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Reactivities of Radiation-protective Aminoalkylisothiuronium Salts. V.[1] Calculation of the Extent of the Transguanylation from pH Drop in the Aqueous Solution of 2-Aminoethyl- and 3-Aminopropyl-isothiuronium Salts

AKIRA HANAKI

National Institute of Radiological Sciences[2]

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2-Aminoethylisothiuronium (AET) and 3-aminopropylisothiuronium (APT) salts undergo, in the presence of alkali, the rapid transguanylation accompanying with the continuous pH drop. Since the liberation of hydrogen ion was resulted undoubtedly from the transguanylation, the calculation of the extent of this transformation was attempted from the pH drop. The extent of the transguanylation, abbreviated as $\boldsymbol{T}$, was expressed briefly as follows.

$$\mathrm{p}(1-\boldsymbol{T})=\Delta\mathrm{pH}$$

This equation can be used in the case of APT, which undergoes the transguanylation alone. AET is cyclized along with the transguanylation, and the cyclization product, 2-aminothiazoline, is able to associate with free hydrogen ion. Therefore, the pH drop observed as a result of a certain extent of the transguanylation is decreased according to the increasing extents of the cyclization. The calculation in the case of AET was performed by using the following equation;

$$\boldsymbol{T}=1-(1-a)K/a[\mathrm{H}^+]+(K-K'')\boldsymbol{C}/(K''+[\mathrm{H}^+])a$$

where $\boldsymbol{C}$, a, K and K'' represent the extent of the cyclization determined experimentally, the equivalent of alkali added, the ionization constants of the transguanylation and the cyclization products, respectively.

Radiation–protective aminoalkylisothiuronium salt, such as 2-aminoethylisothiuronium (AET) or 3-aminopropylisothiuronium (APT) salt, has been shown to undergo the rapid transguanylation to mercaptoalkylguanidine.[3] When caustic alkali is added into an aqueous solution of those compounds, pH is lowered rapidly to a constant level.[4] Since the conjugate base of the isothiuronium salt, namely the ionized species, is very unstable and rapidly transformed to the corresponding mercaptoalkylguanidine, the equilibrium concerning the ionization of the isothiuronium salt is disturbed and thereby free hydrogen ion is liberated.[5] Thus pH drop has been explained as a result of the transguanylation. If the hydrogen ion liberation is related stoichiometrically to the transguanylation, the extent of this transformation may be calculated from pH drop. The present paper concerned with the calculation of the extent of the transguanylation. From the results calculated, the reactivities of AET and APT were discussed relative to the mechanism.

Experimental

Material——AET and APT synthesized in this laboratory were used. All the solutions were prepared from twice distilled water from all glass apparatus.

