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Reactivities of Radiation-protective Aminoalkylisothiuronium Salts. VII.[1] Transguanylation and Cyclization Reactions of 2-Aminoethyl- and 3-Aminopropyl-isothiuronium Salts

Akira Hanaki

National Institute of Radiological Sciences[2]

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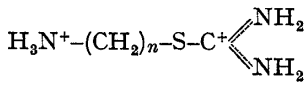
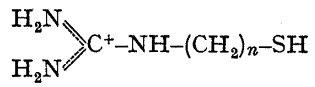
The transformation reactions, *i.e.*, transguanylation and cyclization, of AET and APT were investigated with the potentiometric titration technique. Both reactions, which proceed probably through the similar intermediate, were promoted with the increasing amounts of alkali added initially. In the initial stage of the reaction, the transguanylation occupied larger part in the transformation of AET, as well as in APT. The half life of the conjugate base, which is an active species for the transguanylation, was increased linearly with the reciprocal of the initial concentration of the conjugate base. From the concentration dependence of the half life, measured in the addition of 0.5 equivalent alkali, it was deduced that the transguanylation is second order with respect to the isothiuronium salt. The Arrhenius plots of the rates in AET and in APT gave straight and parallel lines.

Recent studies have revealed that 2-aminoethylisothiuronium (AET) salt, a radiation-protective agent, undergoes in an aqueous solution either transguanylation to yield 2-mercaptoethylguanidine (MEG) or cyclization to 2-amino-2-thiazoline (2-AT) and that 3-aminopropylisothiuronium (APT) salt, another protective agent with an analogous skelton, undergoes transguanylation to 3-mercaptopropylguanidine (MPG) but not cyclization.[3] In both compounds, the transguanylation was initiated by the addition of alkali and its rate was appeared to increase with the amounts of alkali added.[4] This information led to the assumption that the isothiuronium salt, which is a dibasic acid intrinsically,[5] is ionized to a labile conjugate base with its subsequent rapid transformation to the sulfhydryl compound.[6] The cyclization is also promoted by adding alkali, though observed in a weakly acidic medium, and the contribution of the conjugate base to the reaction has been assumed.[7] The present work was attempted to elucidate the mechanism of those transformation reactions by using the potentiometric method, which was presented previously for the determination of the sulfhydryl compound generated from the isothiuronium salt.[8] Since the main reaction is the transguanylation in AET, as well as in APT, and the sulfhydryl product has been assumed to be an active form of those isothiuronium salts as the radiation-protective agent, the emphasis was placed upon the transguanylation.



AET; $n=2$ APT; $n=3$



MEG; $n=2$ MPG; $n=3$

1) Part VI: A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **18**, 399 (1970).
2) Location: *Anagawa-4, Chiba.*
3) J.X. Khym, R. Shapira and D.G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957); **80**, 3342 (1958).
4) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1409 (1968).
5) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **18**, 766 (1970).
6) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 486 (1968).
7) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1146 (1969).
8) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968).

Experimental

Materials——AET and APT, prepared according to the previously reported method,[9] were used after recrystallization from MeOH. Other reagents were commercially available analytical grade chemicals and used without further purification. All the chemicals were dissolved in twice distilled water with all glass apparatus.

Instruments——For the kinetic measurements, a Radiometer TTTlc titrator and SBR2c titrigraph, which were standardized with phthalate and phosphate buffers at an appropriate temperature before the measurement, were used. The glass electrodes 202B and 202C were used for the measurements below and above 20°, respectively.

Kinetic Procedure——The isothiuronium solution, 8.00×10^{-3}M for routine work, was prepared just prior to use by dissolving the salt in 0.1M NaCl. Before each run, the solution was thermostatted in a water jacketted cell for more than 10 min at an appropriate temperature. The reaction was started by adding 0.5 equivalent NaOH rapidly as possible, usually within 7 sec, and the solution was stirred mechanically. Spontaneously after alkali was added, the rapid pH drop was progressed.

a) Transguanylation: After a definite period of time, the reaction was stopped by adding 0.5 equivalent HCl and the solution was titrated with 0.1N NaOH. The titer of NaOH, which corresponds to the amounts of the transguanylation product,[8] was plotted against the reaction time, and the half life of the reactive species was estimated graphically. Some examples were shown in Fig. 1.

