[Chem. Pharm. Bull. 16(3) 486—491 (1968)]

UDC 547.379.1.04 : 541.12

Potentiometric Titration of Aminoalkylisothiuronium Salts

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(Received May 19, 1967)

The reversibilities of the titration curves of 2-aminoethyl- and 3-aminopropyl-isothiuronium salts were discussed. The potentiometric titration was done at various titration speeds and at various temperatures. Though the titration curves of these two compounds showed different behaviors against the rate of alkali addition, it was confirmed that both compounds were transguanylated during the titration. The isothiuronium salt was ionized to the unstable conjugate base, which was in turn transguanylated rapidly to mercaptoalkylguanidine. In 2-aminoethylisothiuronium salt, however, the conjugate base was partly cyclized to 2-aminothiazoline. From the titration curves, the apparent ionization constants were determined graphically; pK_a values at 25° of 2-aminoethyl- and 3-aminopropyl-isothiuronium salts were 7.5 and 8.6, respectively.

In the previous papers, we showed that, when caustic alkali less than one equivalent was added to 2-aminoethylisothiuronium (AET) or 3-aminopropylisothiuronium (APT) salt in aqueous solution, a rapid and continuous drop of pH and transformation of aminoalkylisothiuronium salt to mercaptoalkylguanidine were observed,[2] and that a stoichiometric relation was found between the amounts of alkali added and the sulfhydryl compound produced.[3] Those findings suggest that the isothiuronium salt was ionized at first and thereby the transguanylation is initiated.

The titration curve of the isothiuronium salt appeared to be irreversible at one glance.[2] One reason why the titration curve seems irreversible may result from the rapidity of the transguanylation. Provided that the isothiuronium salt is ionized at first to the conjugate base which is the reactive species of the transguanylation, and that the rate of the transguanylation is extremely rapid, the life of the ionized isothiuronium salt in a titration mixture may be very short. Therefore, the titration curve would appear to be irreversible even though the ionization reaction, which is ordinarily reversible, is involved really in the first step. If those assumptions are correct, the ionization constant, pK_a, should be determined in principle (Chart 1).

Another reaction of the isothiuronium salt encountered in aqueous solution is the cyclization, by which AET is transformed to 2-aminothiazoline (2-AT) and APT to 2-aminopenthiazoline (2-PT). If both the transguanylation and the cyclization proceed through a common cyclic intermediate, as Doherty and his co-workers have proposed,[4] and are accompanied with the ionization reaction, those two reactions might be stimulated in the presence of alkali. Hence, they shoud be considered at the same stand-point in order to elucidate the reversibility of the titration curve of the isothiuronium salt.

For those reasons, it is important and interesting to confirm whether an ionization reaction is involved in the first step of the transguanylation and the cyclization of the isothiuronium salt or not. In this paper, we attempted to titrate AET and APT potentiometrically at various titration speeds and at various temperatures, and discuss on the reversibility of the titration curve.

