A Study of the Kinetics of the Reaction between Thiol Compounds and Chloroacetamide

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It is commonly assumed that the reaction between sulphydryl groups and alkylating agents proceeds via the $RS^-$ form of the thiol compound. This assumption seems necessary in order to account for the pH-dependence of the reaction, but possibly because of technical difficulties of following the course of the reaction at constant pH, quantitative evidence for this hypothesis is lacking. The development of the pH-stat makes such an investigation possible and the present paper gives an account of the study of the kinetics of reaction of chloroacetamide with mercaptoacetic acid, $\beta$-mercaptoethylamine and cysteine. By a study of the pH-dependence of the reaction with the two last-named compounds it was hoped that some light might be shed on their mode of ionization.

EXPERIMENTAL

The mercaptoacetic acid (British Drug Houses Ltd.) was redistilled before use. The mercaptoethylamine hydrochloride was a gift from Evans Chemetics Inc., New York, and the cysteine hydrochloride was prepared by the reduction of cystine with tin and HCl. The chloroacetamide and iodoacetamide were both purified by recrystallization from water before use.

The design of pH-stat used has been described elsewhere (Wood, 1959). Its long-term stability is excellent and for the periods of time occupied by the experiment the drift is completely insignificant. The glass electrode used was a shielded low-resistance type B manufactured by N. L. Jones, Sandringham, Victoria, and no deviation from theoretical slope could be detected between pH 4-0 and 9-0 standard buffers. An external calomel electrode was connected to the reaction vessel by an agar-KCl bridge. The glass reaction vessel of about 30 ml. capacity was double-walled and maintained at constant temperature ($\pm 0-02^\circ$) by water circulated through the jacket from a Colora Ultrathermostat (Lorch, Württ., Germany). In addition to the electrodes the reaction vessel also had a stirrer, thermometer and the burette tip from an Agla microsyringe mounted in it. An inlet tube for introducing a stream of O$_2$-free N$_2$ into the space over the liquid was also provided. For the kinetic work a direct record of alkali consumption versus time was obtained on a chart recorder (Varian G10, Palo Alto, Calif., U.S.A. or Honeywell-Brown, 10 mv span, Philadelphia 44, Pa., U.S.A.).

The general technique used was to titrate 10 ml. of the thiol solution (approx. 3 mm) to the required pH in the pH-stat with 0-2 N-KOH in an atmosphere of N$_2$. Chloroacetamide solution (10 ml.), previously brought to the same temperature (30$^\circ$), was added and the uptake of alkali followed for a suitable period (10-30 min.). For higher pH values (> pH 9-0) it was also found necessary to adjust the chloroacetamide solution to the required pH before adding it to the thiol solution. Varying the concentration of the chloroacetamide solution provided a convenient means of adjusting the reaction rate to the experimental conditions. Over most of the pH range the reaction could be studied with a ratio of chloroacetamide to thiol in the range 1-5:1 to 8:1. Rate constants were calculated with the integrated form of the equation for a second-order reaction. At low pH values it was found more convenient to use very high ratios of chloroacetamide to thiol and treat the reaction as first order. From the pseudo first-order rate constant the second-order constant can be calculated knowing the chloroacetamide concentration. One of the important estimations from the standpoint of accuracy is the determination of alkali uptake at 100% reaction at the pH value of the experiment. From a knowledge of the pK values of all the groups involved this can be calculated, but would obviously be complex with cysteine or $\beta$-mercaptoethylamine since it would involve not only the two pK groups of cysteine but also the pK of the amino group of the reaction product S-carbamidomethylcysteine. This complication was avoided by adding iodoacetamide crystals to the reaction mixture when it was decided from the record that the kinetic study had proceeded sufficiently far. Because of the much greater reaction rate of iodoacetamide compared with chloroacetamide, the reaction went to completion in a very short time. Experimental values of the maximum alkali uptake obtained in this way were used throughout the present work. All rate constants were calculated from the data by the least-squares procedure and other derived data used to calculate pK values were similarly treated.

