[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, WEIZMANN INSTITUTE OF SCIENCE]

Spectrophotometric Titration of α-Amino Acid Copolymers Containing Tyrosine[1]

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Copolymers of L-tyrosine with DL-alanine, L-aspartic acid or L-lysine were prepared and titrated spectrophotometrically. The tyrosine hydroxyl dissociation was decreased by the negatively charged aspartic acid residues in the copolymer with aspartic acid; it was increased by the positively charged lysine residues in the lysine copolymers. The spectrum in solution in the case of all the copolymers investigated remained unchanged on standing or upon the addition of urea. The spectrophotometric titrations were found to be reversible in the entire *p*H range studied. The data obtained indicate that the tyrosine hydroxyls of the copolymers synthesized are free to ionize.

The dissociation of the phenolic hydroxyl group of the tyrosine residue in proteins and tyrosyl peptides has been frequently investigated spectrophotometrically, because the ultraviolet absorption of the ionized phenolic group differs widely from that of the un-ionized group.[2] The apparent dissociation of the tyrosine phenolic group in proteins was found to be dependent on the net charge and on the shape of the native macromolecules investigated.[2-6] In certain proteins it was demonstrated that some of the tyrosine hydroxyl groups are not free to ionize, probably because of their involvement in a hydrogen bond with an appropriate group in an adjacent part of the macromolecule.[3,5,7,8]

In order to elucidate the influence of the various charged and uncharged α-amino acid residues on the ionization of tyrosine in proteins, a number of synthetic tyrosine containing copolymers of α-amino acids were prepared and investigated spectrophotometrically. Recent progress made in the synthesis of poly-α-amino acids,[9-13] permits the preparation of such copolymers practically in any composition and ratio required. Model compounds composed of L-tyrosine and L-aspartic acid, L-lysine and DL-alanine, respectively, were prepared (see Table I). As the β-carboxyl groups of the aspartic acid residues are fully ionized in the *p*H range at which the hydroxyl group of tyrosine ionizes, the copolymers of aspartic acid and tyrosine were expected to illustrate the effect of a constant number of negative charges on the dissociation of the tyrosyl hydroxyls. Crammer and Neuberger[3] have assumed that the phenolic hydroxyls in egg albumin participate in hydrogen bonds with the carboxylate ions of acidic amino acids. It seemed of interest therefore to investigate whether the tyrosyl hydroxyl groups in the above copolymers are free to ionize. As the spectrophotometric titration of polytyrosine was reported previously,[10] the titration of a polymer of tyrosine and alanine could demonstrate the influence of charge dilution on the ionization. The copolymers of lysine and tyrosine were chosen to demonstrate the effect of the positive charges of the ε-ammonium groups on the dissociation of the phenolic group of tyrosine.

TABLE I

No.	Copolymer of	Residue molar ratio	Av. degree of polymerization
I	L-Tyr; L-Asp	1:1	23
II	L-Tyr; L-Asp	1:3	21
III	L-Tyr; L-Asp	1:9	18
IV	L-Tyr; L-Lys[14]	1:3	28
V	L-Tyr; L-Lys	1:9	30
VI	L-Tyr; DL-Ala	1:9	70

Materials and Methods

Syntheses of Copolymers.—The N-carboxy-α-amino acid anhydrides of DL-alanine,[13,15] β-benzyl-L-aspartate,[12] ε,N-carbobenzoxy-L-lysine[11] and O-carbobenzoxy-L-tyrosine[10] were used as starting monomers. The purified monomers were dissolved in anhydrous dioxane in the molar ratio required and the solvent was evaporated *in vacuo*. The solid residue obtained was polymerized in bulk for two hours at 120° *in vacuo* (10^{-4} mm.). The copolymers of O-carbobenzoxy-L-tyrosine and β-benzyl-L-aspartate or ε,N-carbobenzoxy-L-lysine, respectively, were dissolved in dimethylformamide and precipitated with water. The carbobenzoxy or benzyl masking groups of the copolymers were removed by treatment with anhydrous hydrogen bromide in glacial acetic acid[16] for half an hour at room temperature or at 70°, respectively. The copolymers thereupon separated from the reaction mixture. Precipitation was completed with anhydrous ether, and the copolymers obtained were washed with anhydrous ether and dried *in vacuo* over phosphorus pentoxide and potassium hydroxide. As a result of the procedure employed, the lysine–tyrosine copolymers were obtained as the hydrobromide salt. The copolymer of DL-alanine and L-tyrosine (9:1) was purified by dialysis (10 hours).