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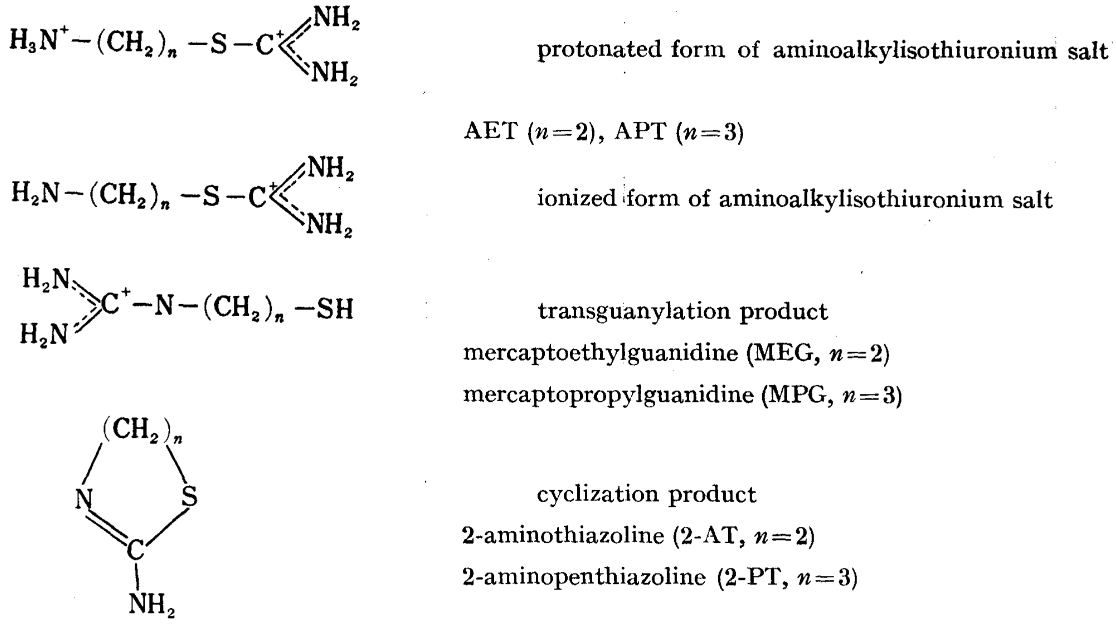
[1] Part IV: A. Hanaki, P. Xumsaeng, T. Hino and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **17**, 677 (1969).

[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, T. Hanaki, K. Oya, A. Andou, T. Hino and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 108 (1966).

[5] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 486 (1968).



protonated form of aminoalkylisothiuronium salt

AET ($n=2$), APT ($n=3$)

ionized form of aminoalkylisothiuronium salt

transguanylation product

mercaptoethylguanidine (MEG, $n=2$)

mercaptopropylguanidine (MPG, $n=3$)

cyclization product

2-aminothiazoline (2-AT, $n=2$)

2-aminopenthiazoline (2-PT, $n=3$)

Chart 1

Potentiometric Determination of the Transguanylation and Cyclization Products——The transguanylation and cyclization products were determined by the potentiometric titration method as described previously.[6] The titration was done with a Radiometer TTTlc titrator and SBR2T titrigraph at an appropriate temperature for the measurement.

Measurement of pH Drop——The measurement was carried out at 5°, 15° and 25° with a Toa-Dempa HM-5A pH meter connected with a ESR-2T recorder. The recording was initiated immediately after adding an appropriate amount of alkali. The detailed procedure was reported previously.[4]

Content of Total SH and Ability of Hydrogen Ion Liberation——The reaction was started by adding 0.2 equivalent NaOH into 250 ml of 2.50×10^{-2}M isothiuronium salt solution, which has been thermostatted at 40°. After 1, 2, 3, 4, 6 and 8 hours, a 10 ml portion of the reaction mixtures was pipetted and neutralized with excess alkali in order to transform the remained isothiuronium salt completely to the SH compound. The SH compound was determined potentiometrically as reported previously.[6] In order to test the ability of hydrogen ion liberation, another 10 ml portion was pipetted from the same reaction mixtures, added into 39 ml of 0.1N NaCl containing 0.5 ml of 0.1N HCl, and thermostatted at 15°. The pH value of the solution was recorded immediately after adding 1.0 ml of 0.1N NaOH, which corresponded to 0.2 equivalent with respect to the total amount of the isothiuronium salt presented initially. As a reference, the variation in the total SH and the ability of hydrogen ion liberation was measured in the absence of alkali.

Calculation and Results

Both AET and APT have been shown to be transguanylated quantitatively, in the presence of excess alkali, to the corresponding mercaptoalkylguanidine.[7] Therefore, if excess alkali is added into the reaction mixtures containing the isothiuronium salt, the transguanylation and cyclization products, the isothiuronium salt is transformed quantitatively to the sulfhydryl compound, which can be determined potentiometrically. The sulfhydryl compound thus determined, which is named tentatively the total SH, includes both the transguanylation product and the isothiuronium salt. The plot of the total SH against the reaction time was presented in Fig. 1. The amount of the total SH apperared to reduce markedly with the reaction time in AET, while did not change significantly in APT. Since the decrease of the total SH corresponds to the formation of the cyclization product, those results indicate that AET is cyclized easily to 2-aminothiazoline(2-AT) but APT is resistant to the cyclization, and the rate of the cyclization in AET was apparently faster in the absence of alkali. It appears from this observation that the cycliza-

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6) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968).