It is not perhaps immediately obvious that the extent of reaction at any time for any given pH is directly proportional to the alkali uptake. This can be demonstrated as follows. Consider a thiol compound, e.g. cysteine or $\beta$-mercaptoethylamine, with two dissociation constants $K_1$ and $K_2$ in the pH region in which we are interested. Then if we originally have $a$ moles of thiol, the number of moles of alkali required to titrate it from the state where no alkali is bound to a pH corresponding to $[H^+]^*$ is

$$aK_1 + aK_2$$

If, at any time $t$, $x$ moles of the thiol have reacted to give a product which now has one dissociation constant $K_3$, then the mol. of alkali required to maintain the same pH is

$$x + (a - x) K_1 + (a - x) K_4 + x K_2.$$  

Bioch. 1960, 74
In this expression the first term represents the acid formed by the reaction, the second and third terms are the alkali bound by the remaining thiol and the fourth term is the alkali bound by the reaction product. The extra alkali (moles) bound as a result of the reaction is thus (2) minus (1)

\[ x = \frac{K_1}{K_2 + [H^+]} - \frac{K_1}{K_2 + [H^+]} \frac{K_3}{K_4 + [H^+]} \]

The extent of reaction is thus directly proportional to the alkali uptake at any fixed pH, and this is also true for even more complex cases where larger numbers of dissociating groups are involved.

RESULTS

Mercaptoacetic acid

Experiments carried out as indicated above with 10 ml. of 4.027 mm-m mercaptoacetic acid and 10 ml. of 7.111 mm-chloroacetamide at 30° gave results which, when plotted for a second-order reaction, gave the family of curves shown in Fig. 1. From these curves apparent second-order velocity constants can be calculated and are tabulated in Table 1.

If the reaction between chloroacetamide and thiol groups proceeds by way of the mercaptide ion, then these rate constants should be proportional to the fraction of the mercaptoacetic acid which is in the form of the RS⁻ ion. A titration curve of mercaptoacetic acid was carried out in the pH-stat at 30° with a similar concentration of the acid to that used in the kinetic experiments. Readings of alkalı bound were taken at intervals of 0-1 pH unit from pH 9.0 to 10.8, and, after correction for the volume of alkali necessary to bring water to the same pH, these were used to calculate the pK from the Henderson–Hasselbalch equation. The mean value obtained from eighteen readings was 10.24 with a standard deviation of 0.015. An alternative way of handling the data is to plot log (x/1-x) (where x is the molar fraction of alkali consumed) against pH. This gives a straight line giving an intercept on the pH axis where pH = pK. Both techniques gave the same result. Incidentally the acid branch of the titration curve gave an equivalence within 0-1% of the expected value, assuming 100% purity. With this value of 10.24 for the pK of the SH group, the molar fraction of the mercaptoacetic acid in the RS⁻ form can be calculated for the various pH values used in the kinetic work. Fig. 2 shows the result of plotting the apparent rate constant against the fraction of the mercaptoacetic acid in the RS⁻ form.

Alternatively the results can be used to estimate the dissociation constant of mercaptoacetic acid. Thus the rate of the reaction should be given by the equation

\[ \text{rate} = k[\text{RS}^-][X], \]

where k is the true second-order rate constant, [RS⁻] is the concentration of mercaptoacetic acid as RS⁻, and [X] is the concentration of chloro-
acetamide. The experimental rate constant \( (k_0) \) is defined by the equation
\[
rate = k_0[X] ([RS^-] + [RSH]),
\]
where \([RS^-] + [RSH]\) is the total concentration of mercaptoacetate.

Equating (4) and (5) we get
\[
k_0([RS^-] + [RSH]) = k[RS^-],
\]
therefore
\[
k_0 = \frac{k[RS^-]}{[RSH] + [RSH]},
\]
But
\[
K = \frac{[RS^-][H^+]}{[RSH]},
\]
where \( K \) is the dissociation constant of the mercaptan group and \([H^+]\) is the hydrogen-ion concentration.

Combining (7) and (6) leads to
\[
k_0 = \frac{kK}{[H^]+K},
\]
or
\[
\frac{1}{k_0} = \frac{1}{kK} + \frac{1}{k}.
\]

A graph of \(1/k_0\) versus \([H^+]\) should thus be a straight line of slope \(1/kK\) and intercept \(1/k\). If the experimental data are treated in this fashion we get Fig. 3, which gives values of \(k\) 7.68 l. moles\(^{-1}\) sec\(^{-1}\) and \(pK\) 10.28.