The amounts of tyrosine, lysine and aspartic acid in the various copolymers were estimated by spectrophotometric analysis,[2] from amino nitrogen (Van Slyke) determination.

(1) Presented at the 3rd International Congress of Biochemistry, August, 1955, Bruxelles, Belgium.

(2) G. H. Beaven and E. R. Holiday, *Advances in Protein Chem.*, **7**, 319 (1952).

(3) J. L. Crammer and A. Neuberger, *Biochem. J.*, **37**, 302 (1943).

(4) C. Fromageot and G. Schnek, *Biochim. Biophys. Acta*, **6**, 113 (1950).

(5) C. Tanford, THIS JOURNAL, **72**, 441 (1950); C. Tanford and G. L. Roberts, *ibid.*, **74**, 2509 (1952).

(6) C. Tanford and J. Epstein, *ibid.*, **76**, 2163 (1954).

(7) D. Shugar, *Biochem. J.*, **52**, 142 (1952); C. Tanford, J. D. Hauenstein and D. G. Rands, THIS JOURNAL, **77**, 6409 (1955).

(8) C. Tanford and M. L. Wagner, *ibid.*, **76**, 3331 (1954).

(9) A review of the literature on the subject up to 1950 is given by E. Katchalski, *Advances in Protein Chem.*, **6**, 123 (1951). See also, W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3009, 3239 (1950); D. Coleman, *ibid.*, 2294 (1951); M. Green and M. A. Stahmann, *J. Biol. Chem.*, **197**, 771 (1952); K. Schlögl, F. Wessely and G. Korger, *Monatsh.*, **83**, 845 (1952); M. Frankel, M. Harnik, Y. Levin and Y. Knobler, THIS JOURNAL, **75**, 78 (1953); H. Tani, H. Yuki, S. Sakakibara and T. Taki, *ibid.*, **75**, 3042 (1953); M. Sela and E. Katchalski, *ibid.*, **76**, 129 (1954); A. Patchornik, M. Sela and E. Katchalski, *ibid.*, **76**, 299 (1954); J. Noguchi and T. Hayakawa, *ibid.*, **76**, 2846 (1954); A. Berger, J. Kurtz and E. Katchalski, *ibid.*, **76**, 5552 (1954); V. Bruckner, K. Kovács, J. Kovács and A. Kótai, *Acta Chim. Hungar.*, **5**, 267 (1955).

(10) E. Katchalski and M. Sela, THIS JOURNAL, **75**, 5284 (1953).

(11) E. Katchalski, I. Grossfeld and M. Frankel, *ibid.*, **70**, 2094 (1948).

(12) A. Berger and E. Katchalski, *ibid.*, **73**, 4084 (1951).

(13) M. Sela and A. Berger, *ibid.*, **77**, 1893 (1955).

(14) A copolymer of L-tyrosine and L-lysine in a molar ratio of 1:10 was described previously (M. Sela and E. Katchalski, *ibid.*, **77**, 3632 (1955)). F. Michael and C. Berding recently described the preparation of L-tyrosine-DL-lysine copolymers (*Ber.*, **88**, 1062 (1955)).

(15) J. L. Bailey, *J. Chem. Soc.*, 3461 (1950).