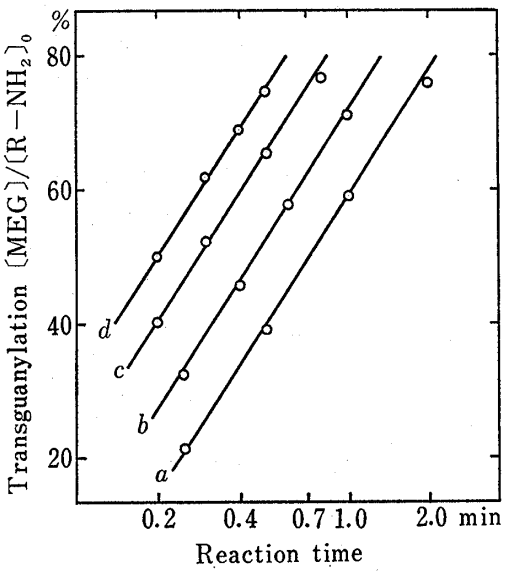


Fig. 1-A. Plot of the Extent of the Transguanylation against the Reaction Time

concentration of AET
a: 5.00×10^{-3}M b: 8.00×10^{-3}M
c: 1.25×10^{-2}M d: 2.00×10^{-2}M
alkali: 0.5 equivalent, temperature: 5°

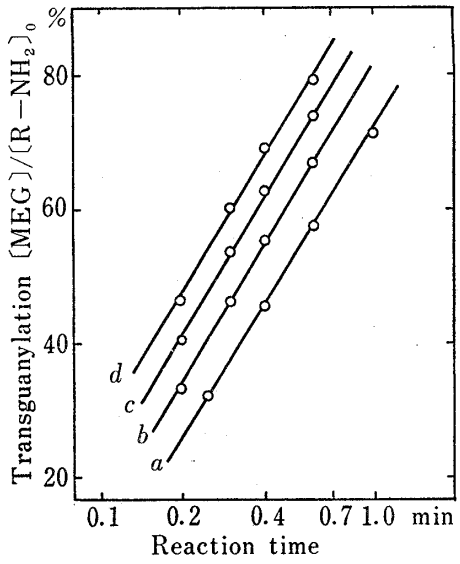


Fig. 1-B. Plot of the Extent of the Transguanylation against the Reaction Time

temperature
a: 5° b: 10° c: 15° d: 20°
concentration of AET: 8.00×10^{-3}M
alkali: 0.5 equivalent

b) Cyclization: The reaction was stopped by adding another 1.0 equivalent NaOH. This treatment induced the quantitative transguanylation of all the isothiuronium salt remained in the reaction medium. The solution was then neutralized with 1.5 equivalent HCl and was titrated with 0.1N NaOH. The extent of the cyclization was evaluated from the difference between the amounts of the initial isothiuronium salt and of free acid titrated.

Result and Discussion

The transguanylation was promoted progressively with the increasing amounts of alkali added.[4] The cyclization, which proceeds rather slowly, was also enhanced in parallel with

9) T. Hino, K. Tana-ami, K. Yamada and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 1139 (1966).

the increase of the alkali concentration as shown in Fig. 2. The isothiuronium salt is ionized first to the conjugate base, and its concentration is expressed briefly by Eq. (1);

$$[R\text{–}NH_2]=[\text{Alkali}]+[H^+]-[OH^-]$$
$$=a[R]_0+[H^+]-[OH^-] \quad (1)$$

where a, $[R\text{–}NH_2]$ and $[R]_0$ represent equivalent alkali with respect ot the isothiuronium salt, the concentrations of the conjugate base and the total isothiuronium salt, respectively. Therefore, the results described above support that both reactions may proceed through the similar reactive species; the conjugate-base. The half life of the conjugate base for the transguanylation, as estimated from Fig. 1, was extremely short under any condition, while the extent of the cyclization was a little under the corresponding condition. The temperature effect of the cyclization was pictured in Fig. 3. From those facts, it is possiblycons idered that the transformation reaction in the first stage is composed of the transguanylation. The half life of the conjugate base was shortened in accordance with the increase of alkali added and was related linearly to the reciprocal of the concentration of the conjugate base produced immediately after adding alkali. The relationship was presented in Fig. 4.

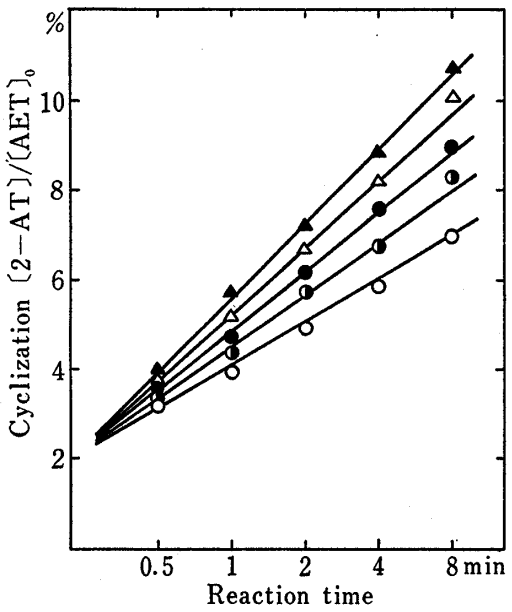


Fig. 2. The Cyclization of AET in the Presence of Various Amounts of Alkali

alkali
—○—: 0.125 eq. —◑—: 0.25 eq.
—●—: 0.375 eq. —△—: 0.5 eq.
—▲—: 0.625 eq.
total concentration of AET: 8.00×10^{-3}M
temperature: 5°