\(\beta\)-Mercaptoethylamine hydrochloride

Amperometric titration of this compound by Dr. S. J. Leach suggested that the sample was at least 98\% pure and this was confirmed by titration data. In order to maintain reasonably constant ionic strength the compound (at 3.25 mm) was titrated at 30° in the presence of 0.1 \text{M} potassium chloride.

Apparent second-order rate constants for the reaction of the mercaptoethylamine and chloroacetamide in the presence of 0.1 \text{M} potassium chloride were then obtained at intervals of 0.25 pH unit from pH 7.0 to 10.5. The values obtained are listed in Table 2. The interpretation of these results will be discussed jointly with those on cysteine. By the method of Benesch & Benesch (1957) it was shown that reaction of the amino group with chloroacetamide was insignificant under the experimental conditions even at pH 10.5.

Table 2. pH-Dependence of the apparent second-order rate constant \( (k_0) \) for the reaction between \(\beta\)-mercaptoethylamine and chloroacetamide at 30° and in the presence of 0.1 \text{M} potassium chloride

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Table 3. pH-Dependence of the apparent second-order rate constant \( (k_0) \) for the reaction between cysteine and chloroacetamide at 30° and in the presence of 0.1 \text{M} potassium chloride

<table>
<thead>
<tr>
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Fig. 3. Plot of the reciprocal of the apparent second-order rate constant \( (1/k_0) \) against \([H^+]\) ion concentration \([H^+]\) for the reaction between mercaptoacetic acid and chloroacetamide at 30°.
Cysteine hydrochloride

Precisely similar data were collected for cysteine. The sample was shown to be 96.5% pure, calculated as cysteine hydrochloride monohydrate. Titration pH values (ignoring the carboxyl group) were 8-10 and 10-36 in the presence of 0.1 M KCl. Table 3 gives the apparent second-order rate constants for cysteine and chloroacetamide at 30°C in the presence of 0.1 M KCl at intervals of 0.25 pH unit from pH 6.5 to 10.5. Reaction of the amino group of cysteine with chloroacetamide was found to be insignificant.

Interpretation of the results on cysteine and \( \beta \)-mercaptoethylamine

The ionization of cysteine at alkaline pH values was originally postulated as shown in Scheme 1 (see, for example, Cohn & Edsall, 1943). Calvin (1954) later suggested that the order of ionization of the two groups be inverted, i.e. that the first pK was associated with the ionization of the thiol group, as in Scheme 2.

Edsall (quoted by Rykland & Schmidt, 1944) postulated that both ionization mechanisms could proceed simultaneously and that the dissociation constants \( K_1 \) and \( K_2 \) calculated from titration data were composite constants and not directly attributable to any specific group. Edsall’s ionization scheme can be formulated as in Scheme 3.

For this complex ionization scheme the following relationships hold between the four dissociation constants \( K_A, K_B, K_C \) and \( K_D \), and the two titration constants \( K_1 \) and \( K_2 \).

(i) \( K_1 = K_A + K_B \),

(ii) \( K_1K_2 = K_AK_C = K_BK_D \),

(iii) \( \frac{1}{K_2} = \frac{1}{K_C} = \frac{1}{K_D} \).

Cohn & Edsall’s formulation (Scheme 1) is a particular case of this general formulation for which \( K_1 = K_B, K_3 = K_C \) and \( K_A \) approaches 0, and Calvin’s proposal (Scheme 2) is another special case in which \( K_1 = K_A, K_3 = K_B \) and \( K_A \) approaches 0. Hence a kinetic expression based on Scheme 3 will include (1) and (2) as special cases. With Scheme 3 as the basis for discussing both cysteine and \( \beta \)-mercaptoethylamine and assuming that only RS\(^-\) forms react we have

rate of reaction = \( k_1[B][X] + k_2[D][X] \), (10)
where $k_1$ and $k_2$ are the specific rate constants for the two $\text{RS}^-$ forms in scheme (3).