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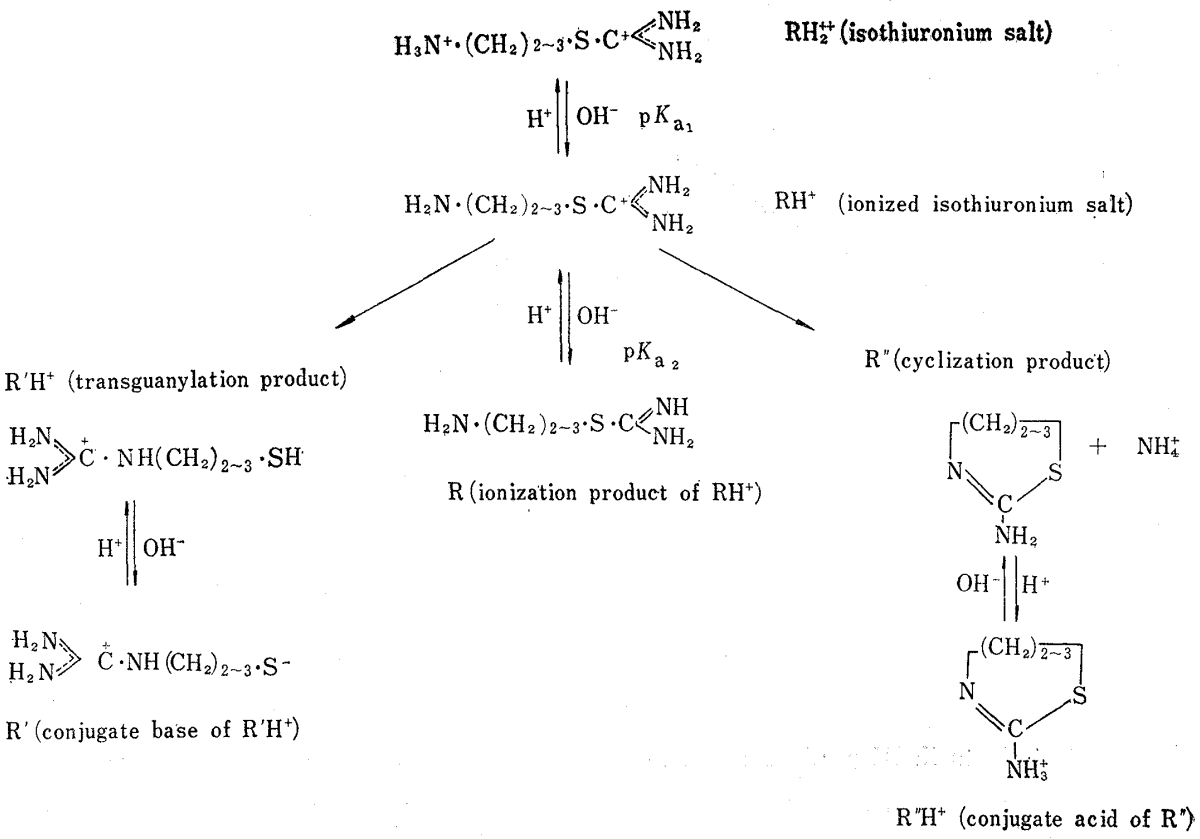

Chart 1. Reaction Pathway of Aminoalkylisothiuronium Salt

Experimental

Potentiometric Titration——pH measurements were done with a Toa–Dempa HM–5A pH meter equipped with an HG–2005 type glass electrode. The instrument was standardized with phthalate and phosphate buffers. The isothiuronium salt (aminoalkylisothiuronium bromide hydrobromide) dissolved in 0.5 N NaCl was titrated with 0.1 N decarbonated NaOH at 5° and 25°. The titrations were done at the rates of 0.1 equivalent NaOH per 5, 10, 15, 20, 30, 40 and 60 sec, respectively. The concentration and the total volume of the isothiuronium salt solution were respectively 2.00×10^{-3} M and 100 ml at the half neutralization point. During the titration, pH values were recorded with a Toa–Dempa EPR–2T recorder.

Retitration of the Isothiuronium Salt——0.5 Equivalent NaOH was added all at once into 2.00×10^{-3} M AET or APT solution which had been thermostatted at 5°. After standing for 0.5, 1, 2, 4 or 8 min, 0.5 equivalent HCl was added into this solution which was expected to contain both the isothiuronium salt and the transformation products. Then, the solution was titrated with 0.1 N NaOH at 5°. The titration was done at the rate of 0.1 equivalent NaOH per 10 sec.

Results and Discussion

The titration curves of the isothiuronium salt were influenced remarkably by varying the rates of alkali addition. In APT, the curves were shown to move monotonously to acidic side in accordance with the decreasing speeds of titration (Fig. 1). On the other hand, the behavior of the curve in AET appeared rather complicate (Fig. 2). In the region below half neutralization point, the curves moved to acidic side. Beyond half neutralization point they moved monotonously to alkaline side with the decreasing speeds of titration. The curves at various titration speeds intersected at a point between 0.6 and 0.7 equivalent alkali per AET. It is doubtless that the monotonous shift of the titration curve shown in APT is attributed to the property that the isothiuronium salt liberates hydrogen ion by adding alkali. Since pH drop was postulated to be related to the transguanylation, the titration

curve of APT might indicate that the transguanylation to mercaptopropylguanidine (MPG) occurred during the titration. The titration curve of AET suggests the contribution of another reaction as well as the transguanylation.