(16) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

and from potentiometric titration.[12] The amino acid content of the various polymers was also determined chromatographically[17] after hydrolysis with boiling 6 *N* hydrochloric acid for 16 hours. Good agreement was found between the analyses of the intact polymers and their hydrolysates. In all cases the initial ratio of monomers used in the synthesis of the required copolymers was retained in the final high molecular weight products. The average molecular weights of the masked copolymers were obtained from end group analyses.[13]

Spectrophotometric Measurements.—Measurements were made on a Hilger Uvispek photoelectric spectrophotometer H 700/303, at approximately 25°, using one centimeter quartz cells. The tyrosine content of the copolymers was calculated from the absorption, at *p*H 13, at 2935 Å. where there is a characteristic peak. A molar extinction coefficient of $\epsilon = 2330$ per tyrosine residue was assumed at this *p*H.

As copolymers I, II and III at *p*H 6 gave an extinction $\epsilon = 140$ per tyrosine residue at 2935 Å., the degree of ionization, α, of the tyrosine hydroxyl groups of these copolymers at any *p*H was calculated from the relation $\alpha = (\epsilon_{2935\text{ Å.}} - 140)/2190$. Similarly in the case of the copolymers IV, V and VI, α was calculated from the relation $\alpha = (\epsilon_{2935\text{ Å.}} - 70)/2260$.

In the range 2500 to 3000 Å. no selective absorption is shown by poly-L-aspartic acid[11] ($n = 20$; *p*H 12.0), by poly-DL-alanine[13] ($n = 25$; *p*H 7.0) and by poly-L-lysine[11] ($n = 20$; *p*H 2.0 or 12.0). The molar extinction coefficient per amino acid residue did not in any of the measurements made exceed $\epsilon = 17$.

Except when otherwise stated all measurements were carried out at an ionic strength of 0.1 using glycine (*p*H 8.2 to 10.1), carbonate (*p*H 10.2 to 11.3) or phosphate (*p*H 11.0 to 12.0) buffers.[18]

Turbidimetric measurements were carried out in a Klett-Summerson photoelectric colorimeter.

Measurements of *p*H.—Measurements of *p*H were made on a model G Beckman *p*H meter. A standard phosphate buffer (*p*H 7.00) was used for calibration. A Beckman Type E high *p*H glass electrode, standardized against a borate buffer (*p*H 10.00), was used for readings above *p*H 10.

Results and Discussion

The spectrophotometric titration of the tyrosine residues in copolymers III, VI and V is given in Fig. 1 (curves 1, 2 and 3, respectively). The spectrophotometric titrations were reversible in all cases. None of the solutions showed any change in spectrum on standing, nor did urea have any effect. Half ionization of the phenolic tyrosine groups ($\alpha = 0.5$) is achieved in the case of the copolymers V, VI and III at *p*H values of 9.75, 10.35 and 11.00, respectively. The phenolic hydroxyl groups of copolymer V, containing the positively charged ε-ammonium groups of lysine, obviously ionize more readily than those of copolymer VI containing neutral alanine. In copolymer III, on the other hand, dissociation of the phenolic hydroxyls is retarded by the negatively charged β-carboxylate groups of the aspartic acid residues.

As the dissociation of the tyrosyl hydroxyls occurs in a *p*H range in which practically all the free carboxyl groups of copolymer III are ionized, it can be assumed that in this *p*H range the number of negative charges on the copolymer molecule equals $m + \alpha n$, where m denotes the average number of aspartic acid residues, n is the average number of tyrosine residues per copolymer molecule, and α is the degree of ionization of the tyrosine hydroxyl groups. Each molecule of copolymer III contains a relatively high negative charge at the beginning of the tyrosine dissociation. It may therefore be assumed that the molecule is already stretched at the beginning of the titration, and that during the titration of the phenolic hydroxyls no significant change in its shape is produced. Since the copolymer molecules are relatively small it is reasonable to apply as a first approximation formula (1), used by Cannan[19] and Scatchard[20] to describe the titration of various proteins

$$p\mathrm{H} = pK_0 - \log \frac{1-\alpha}{\alpha} + 0.868w(m + n\alpha) \quad (1)$$

K_0 is the intrinsic ionization constant of the phenolic hydroxyl groups and w is the usual electrostatic factor.