Since
\[
K_c = \frac{[D][H^+]}{[B]},
\]
the rate of reaction is given by
\[
rate = \frac{[B][X]}{[H^+]}(k_1[H^+] + k_2K_c).
\] (11)

The apparent rate constant $k_0$ as observable experimentally is defined by the equation
\[
rate = k_0[X][A + B + C + D].
\] (12)

Equating (11) and (12) and substituting for $A$, $C$ and $D$ in terms of $B$, $K_A$, $K_B$, $K_C$ and $K_D$ from the various equilibria and making use of relationships (i) and (ii) above leads to the relationship
\[
k_0 = \frac{K_A k_1[H^+] + k_2 K_A K_B}{[H^+]^2 + K_1[H^+] + K_1 K_2}.
\] (13)

For Cohn & Edsall's scheme (1), $K_A = 0$, and hence
\[
k_0 = \frac{k_2 K_1 K_2}{[H^+]^2 + K_1[H^+] + K_1 K_2}.
\] (14)

For Calvin's Scheme 2, $K_A = K_1$, and hence
\[
k_0 = \frac{K_1 k_1[H^+] + k_2 K_2}{[H^+]^2 + K_1[H^+] + K_1 K_2}.
\] (15)

Equation (13) can be inverted to give
\[
\frac{1}{k_0} = \frac{[H^+]^2 + K_1[H^+] + K_1 K_2}{k_2 K_1 K_2}.
\] (16)

From the titration data we know that for both cysteine and $\beta$-mercaptoethylamine $pK_1$ and $pK_2$ are well separated. Hence at pH values $< 8.5$, $K_1 K_2$ can be neglected in comparison with $[H^+]^2$ and $K_1[H^+]$. Also, unless $k_2 \gg k_1$ and $K_A \ll K_1$, $k_2 K_1 K_2$ can be neglected in comparison with $k_1 K_1 K_2$ [H^+]. At low pH values equation (16) therefore approximates to
\[
\frac{1}{k_0} = \frac{[H^+]^2 + K_1}{K_1 K_A + K_1 K_A}.
\] (17)

A plot of $1/k_0$ versus $[H^+]$ should thus be a straight line of slope $1/k_1 K_A$ and intercept $K_2/k_1 K_A$, whence it is possible to determine $k_1 K_A$ and $K_1$. No estimate of $k_2$ can thus be obtained in the absence of data for $K_A$. Calvin's formulation (Scheme 2) is such a case since $K_A = K_1$. However, in the absence of any data on $K_A$ only the composite constant $k_1 K_A$ can be obtained from kinetic data.

For Cohn & Edsall's formulation (Scheme 1) inversion of (14) gives
\[
\frac{1}{k_0} = \frac{[H^+]^2 + K_1}{k_2 K_1 K_2 + k_2 K_2 + k_2} = \frac{1}{k_2 K_1 K_2} \left[ \frac{[H^+]^2 + [H^+] + 1}{K_2} \right].
\] (18)

Hence for this case at low pH values a plot of $1/k_0$ versus $[H^+]$ would not be linear, and would become linear only at higher pH values where $[H^+]^2/K_1 K_2$ is negligible compared with $[H^+]$.

Figs. 4 and 5 show the result of plotting $1/k_0$ against $[H^+]$ for low pH values for $\beta$-mercaptoethylamine and cysteine respectively with the data of Tables 2 and 3. Both sets of data yield straight lines and hence we can conclude that Scheme (1) of Cohn & Edsall cannot be used to interpret the present results on cysteine or $\beta$-mercaptoethylamine. The graph also permits us to obtain estimates of $K_1$ for both compounds directly from the kinetic data. The values obtained in this way...

Fig. 4. Plot of the reciprocal of the apparent rate constant $(1/k_0)$ against $[H^+]$ for the reaction of chloroacetamide with $\beta$-mercaptoethylamine (30°C, 0.1 M-KCl; pH values below 8.5).

Fig. 5. Plot of the reciprocal of the apparent rate constant $(1/k_0)$ against $[H^+]$ for the reaction of chloroacetamide with cysteine (30°C, 0.1 M-KCl; pH values below 8.5).
expressed as pK values are 8-11 for cysteine and 8-11 for β-mercaptoethylamine, in excellent agreement with the values obtained by titration. If Calvin’s view is correct and $K_1 = K_4$, then values of $k_1$ 0-119 l. moles$^{-1}$ sec.$^{-1}$ and 0-0959 l. moles$^{-1}$ sec.$^{-1}$ are obtained for β-mercaptoethylamine and cysteine respectively.