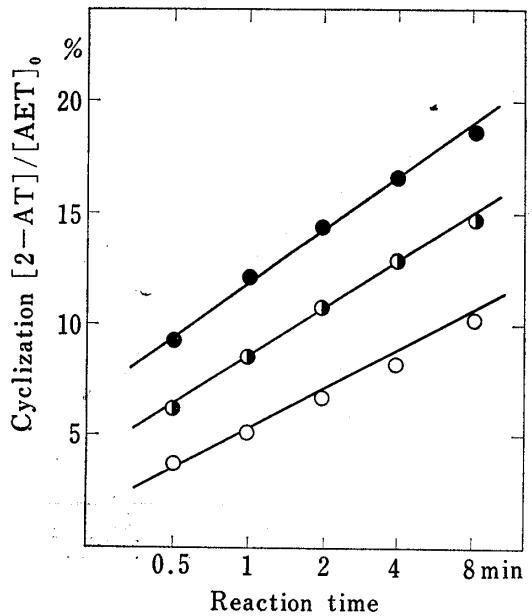


Fig. 3. The Cyclization of AET at Different Temperature

temperature
—○—: 5° —◑—: 15° —●—: 25°
concentration of AET: 8.00×10^{-3}M
alkali: 0.5 equivalent

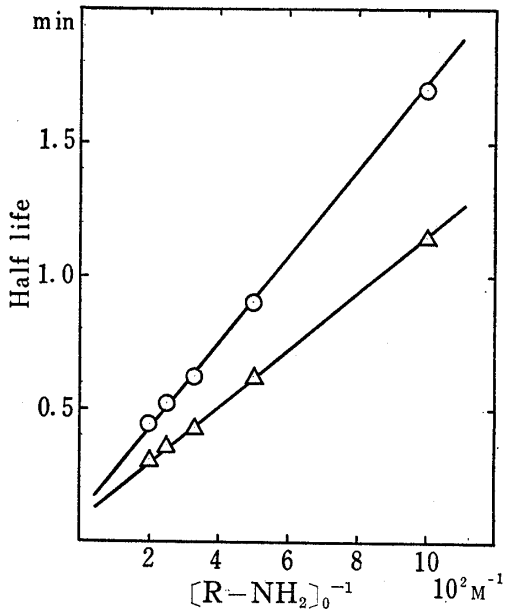


Fig. 4. The Relationship between the Half Life and the Initial Concentration of the Conjugate Base

—○—: AET —△—: APT
total concentration of the isothiuronium salt: 8.00×10^{-3}M
alkali: variable from 0.125 eq. to 0.625 eq.
temperature: 5°

With the increase of the alkali concentration, the rate of the transguanylation and the initial pH value of the medium were increased. The pH value of the medium was lowered progressively in parallel with the extent of the transguanylation. The range of pH drop for the 50% transguanylation was calculated as 0.3 under the condition where less than one equivalent alkali was added initially.[7] Thus, the concentration of hydrogen ion, hence that of hydroxide ion, was varied during the reaction. Since hydroxide ion might contribute to the acceleration of the reaction, the measurement was nessecitated to be done at the constant pH, in order to elucidate and clarify the reaction mechanism. As far as the potentiometric technique presented here was employed, the measurement at the constant pH was impossible. However, as far as the half life was accepted as an index for the rate and as far as a fixed equivalent alkali was added into the medium, the initial pH, decided by pK_a and equivalent alkali added, and the range of pH variation, decided by the life of the active species, were fixed constant. It means that the contribution of hydroxide ion can be kept constant even if the concentration of hydroxide ion and the total concentration of the isothiuronium salt are varied. In the present work, the transguanylation was measured in the presence of half equivalent alkali, where the initial pH corresponds to pK'_a.