Applying the Debye–Hückel approximation to a sphere of constant radius, b, with a charge spread uniformly on its surface one finds

$$w = \frac{e^2}{2DkT}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) \quad (2)$$

where D is the dielectric constant of the medium, k is the Boltzmann constant, T is the absolute temperature, e is the electronic charge, a is the distance of closest approach, and κ has its usual significance in the Debye theory.

Curve 1 of Fig. 1 which closely fits the experimental data at ionic strength 0.1 was computed from formula (1) by introducing the numerical val-

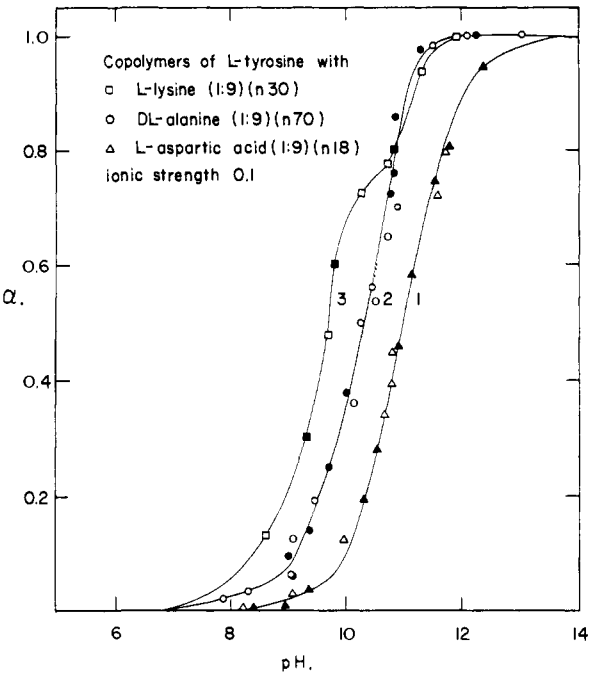

Fig. 1.—Spectrophotometric titrations, at an ionic strength of 0.1, of: △, copolymer III (Tyr–Asp, 1:9 mole/mole); ○, copolymer VI (Tyr–Ala, 1:9 mole/mole); and □, copolymer V (Tyr–Lys, 1:9 mole/mole). The full points represent back-titrations, *i.e.*, results obtained after previous exposure of the copolymers to 0.1 *N* sodium hydroxide.

(17) R. A. Boissonas, *Helv. Chim. Acta*, **33**, 1975 (1950).

(18) H. T. S. Britton, "Hydrogen Ions," Chapman & Hall Ltd., London, 1932, p. 217.

(19) R. K. Cannan, A. Kibrick and A. H. Palmer, *Ann. N. Y. Acad Sci.*, **41**, 243 (1941); R. K. Cannan, *Chem. Revs.*, **30**, 395 (1942).

(20) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

ues $m = 16.2$, $n = 1.8$, $pK_0 = 9.5$ and $w = 0.100$.[21] The intrinsic ionization constant, $pK_0 = 9.5$, used closely approaches the value $pK_0 = 9.6$ estimated as the intrinsic constant of the phenolic groups of tyrosine.[6] This value was also used successfully to describe the spectrophotometric titration of polytyrosine.[10] From the above value for w the approximate radius of the assumed sphere, b, and the distance of closest approach $a = b + 2$ Å. were calculated for copolymer III from equation 2, as $b = 15.4$ Å. and $a = 17.4$ Å., respectively.