Although the data do not enable us to differentiate between ionization Schemes 2 and 3 we can fix limits to the value of $K_A$ with some degree of confidence. The reactivity of the nucleophilic RS$^-$ ion towards reagents such as chloroacetamide will depend in part on the electron density near the sulphur atom. The simultaneous presence of a NH$_3^+$ group in the molecule will have some tendency to withdraw electrons from the sulphur atom. Thus it seems probable that a compound of this type will react more slowly with chloroacetamide when it is in the form containing a NH$_3^+$ group than when the amino group is present in the uncharged NH$_2$ form. Hence we can predict that $k_1 > k_2$ and the condition $k_1 = k_2$ would be limiting. Applying this relationship allows us to predict maximum values for $pK_A$. Since $K_1 = K_A$ corresponds to minimum values of $pK_A$, we can place rather broad limits on $pK_A$ for cysteine and β-mercaptoethylamine if we know $k_2$.

The calculation of $k_2$ and $K_2$ from the data presents practical problems. If the kinetic studies could be extended to sufficiently high pH values, $k_0$ would gradually approach $k_2$ and an estimate could be made on this basis. Alternatively, at very high pH values where [H$^+$]$^3$ could be neglected in comparison with $K_1$ [H$^+$], and $K_1 K_2$ and $K_1 K_2$ [H$^+$] can be neglected in comparison with $k_2 K_1 K_2$, equation (16) can be written as

$$\frac{1}{k_0} = \frac{K_1 [H^+] + K_1 K_2}{k_2 K_1 K_2} = \frac{[H^+]}{k_2 K_1 K_2 + k_2}. \quad (19)$$

However, it is not possible experimentally to extend the study to pH values much greater than 10-5 because the amount of acid liberated in the reaction tends to decrease very markedly, and the buffering power of the system becomes considerable so that determination of alkali uptake is subject to considerable error. The only straightforward method of calculating both $k_2$ and $K_2$ is thus to substitute $k_0$, $k_1 K_A$, $K_4$ and [H$^+$] in equation (13) and use pairs of values to solve for $k_2$ and $K_2$. The difficulty with this procedure is that it is unsystematic and difficult to handle statistically. Alternatively, in the present case we can use the experimental value of $K_2$ from titration data and solve directly for $k_2$. As $k_2$ is the value which has most significance in the present work the latter procedure was adopted. Table 4 summarizes all data obtained on β-mercaptoethylamine and cysteine in this way.

As will be mentioned in the Discussion there have been various estimates of $pK_A$ for cysteine in the literature. Values reported for $pK_A$ (not corrected for ionic strength) are 8-66 (Ryklan & Schmidt, 1944), 8-65 (Grafius & Neilands, 1955) and 8-53 (Benesch & Benesch, 1955), whereas Gorin (1956) quotes data which, interpreted with the corrected value of 8-26 for $pK_4$ found in the present investigation, lead to an upper limit of 8-72 for $pK_A$ at zero ionic strength.

**DISCUSSION**

There have been a number of studies which have attempted to determine the sequence of ionizations of cysteine. The earlier attempts (Ryklan & Schmidt, 1944; Grafius & Neilands, 1955) made use of model compounds related to cysteine in which either the SH or NH$_3^+$ group was modified by alkylation so as to prevent its ionization. From Wgscheider’s principle of equivalence the pK of the remaining group is assumed to be unaffected, and this value was used to calculate the remaining dissociation constants in Edsall’s formulation. This is obviously only an approximation.