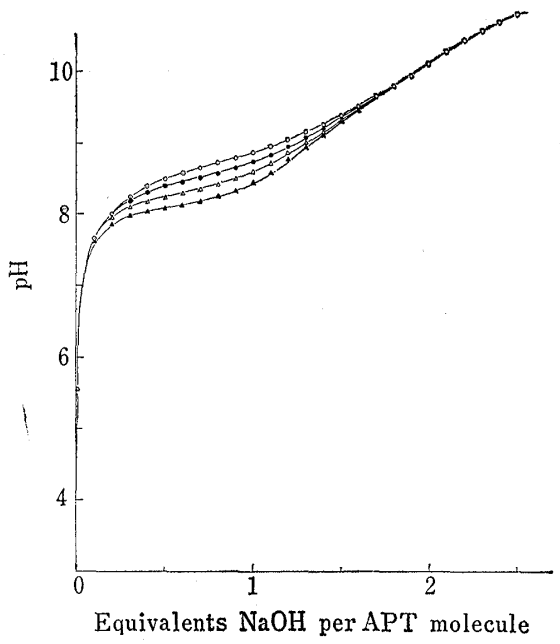

Fig. 1. Potentiometric Tirtation of APT at 25° in 0.1N NaCl

concentration; 2.00×10^{-3}M
Titration Speed;
—○—○— 0.1 eq/5 sec —●—●— 0.1 eq/10 sec
—△—△— 0.1 eq/20 sec —▲—▲— 0.1 eq/40 sec

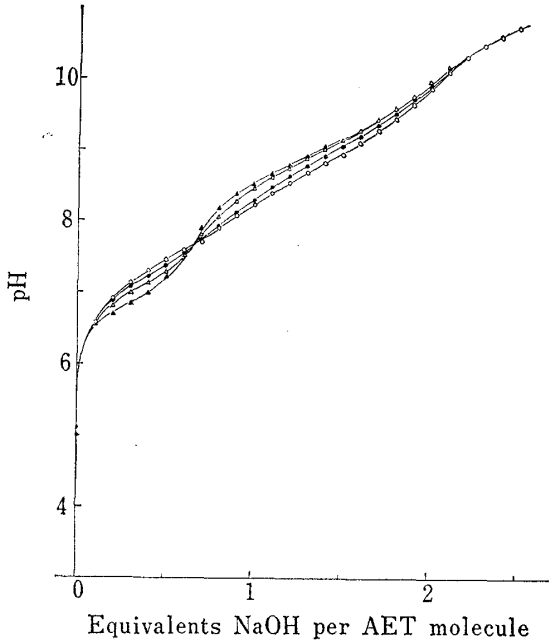

Fig. 2. Potentiometric Titration of AET at 25° in 0.1N NaCl

Concentration; 2.00×10^{-3}M
Titration Speed;
—○—○— 0.1 eq/5 sec —●—●— 0.1 eq/15 sec
—△—△— 0.1 eq/1 min —▲—▲— 0.1 eq/5 min

As mentioned in the preceeding paper,[2] the titration curve of the isothiuronium salt seemed irreversible at room temperature. However, if an ionization is involved really in the first step of the transguanylation, the titration cuve measured in such a condition as the transguanylation proceeds little should become reversible even though the molecular species concerned with the ionization is extremely unstable. In order to prevent the reaction of the isothiuronium salt, the titration was attempted at low temperature and at a rapid speed.