The spectrophotometric titrations of copolymers I and II at 0.1 ionic strength were best described by theoretical curves computed from equation 1 with $pK_0 = 9.5$ and in the case of copolymer I the values $m = 11.5$, $n = 11.5$ and $w = 0.115$ (corresponding to $b = 14.2$ Å.) and in the case of copolymer II the values $m = 15.75$, $n = 5.25$ and $w = 0.093$ ($b = 15.6$ Å.). The variation of the degree of ionization α with ionic strength at a constant pH is given for copolymer II in Fig. 2. The theoretical curve was computed from eq. 1, introducing for w the values evaluated, for various ionic strengths, from the calculated radius with the aid of eq. 2.

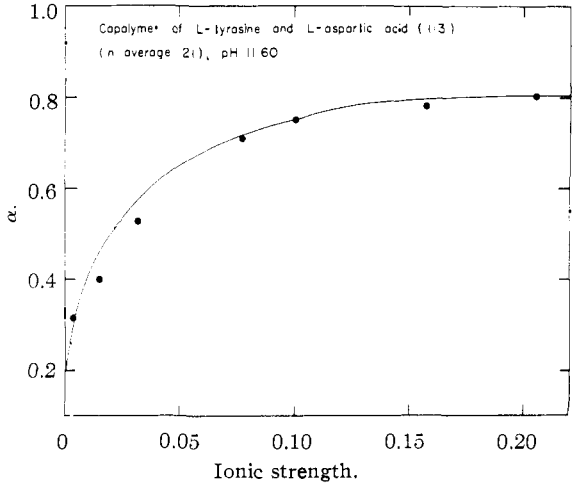

Fig. 2.—Dependence of the degree of ionization of the phenolic groups of copolymer III on the ionic strength at pH 11.60. The full curve was computed from eq. 1.

A comparison of the spectrophotometric titration of the alanine–tyrosine copolymer VI (curve 2 in Fig. 1) with that obtained previously for polytyrosine[10] shows that the curve of the copolymer is considerably steeper than that of polytyrosine. Thus in the case of polytyrosine (n average 30) a variation from $\alpha = 0.2$ to 0.9, at 0.2 ionic strength, was accompanied by a change of 2.8 pH units, while a similar variation in the alanine–tyrosine copolymer, at 0.1 ionic strength, caused a change of only 1.4 pH units. The relative steepness of the curve of the copolymer VI shows that the spectrophotometric titration in this case more closely resembles the spectrophotometric titration of monomeric tyrosine than of polytyrosine. This obviously is the result of the weaker electrostatic field surrounding the ionized copolymer molecules, which contain only one ionizable group per ten amino acid residues, as compared with polytyrosine. An attempt to calculate a theoretical curve to fit the experimental data has shown that when the intrinsic ionization constant $pK_0 = 9.5$ was introduced into eq. 1 assuming $m = 0$ and $n = 7$, the value of w varied from $w = 0.49$ for $\alpha = 0.20$ to $w = 0.165$ for $\alpha = 0.80$. The drop in w with the increase in α seems to indicate that the apparent radius of the molecule increases considerably with ionization.

Copolymer V of lysine and tyrosine, whose spectrophotometric titration is given in curve 3 of Fig. 1, is a typical polyampholyte[22,23] which contains in each molecule both basic ϵ-amino groups and acidic phenolic groups. As the intrinsic dissociation constant of the ϵ-ammonium groups ($pK_0 = 9.4$–10.6[24]) is similar to that of the intrinsic dissociation constant of the hydroxyl groups of tyrosine ($pK_0 = 9.5$), the addition of alkali in this pH range obviously will increase the number of negative charges and decrease the number of positive charges in the copolymer molecule. At the isoelectric point the number of positive ϵ-ammonium groups will equal the number of phenolate groups, *i.e.*

$$\alpha_{is} n = \beta_{is} m \qquad (3)$$

where β_{is} is the degree of the ionization of the basic groups at the isoelectric point and m is the average number of lysine residues in the copolymer molecule.