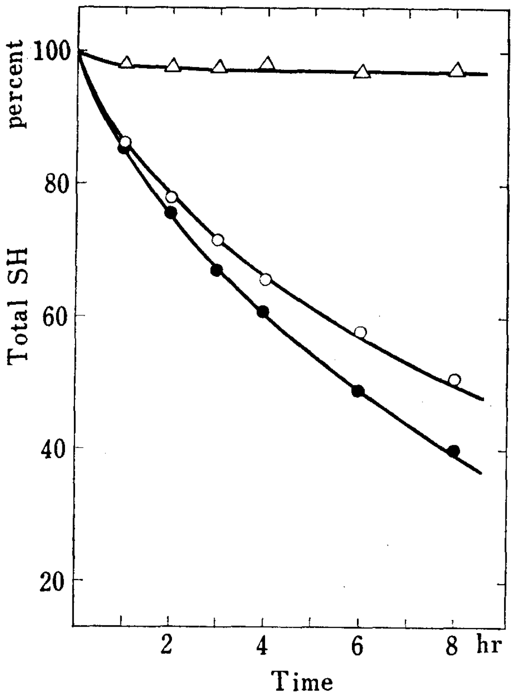

Fig. 1. Plot of the Total SH against the Reaction Time

total concentration: 2.50×10^{-2}M temperature: 40°

—○—: AET —△—: APT

in the presence of 0.2 equivalent NaOH

—●—: AET in the absence of NaOH

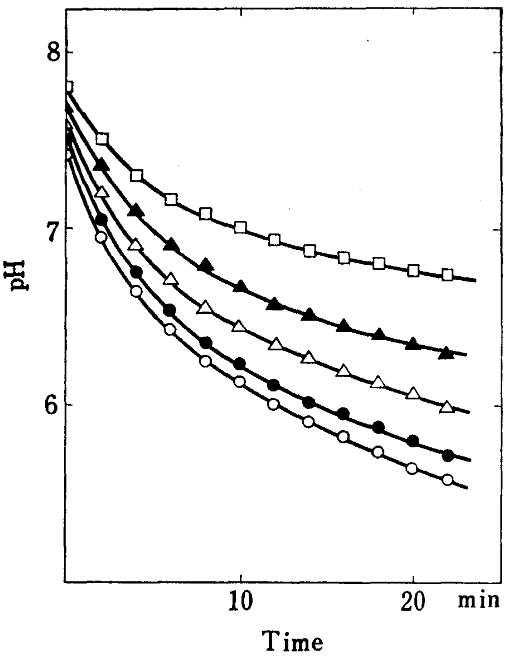

Fig. 2. Hydrogen Ion Liberation from the AET Solution Thermostatting for Different Hours

The AET solution was thermostatted at 40° in the presence of 0.2 equivalent NaOH. After 1 (○), 2 (●), 4 (△), 6 (▲) and 8 (□) hours, the recording of the pH drop, measured at 15°, was initiated by adding another 0.2 equivalent NaOH. The detailed procedure was described in experimental section.