The possible reactions of the isothiuronium salt in aqueous solution are described as follows (Chart 1):

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

$$RH^+ \rightleftarrows R + H^+ \quad (2)$$

$$RH^+ \longrightarrow R'H^+ \quad (3)$$

$$R'H^+ \rightleftarrows R' + H^+ \quad (4)$$

$$RH^+ \longrightarrow R'' + NH_4^+ \quad (5)$$

$$R'' + H^+ \rightleftarrows R''H^+ \quad (6)$$

where RH_2^{++}, RH^+, R, $R'H^+$, R', R'' and $R''H^+$ represent the isothiuronium salt, its first and second ionization products, the transguanylation product, its conjugate base, the cyclization product and its conjugate acid, respectively. In the presence of alkali less than one equivalent, the contribution of reaction (2) to over-all reaction may be almost neglected, because the

second ionization constant, pK_{a_2}, may be greatly larger than pK_{a_1}[5] and pH of the solution is always lowered during the titration. If the rates of reactions (3) and (5) are very slow, the retitration curve, which is measured after standing the isothiuronium salt for a certain period in the presence of alkali, may be overlapped with the titration curve due to reactions (1) and (2). If reaction (3) proceeds rapidly, the equilibrium in reaction (1) is to be shifted to right-hand side and free hydrogen ion will be accumulated, because the product, $R'H^+$, has no ability to associate with hydrogen ion. Therefore, the retitration curve is to show the presence of free hydrogen ion, and the concentration of the free hydrogen ion may increase in accordance with the extent of the transguanylation. The curves shown in Figs. 3 and 4 indicate clearly that both AET and APT are transguanylated in the presence of alkali. The half lives of the ionized APT and AET at 5° were approximately within one and two min, respectively. The extent of the transguanylation could not exceed the alkali equivalent added, which corresponded to the amounts of the ionized isothiuronium salt. Those facts indicate that by adding alkali the isothiuronium salt is ionized to the unstable conjugate base which is in turn transguanylated to the sulfhydryl compound.

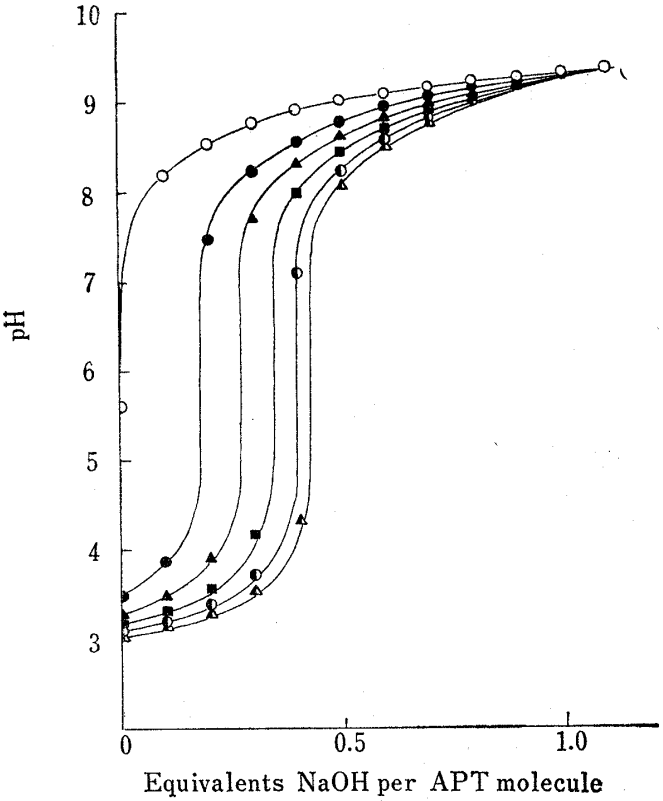

Fig. 3. Retitration of APT at 5° in 0.1N NaCl

Before the titration, APT solution was thermostatted respectively for 0 (—○—○—), 0.5 (—●—●—), 1 (—▲—▲—), 2 (—■—■—), 4 (—◐—◐—) and 8 min (—◭—◭—).
The detailed procedure was presencted in **Experimental**

