrogen ion liberation was observed. In the presence of alkali more than one equivalent, hydrogen ion liberation was not occured. This reaction was attributed to the intramolecular transguanylation which was accompanied with the acid dissociation of AET and APT. The rate of hydrogen ion liberation was more than twenty times fast in AET. The ratio of activation energy was 20 or 30 per cent larger in AET than in APT. In the absence of alkali, AET was transformed to 2-aminothiazoline and ammonium ion was splitted off. While the cyclization of APT was scarcely observed in the same condition. (Received June 26, 1965)

*6 The discussion in detail will be published in the following papers.