Table I. The Cyclization of AET in the Presence and Absence of Alkali

Time hr	Cyclization $[2\text{-AT}]/(1-a)[\text{AET}]_0$ $a=0$	$a=0.2$	Time hr	Cyclization $[2\text{-AT}]/(1-a)[\text{AET}]_0$ $a=0$	$a=0.2$
1	0.150	0.175	4	0.392	0.432
2	0.246	0.278	6	0.512	0.525
3	0.332	0.358	8	0.600	0.612

$[\text{AET}]_0$: 2.50×10^{-2}M temperature: 40°

Table II. Relation between the Concentration of AET and the pH Drop after Adding Alkali

Time hr	$[\text{AET}]_t$[a) 10^{-3}M	ΔpH/10min	$[\text{APT}]_t$[a) 10^{-3}M	ΔpH/10min
1	3.30	1.29	3.93	1.22
2	2.89	1.25	3.87	1.23
3	2.57	1.20	3.88	1.23
4	2.27	1.15	3.90	1.24
6	1.90	1.02	3.85	1.25
8	1.55	0.80	3.86	1.24

a) $[\text{AET}]_t$ and $[\text{APT}]_t$ indicate, respectively, the concentration of AET and APT after *t* hr.

The concentration of the isothiuronium salt at the start, which corresponds to $(1-a)$ $[\text{Isothiuronium}]_0$ is 4.00×10^{-3}M. After 1,2,3,4,6 and 8 hr, NaOH (0.2 equivalent with respect to $[\text{Isothiuronium}]_0$) was added newly into the reaction mixtures.

tion might be retarded in the presence of alkali. However, since the transguanylation is completed really within a few minute after adding alkali[8] and the extent of this transformation is correlated to the equivalent of alkali added first,[9] the extent of the cyclization should be expressed relative to the real concentration of the isothiuronium salt immediately after adding alkali. The concentration of the ionized AET, immediately after a equivalent alkali has been added,[10] is expresses approximately as $(1-a)\,[\mathrm{AET}]_0$, where $[\mathrm{AET}]_0$ indicates the total concentration of AET. The extent of the cyclization thus calculated in the presence and absence of alkali was shown in Table I. In the presence of alkali, where the concentration of the ionized AET becomes large with the increasing amounts of alkali, the rate of the cyclization was slightly faster, and this fact agreed with the postulated mechanism that the cyclization proceeds also through the ionized AET.

AET was also cyclized even in the absence of alkali as shown in Table I. Since the ionized species of AET, abbreviated as $\mathrm{RNH_2}$, is present little in the absence of alkali, this fact might indicate the possibility that the protonated species, as well as the ionized AET, is also cyclized. On the other hand, the ionized APT does not undergo the cyclization.

The pH value in the reaction mixtures was dropped continuously during the course of the transguanylation. If alkali was added newly into the reaction mixtures after a certain period, the rapid pH drop was initiated again. The pH drop observed newly appeared to depend on the concentration of the isothiuronium salt remained in the solution. The relation between the concentration of the isothiuronium salt and the pH drop was presented in Table II. In APT, which is stable against the cyclization, ΔpH/10 min was nearly constant irrespective of the reaction time. On the other hand, the pH drop in AET was diminished according to the time, and was parallel with the concentration of the remained AET. The profile of the pH drop in AET, which had been thermostatted at 40° for different hours, were shown in Fig. 2. Thus, the pH drop is related undoubtedly to the amount of the isothiuronium salt, which is capable of being transguanylated further by adding alkali.

The transguanylation is initiated by the ionization of the amino group of the isothiuronium salt. The cyclization proceeds through protonated species as well as its ionization product, though the latter might be more susceptible to this reaction. Then, the reactions of the isothiuronium salt in the aqueous solution can be pictured as follows;

$$\mathrm{RNH_3^+} \rightleftarrows \mathrm{RNH_2 + H^+} \qquad \text{i)}$$

$$\mathrm{RNH_2} \longrightarrow \mathrm{R'SH} \qquad \text{ii)}$$

$$\mathrm{RNH_2} \longrightarrow \mathrm{R''NH_2} \qquad \text{iii)}$$

$$\mathrm{RNH_3^+} \longrightarrow \mathrm{R''NH_3^+} \qquad \text{iv)}$$

$$\mathrm{R''NH_2 + H^+} \rightleftarrows \mathrm{R''NH_3^+} \qquad \text{v)}$$

where $\mathrm{RNH_3^+}$, $\mathrm{RNH_2}$, $\mathrm{R'SH}$, $\mathrm{R''NH_2}$ and $\mathrm{R''NH_3^+}$ represent, respectively, the isothiuronium salt, its conjugate base, the transguanylation product, the cyclization product and its conjugate acid. The ionization constants of the isothiuronium salt and the cyclization product are represented respectively by equations (1) and (2).