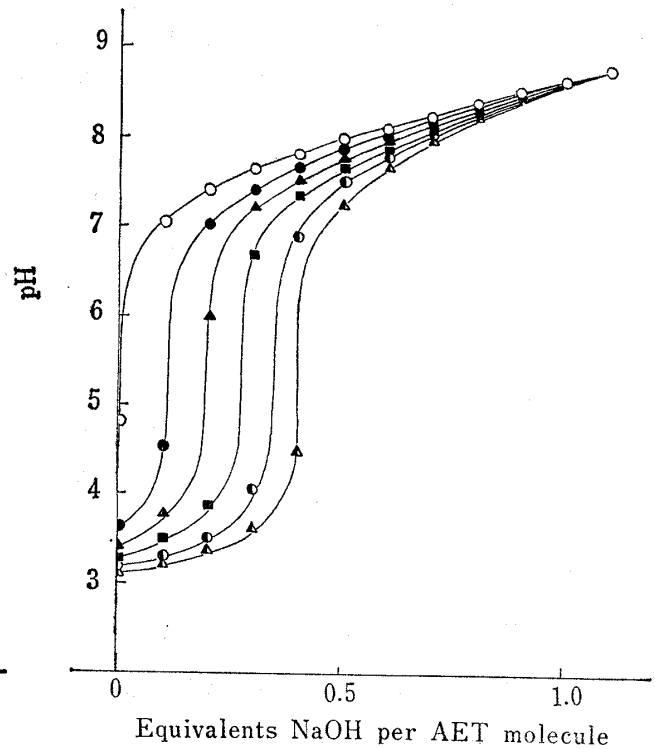

Fig. 4. Retitration of AET at 5° in 0.1N NaCl

Before the titration, AET solution was thermostatted respectively for 0 (—○—○—), 0.5 (—●—●—), 1 (—▲—▲—), 2 (—■—■—), 4 (—◐—◐—) and 8 min (—◭—◭—).
The detailed procedure was presented in **Experimental**

In the case when the cyclization, reaction (5), proceeds selectively, the retitration curve does not show the presence of free hydrogen ion because of an ability of R'' to associate with hydrogen ion. The difference between the titration and retitration curves are explained by considering the pK_a values of RH_2^{++} and $R''H^+$. If the cyclization proceeds significantly during the titration and pK_a of the product is higher than that of the isothiuronium salt,

5) In an isothiuronium salt, 2-(2-aminoethylthio)-3,4,5,6-tetrahydropyrimidine dihydrobromide, which is hardly transguanylated, the ratio of pK_{a_2} to pK_{a_1} is more than 2 (unpublished result).

the retitration curve may shift to alkaline side with the increasing extents of the cyclization and with the difference of pK_a values between RH_2^{++} and $R''H^+$.

Since it was confirmed that the isothiuronium salt was ionized at first by adding alkali, the calculation of the ionization constant was attempted from the titration curves shown in Figs. 1 and 2. Though the isothiuronium salt is a dibasic acid theoretically, it is almost impossible to determine the second ionization constant, because of the instability of RH^+. The determination of accurate pK_{a1}^{λ} value seems also difficult. In order to make the calculation simple, it was treated tentatively that the isothiuronium salt behaves like a monobasic acid in the region of low alkali concentration in the titration curve. The approximate pK_{a_1}' thus calculated at several points of a titration curve appeared to decrease with the increasing alkali equivalent. In Figs. 5 and 6, the plots of the approximate pK_{a_1}' against alkali equivalent are shown. In APT, pK_{a_1} decreases steeply with the increasing alkali equivalents. Considering that the isothiuronium salt liberates hydrogen ion by adding alkali and the pH value in the presence of definite amount of alkali drops with time, the behavior of the curves shown in Figs. 5 and 6 can be understood. An intrinsic ionization constant is probably higher than any value calculated from the titration curve. However, it is expected that the approximate value calculated at a possible lower alkali equivalent of the curve is close nearly to the intrinsic value, because in that condition the extent of the transguanylation is little. Reliable values; *i.e.* 7.5 for AET and 8.6 for APT, were determined by extrapolating the approximate value to a point of zero alkali equivalent.