Katchalsky and Miller[23] have recently carried out a spectrophotometric study of copolymers of 2-vinylpyridine and methacrylic acid. All the spectrophotometric titration curves showed a jump in pH at the isoelectric points of the polyampholytes studied. This jump was explained by the assumption that the polyampholytic molecule is hypercoiled at the isoelectric pH, where the number of positive and negative charges is equal, and extended considerably above and below this pH. A similar jump occurs in curve 3 of Fig. 1 around pH 10.60, corresponding to $\alpha = 0.76$. If this pH represents the isoelectric point of copolymer V, the intrinsic ionization constant pK_0 of the phenolic groups of the copolymer may be calculated from equation 4a, which holds for the isoelectric point.[22]

$$p\mathrm{H}_{is} = pK_0 - \log \frac{1 - \alpha_{is}}{\alpha_{is}} \qquad (4a)$$

The value obtained, $pK_0 = 10.0$, is higher than the value used to describe the ionization of the phenolic hydroxyls in polytyrosine[10] and in the copolymers I, II and III. β_{is} may be calculated by means of eq. 3 assuming $\alpha = 0.76$, $n = 3$ and $m = 27$. The intrinsic pK_0' of the ϵ-ammonium groups of copolymer V may then be calculated from equation 4b.

$$p\mathrm{H}_{is} = pK_0' - \log \frac{\beta_{is}}{1 - \beta_{is}} \qquad (4b)$$

(21) It should be remarked that an identical theoretical titration curve for the tyrosine residues in copolymer III may be obtained by assuming a constant end-to-end distance, h, in the formula given by A. Katchalsky, O. Künzle and W. Kuhn (*J. Polymer Sci.*, **5**, 283 (1950)) to describe the potentiometric titration of a polyelectrolyte.

(22) The potentiometric titration of synthetic polyampholytes was described in several cases. *Cf.* T. Alfrey, Jr., and H. Morawetz, THIS JOURNAL, **74**, 436 (1952); T. Alfrey, Jr., R. M. Fuoss, H. Morawetz and H. Pinner, *ibid.*, **74**, 438 (1952); G. Ehrlich and P. Doty, *ibid.*, **76**, 3764 (1954); G. van Paesschen and G. Smets, *Bull. soc. chim. Belg.*, **64**, 173 (1955).

(23) A. Katchalsky and I. R. Miller, *J. Polymer Sci.*, **13**, 57 (1954).

(24) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 445.

The value obtained, $pK_0' = 9.8$, is in good agreement with the values found for the ionization of the ϵ-amino groups of polylysine ($pK_0' = 10.04$[25]) and of proteins.[24]

The spectrophotometric titration curve, at ionic strength 0.1, of the lysine–tyrosine copolymer IV resembles that of copolymer V. No measurements could be performed between pH 9.60 and pH 11.00, where the solutions became turbid. The maximum turbidity occurred at pH 10.20. Assuming that this pH corresponds to the isoelectric point, $\beta_{is} = 0.286$ is obtained from eq. 4b, introducing $pK_0' = 9.8$. Equation 3 gives 0.86 for α_{is}, from which 9.4 is calculated for pK_0 from eq. 4a.

The results described above clearly show that copolymers of α-amino acids with polar side groups may serve as suitable model compounds for the study of the influence of different factors such as over-all electric charge, size and shape of the relatively high molecular weight peptides on the dissociation of specific groups in the chain. Such studies may be of importance in the detailed analysis of the potentiometric titrations of natural peptides as well as of proteins. As the spectrophotometric titrations of the tyrosine residues in the copolymers investigated were found to be reversible, and no change in spectrum occurred on addition of urea, it is reasonable to assume that all the tyrosine hydroxyl groups of the copolymers synthesized are free to ionize. In the various tyrosine-containing copolymers investigated no hydrogen bonds involving tyrosine seem therefore to be present, at least in the pH range over which the phenolic hydroxyls ionize.