$$K=[\mathrm{RNH_2}][\mathrm{H^+}]/[\mathrm{RNH_3^+}] \qquad (1)$$

$$K''=[\mathrm{R''NH_2}][\mathrm{H^+}]/[\mathrm{R''NH_3^+}] \qquad (2)$$

In the reaction mixtures, the following equations are satisfied,

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7) A. Hanaki, T. Hino and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1446 (1967).

8) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1393 (1968).

9) A. Hanaki, unpublished data.

10) $a=[\mathrm{alkali}]/[\mathrm{AET}]_0$.

$$[\mathrm{R}]_0=[\mathrm{RNH_3^+}]+[\mathrm{RNH_2}]+[\mathrm{R'SH}]+[\mathrm{R''NH_2}]+[\mathrm{R''NH_3^+}] \qquad (3)$$

$$[\mathrm{NaOH}]=a[\mathrm{R}]_0 \qquad (4)$$

$$[\mathrm{R'SH}]=a[\mathrm{R}]_0\boldsymbol{T} \qquad (5)$$

where $[\mathrm{R}]_0$ and [NaOH] represent the total concentrations of the isothiuronium salt and alkali added, respectively. The terms $\boldsymbol{a}$ and $\boldsymbol{T}$ indicate alkali equivalent and the extent of the transguanylation, respectively. As to the cyclization, let us accept the following equation for simplicity,

$$[\mathrm{R''NH_2}]+[\mathrm{R''NH_3^+}]=[\mathrm{R}]_0\boldsymbol{C} \qquad (6)$$

where $\boldsymbol{C}$ represents the extent of the cyclization. For electroneutrality requirment, equation (7) should be satisfied.

$$[\mathrm{RNH_3^+}]+a[\mathrm{R}]_0+[\mathrm{R''NH_3^+}]+[\mathrm{H^+}]=[\mathrm{R}]_0+[\mathrm{OH^-}]$$
$$=[\mathrm{RNH_3^+}]+[\mathrm{RNH_2}]+(a\boldsymbol{T}+\boldsymbol{C})[\mathrm{R}]_0+[\mathrm{OH^-}] \qquad (7)$$

From equation (7), equations (8) and (9) can be deriven.

$$[\mathrm{RNH_3^+}]=(1-a)[\mathrm{R}]_0-([\mathrm{H^+}]-[\mathrm{OH^-}])-[\mathrm{R''NH_3^+}] \qquad (8)$$

$$[\mathrm{RNH_2}]=a[\mathrm{R}]_0-(a\boldsymbol{T}+\boldsymbol{C})[\mathrm{R}]_0+([\mathrm{H^+}]-[\mathrm{OH^-}])+[\mathrm{R''NH_3^+}] \qquad (9)$$

By combining equations (2) and (6), equation (10) can be obtained.

$$[\mathrm{R''NH_3^+}]=[\mathrm{H^+}][\mathrm{R}]_0\boldsymbol{C}/(K''+[\mathrm{H^+}]) \qquad (10)$$

By introducing equations (8), (9) and (10) into (1), the following equation indicating the relation between the extent of the reactions and the concentration of hydrogen ion can be deriven.

$$\boldsymbol{T}=1-(1-a)K/a[\mathrm{H^+}]+(K-K'')\boldsymbol{C}/(K''+[\mathrm{H^+}])a$$
$$+([\mathrm{H^+}]-[\mathrm{OH^-}])([\mathrm{H^+}]+K)/a[\mathrm{H^+}][\mathrm{R}]_0 \qquad (11)$$