The half life thus estimated decreased with the increasing concentrations of the isothiuronium salt. Provided that the transguanylation is first order with respect to the isothiuronium salt, the half life should be constant irrespective of the concentration. The plot of the half life against the reciprocal of the initial concentration of the conjugate base was shown in Fig. 5. There existed a linear relationship between those two variables, indicating a possibility of the second order contribution of the isothiuronium salt in the transguanylation. The second order contribution of the isothiuronium salt might sound as if the transguanylation is a bimolecular, *i.e.*, intermolecular, migration. The transguanylation in APT, depending on the hydroxide ion concentration,[10] is proposed to be a base catalyzed reaction. Since the conjugate base of AET possesses the basic character and the concentration of hydroxide ion under the condition

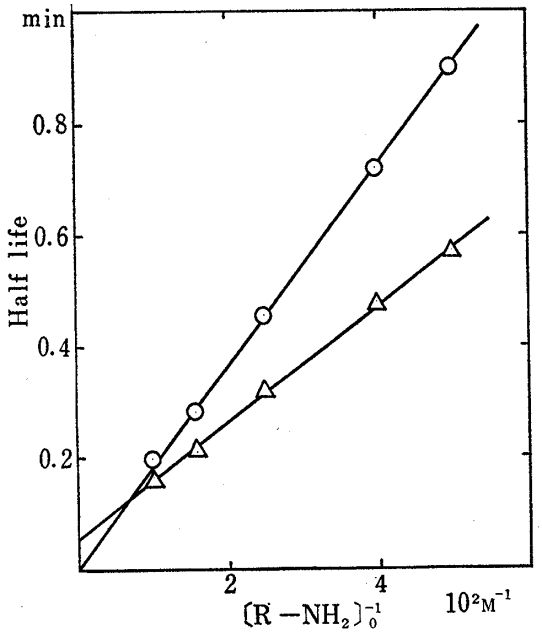


Fig. 5. Concentration Dependence of the Half Life

—○—: AET —△—: APT
$[R\text{-}NH_2]$: $0.5[R]_0$
temperature: 5°

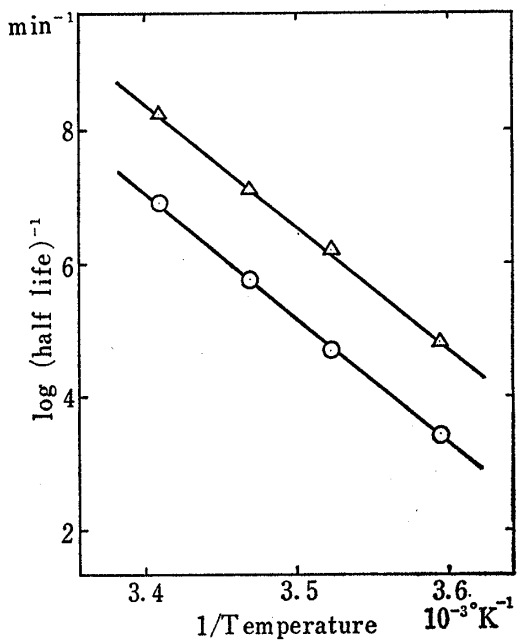


Fig. 6. Arrhenius Plot of the Rate of the Transguanylation

—○—: AET —△—: APT
initial concentration of the conjugate base: 4.00×10^{-3}M

10) A. Hanaki, unpublished data.

Table I. Proton Ionization Constant of AET and APT

	pK_a[a]			
	5°	15°	25°	35°
AET	8.32	7.97	7.63	7.26
APT	9.52	9.16	8.76	8.48

a) ionic strength: 0.1 with KNO_3

examined is extremely low as compared with that of the conjugate base, the transguanylation in AET might be catalyzed by its conjugate base and be considered to be a base catalyzed, intramolecular, migration.

The temperature dependence of the rate, expressed by the reciprocal of the half life, was presented in Fig. 6. In both compounds, there existed a linear Arrhenius-like plot, indicating the constant enthalpy. The slope of both curves was appeared to be parallel, indicating the similar activation energy over the range examined. The rates in Fig. 6 were measured at the same ratio of $[R\text{–}NH_2]/[R]_0$ and under the different concentration of hydroxide ion. Considering that APT possesses a higher pK_a value than AET,[5] one reason why APT is less stable may be explained from the larger contribution of hydroxide ion: at the same ratio of $[R\text{–}NH_2]/[R]_0$, the contribution is approximately 15 times larger in APT than in AET. If the reaction was followed at the same pH, where the ratio of $[R\text{–}NH_2]/[R]_0$ is different, AET may be transguanylated more rapidly. For instance, if the measurement was done at pH 8 and at 5°, the half lives were calculated, from pK_a values in Table I and from Fig. 4, to be 0.5 min for AET and 2 min for APT. The enthalpy change for the ionization in both compounds was shown to be similar, approximately 14 kcal/mole.[5] This means that the difference of pK_a between those two compounds is kept constant when the circumstance becomes changed. Therefore, under the physiological condition, AET is expected to be more susceptible to the transguanylation as compared with APT.

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