(25) A. Katchalsky, N. Shavit and H. Eisenberg, *J. Polymer Sci.*, **13**, 69 (1954).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Exchange of Tritium between TCl and Toluene with and without Catalyst

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Mixtures of tritium-labeled HCl with toluene showed no isotopic exchange when heated at 140° for 40 hr. The addition of either NO_2 or $SnCl_4$ resulted in exchange even at room temperature. The rates at 25° appeared to be in agreement with the expression $d[TCl]/dt = -k[TCl][SnCl_4 \text{ or } NO_2]$ where $k = ca.\ 4 \times 10^{-4}$ l. moles^{-1} sec.$^{-1}$ for $SnCl_4$, and is somewhat higher for NO_2. The catalyzed exchange proceeded faster at 140°, with an apparent activation energy of 10 ± 4 kcal./mole in each case. Mixtures of tritium-labeled HCl with mesitylene at 140° gave erratic exchange, the rate in the absence of impurities always being less than 0.03% per sec. Solutions of $SnCl_4$ in toluene absorb ultraviolet light at higher wave lengths than either component alone and the spectrum is not altered by the addition of HCl.

Introduction

The isotopic exchange of hydrogen between deuterium chloride and benzene has been shown to occur in the presence of $AlCl_3$,[1-3] while fragmentary evidence on the reaction in the absence of catalysts[3,4] suggests that slow exchange may possibly occur at 50°. Brown and Brady[5] have investigated the interaction of HCl with aromatic hydrocarbons at −78°, interpreting their data in terms of weak π-complexes which would not lead to hydrogen exchange, in contrast to the σ-complexes proposed for the aromatic–HCl–$AlCl_3$[6] and aromatic–HF–BF_3[7] systems, in which the hydrogen atom of the hydrogen halide is bonded to a particular carbon atom. They also proposed that the formation of σ-complexes requires an appreciable activation energy. On the basis of studies of the exchange of hydrogen between deuterated aromatic compounds and aqueous acids Gold and Satchell[8-10] have postulated rapid formation of a conjugate acid, $(C_6H_5D)H^+$ ("outer complex," "π-complex") between the aromatic molecule and a proton, followed by a slow intramolecular rearrangement to $(C_6H_6)D^+$ with resultant exchange.

In the light of these hypotheses and the available data[3,4] it seemed possible that the hydrogen from HCl might be induced to form σ-complexes and give measurable rates of exchange with aromatic compounds even in the absence of a catalyst if sufficiently high temperatures and long times of reaction were used. If such exchange occurred the relative activation energies for reaction with hydrocarbons of different basicity (*e.g.*, benzene, toluene, xylene, mesitylene) would be useful in considering the relative nature of π and σ-complexes. The work reported here was initiated to test these speculations by studying the isotopic exchange of tritium between TCl and aromatic compounds. It was extended to obtain information on the exchange of tritium between TCl and toluene, with $SnCl_4$ present as a catalyst. Stannic chloride was chosen because it forms single phase systems with toluene and HCl. This property made it possible to determine whether exchange could occur in the absence of a separate, highly polar, catalyst phase, and to make experiments on the effect of catalyst concentration on the reaction rate.

(1) A Klit and A. Langseth, *Z. physik. Chem.*, **A176**, 65 (1936).
(2) A. Klit and A. Langseth, *Nature*, **135**, 956 (1935).
(3) J. Kenner, M. Polanyi and P. Szego, *ibid.*, **135**, 267 (1935).
(4) H. Hart, THIS JOURNAL, **72**, 2900 (1950).
(5) H. C. Brown and J. D. Brady, *ibid.*, **74**, 3570 (1952).
(6) H. C. Brown and H. W. Pearsall, *ibid.*, **74**, 191 (1952).
(7) D. A. McCaulay and A. P. Lien, *ibid.*, **73**, 2013 (1951).
(8) V. Gold and D. P. N. Satchell, *Nature*, **176**, 602 (1955).
(9) V. Gold and D. P. N. Satchell, *J. Chem. Soc.*, 3609 (1955).
(10) V. Gold and D. P. N. Satchell, *ibid.*, 3619 (1955).