In equation (11), the fourth term of the right-hand side, which is very small as compared with others, can be neglected. The third term, which is a correction term due to the cyclization is always positive, because K is usually larger than K''. If the isothiuronium salt does not undergo the cyclization, where the third term becomes zero, the extent of the transguanylation can be calculated from the hydrogen ion alone. At a definite concentration of hydrogen ion, the value of $\boldsymbol{T}$ increases with $\boldsymbol{C}$. The relation between $\boldsymbol{T}$ and $\boldsymbol{C}$, when a definite pH drop is observed, is presented Fig. 3. This relation indicates in other words that, when a certain extent of the transguanylation is observed, the pH drop decreases in accordance with the increasing extent of the cyclization. In the case of APT, the third term may be neglected, because this compound is resistant to the cyclization.

By using equation (11), the calculation of the extent of the transguanylation was attempted at various temperatures. The calculated values shown in Table III were agreed satisfactorily with the experimental values, which were obtained by the potentiometric titration. The correction term due to the cyclization in AET was shown in the last column of Table III.

Discussion

In order to evaluate approximately the extent of the transguanylation, the following equation may be used,

$$\boldsymbol{T}=1-(1-a)K/a[\mathrm{H^+}] \qquad (12)$$

TABLE III. The Calculated Values for the Extent of the Transguanylation at Various Temperatures

APT at 5°, pK=9.50

Time min	pH	T	
		Calcd.	Obsd.
0.25	9.29	0.383	0.384
0.5	9.11	0.593	0.591
1	8.86	0.771	0.753
2	8.59	0.877	0.855
4	8.34	0.931	0.913

APT at 15°, pK=9.10

Time min	pH	T	
		Calcd.	Obsd.
0.25	8.79	0.510	0.525
0.5	8.51	0.743	0.741
1	8.23	0.864	0.856
2	7.94	0.931	0.951
4	7.61	0.968	0.951

APT at 25°, pK=8.70

Time min	pH	T	
		Calcd.	Obsd.
0.25	8,23	0.661	0.649
0.5	7.92	0.834	0.826
1	7.60	0.921	0.940
2	7.28	0.962	0.962
4	7.01	0.980	0.975

AET at 5°, pK=8.35, pK''=9.47

Time min	pH	C	T		Correction term
			Calcd.	Obsd.	
0.25	8.23	0.023	0.271	0.278	0.030
0.5	8.07	0.038	0.510	0.499	0.035
1	7.87	0.054	0.701	0.673	0.032
2	7.63	0.069	0.833	0.800	0.024
4	7.35	0.084	0.915	0.893	0.015

AET at 15°, pK=7.95, pK''=9.10

Time min	pH	C	T		Correction term
			Calcd.	Obsd.	
0.25	7.72	0.042	0.455	0.437	0.044
0.5	7.50	0.064	0.686	0.671	0.041
1	7.22	0.085	0.843	0.820	0.029
2	6.87	0.105	0.933	0.948	0.016
4	6.51	0.127	0.973	0.970	0.009

AET at 25°, pK=7.60, pK'' =8.75

Time min	pH	C	T		Correction term
			Calcd.	Obsd.	
0.25	7.30	0.069	0.561	0.551	0.062
0.5	7.03	0.097	0.779	0.775	0.048
1	6.68	0.122	0.907	0.896	0.027
2	6.31	0.147	0.963	0.947	0.014
4	5.81	0.170	0.989	0.989	0.005