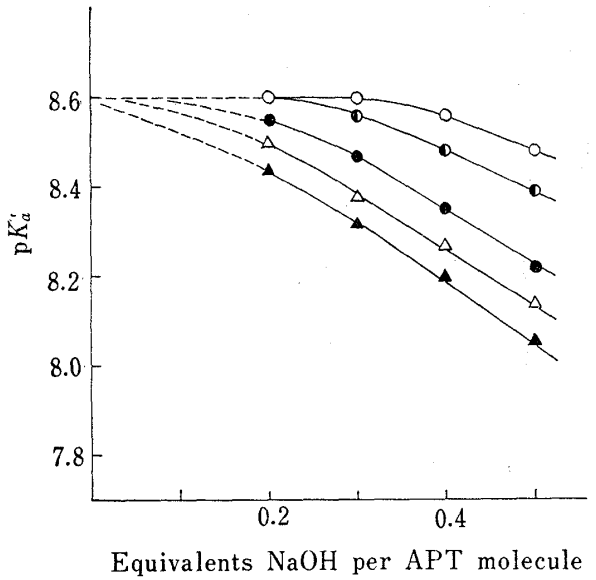

Fig. 5. Plot of Approximate pK_a' of APT against Equivalent NaOH

Concentration; 2.00×10^{-3}M
Titration Speed;
—○—○— 0.1 eq/5 sec —◐—◐— 0.1 eq/10 sec
—●—●— 0.1 eq/20 sec —△—△— 0.1 eq/30 sec
—▲—▲— 0.1 eq/40 sec
Temperature; 25° Ionic Strength; 0.1N NaCl

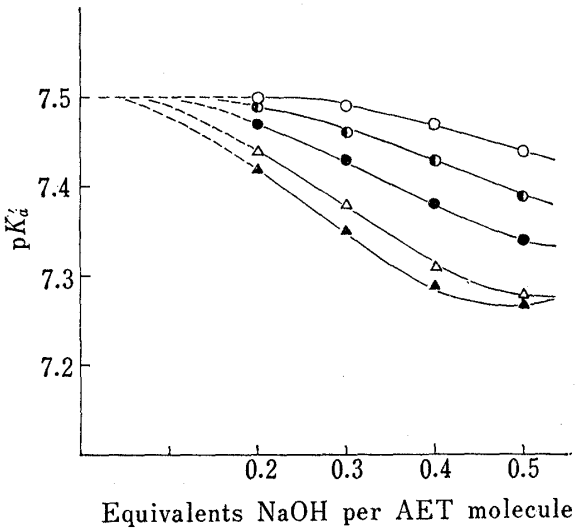

Fig. 6. Plot of Approximate pK_a' of AET against Equivalent NaOH

Concentration; 2.00×10^{-3}M
Titration Speed;
—○—○— 0.1 eq/5 sec —◐—◐— 0.1 eq/10 sec
—●—●— 0.1 eq/20 sec —△—△— 0.1 eq/30 sec
—▲—▲— 0.1 eq/60 sec
Temperature; 25° Ionic Strength; 0.1N NaCl

The plot of pK_{a_1}' in AET showed different pattern; the decrease of the curve was slow as compared with APT. Since the rate of pH drop was not greatly different between AET and APT, the plot of AET might indicate that some component possessing an ability to prevent the accumulation of hydrogen ion was partly produced during the titration. This component was identified with 2-AT. If the cyclization proceeds significantly during the titration, the approximate pK_{a_1}' calculated from the titration curve will increase with the increase of alkali equivalent, because of higher pK_a of 2-AT (8.75) than AET (7.5), and the increase of the

curve will depend on the extent of the cyclization. Therefore, the titration curve observed indicates that AET is transformed mainly to MEG and partly to 2-AT and the ratio of the transguanylation to the cyclization is related to the titration speed.

As mentioned above, the ionized isothiuronium salt was transguanylated at an extremely fast rate. The ionized isothiuronium salt may be transguanylated preferably to the sulfhydryl compound without undergoing further ionization. The titration curve can be explained as a binary system composed of the ionizations of the amino group of the isothiuronium salt and the sulfhydryl group of the transguanylation product. The quantitative explanation on the titration curve will be presented in future.

Acknowledgement The author wishes to thank Drs. S. Akaboshi and T. Hino for several helpful discussions concerning this work.