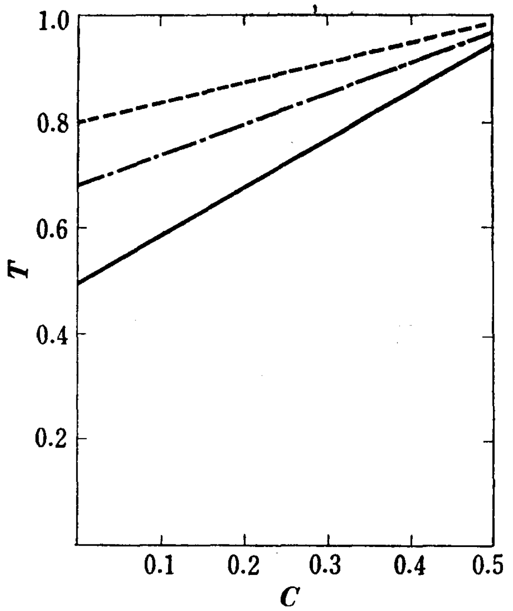

Fig. 3. Relation between *T* and *C*

ΔpH
——— : 0.3
– – – : 0.5
—·—·— : 0.7
a : 0.5 equivalent

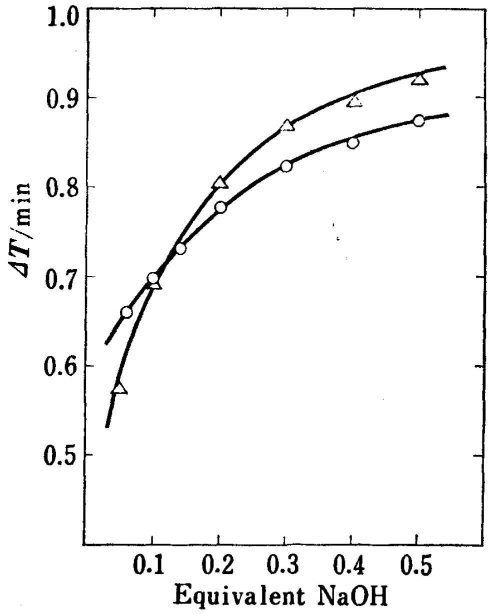

Fig. 4. Dependence of the Rate of the Transguanylation on the Equivalent of Alkali Added

total concentration : 5.00×10^{-3}M
temperature: 25°
—○— : AET —△— : APT

where the term $(1-a)/a$ can be considered constant relative to a definite amount of alkali. Equation (12) can be rewritten as follows.

$$p(1-T) = pK - pH - \log(1-a)/a \qquad (13)$$

The isothiuronium salt is intrinsically a dibasic acid. However, it may be considered that the ionization of the two groups, *i.e.*, amino and amidine groups, of the isothiuronium salt occurs separately. Especially, in the region where alkali added is less than 0.8 equivalent, the ionization of amidine group does not occur, because the second ionization constant, K_2, is approximately 10^{-2} K_1. Therefore, the isothiuronium salt may be treated as a monobasic acid in this region. If the compound is not transguanylated, pH of the solution indicates a definite value relative to the equivalent of alkali added. In this case, the pH value can be expressed as follows.

$$pH = pK - \log(1-a)/a = pH_0 \qquad (14)$$

If the compound is transguanylated, the pH drop is observed and then pH_0 indicates the pH value immediately after adding *a* equivalent alkali. By introducing equation (14) into (13), the following relation can be deriven.

$$p(1-T) = pH_0 - pH = \Delta pH \qquad (15)$$

Thus, the pH drop resulted from a definite extent of the transguanylation indicates a constant value irrespective of the amount of alkali added, if the compound does not undergo the cyclization.

By using equation (15), the susceptibility of the isothiuronium salt to the transguanylation can be estimated. As an example, the relation between the reaction rate and the amount of alkali added was presented in Fig. 4, where the rate was expressed as the extent of this transformation during the initial one minute. Since the stoichiometric relation exists between the equivalents of alkali added and the ionized isothiuronium salt, it is supported that the reactive species for the transguanylation is the ionized form of the isothiuronium salt.

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