More recently the problem has been tackled by making use of the ultraviolet-absorption band of the RS$^-$ ion (de Deken, Broekhuyzen, Béchet & Mortier (1956), Benesch & Benesch (1955) and Gorin (1956)). It is perhaps significant that although all three groups of workers agree closely on their data, they differ markedly in their interpretations. Thus de Deken et al. stress the shift in wavelength of the absorption maximum with pH, which they interpret as indicative of hydrogen bonding. In view of this and other evidence they

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<th>Cysteine</th>
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<td>$k_1 K_A$ (l. mol$^{-1}$ sec.$^{-1}$)</td>
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<td>$k_2$ (l. mol$^{-1}$ sec.$^{-1}$)</td>
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<td>$pK_4^*$ (by titration)</td>
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<td>$pK_4^*$ (by kinetic data)</td>
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</tbody>
</table>

* These values have been approximately corrected to zero ionic strength by the factor 0-505 V/I.
reject all the three ionization schemes discussed earlier and propose that the only intermediate ionic species can be represented as (I).

\[
\begin{align*}
&\text{CO}_2^- \\
&\text{H} - \text{C} - \text{CH}_2 \\
&\text{NH}_2 - \text{S} \\
&\text{H} \\
\end{align*}
\]

(I)

On this view the titration constants are also intrinsic dissociation constants.

Benesch & Benesch, on the other hand, interpret their results in terms of Edsall's scheme and whilst mentioning the shift in absorption maximum with pH make no attempt to interpret it. Their mathematical formulation can be interpreted as identical with equation (13) of the present paper with extinction coefficients replacing rate constants. Although they do not express it in this way, ultimately they arrive at precisely the same situation as has arisen in the present work: they can determine the extinction coefficient of the \( R\text{NH}_3^+ \) form, but the only other parameter directly calculable from their data is a product of \( K_A \) and an extinction coefficient. Benesch & Benesch resolve this situation by assuming that the two extinction coefficients are equal and quote evidence to support this assumption. However, as has since been pointed out by Edsall, Martin & Hollingworth (1958), this assumption is not valid though it may be nearly correct. Benesch & Benesch's results could thus be interpreted equally well on Calvin's ionization scheme by assuming an appropriate value for the extinction coefficient of the \( R\text{NH}_3^+ \) species. A strict interpretation of their results is thus that they have set limits to the value of \( K_A \). [Although Benesch & Benesch solve their results independently for \( K_A \), \( K_B \) and \( K_D \), only one of these can be considered truly independent because of interrelationships (i), (ii) and (iii) above.]

This was realized by Gorin (1956), who took the view that it is impossible to draw any definite conclusions from the study of variation of ultraviolet spectra with pH because the extinction coefficient of the intermediate ionic species is not accessible to experimental measurement.

The failure of all methods so far employed to give an unequivocal answer to the problem of the precise pattern of ionization of cysteine raises the question whether any solution is possible. Looked at from this standpoint it appears that the failure of all methods so far is due to the fact that none of them gives a measure solely of either of the intermediate ionic species. Both the ultraviolet-absorption work and the present kinetic data are basically ways of measuring \( RS^- \) but cannot differentiate between

\[
R\text{S}^- \quad \text{and} \quad R\text{NH}_2^+.
\]

Until the variation in concentration of a single one of these two ionic species with pH can be followed any conclusions drawn must be based on arbitrary assumptions.

For most purposes this conclusion is not quite so unfortunate as might appear at first sight. The work of Benesch & Benesch shows that equilibrium processes for cysteine, as for other polyacids (Adams, 1916), can be interpreted mathematically merely on the basis of titration constants, and the present work shows that this is true also for kinetic data.

Although it is possible to describe satisfactorily the behaviour of cysteine in terms of titration constants for many situations, nevertheless there are problems which cannot be solved without a knowledge of the intrinsic constants. Thus there would be little point in extending the present work to differing temperatures since both \( k_1 \) and \( pK_A \) could be temperature-dependent and cannot be separately determined. Moreover a knowledge of intrinsic \( pK \) values for groups in proteins would enable the distances apart of charged groups to be estimated.

The problem thus has a definite significance but it should be realized that at present no completely unequivocal solution is possible. Fundamentally all that this or previous work has established are limits for the value of \( pK_A \). If analogous work could be done making use of the amino group, limits could be similarly set for \( pK_B \). Combined with the relationship (i) \( K_1 = K_A + K_B \) this would permit much closer limits to be set for \( K_A \) and \( K_B \). With regard to the present technique any extension of this kind is limited by lack of a suitable reaction which is specific for \( R\text{NH}_3^+ \) or \( R\text{NH}_2 \) in the presence of thiol groups, and spectroscopy of some kind seems the most hopeful possibility.

SUMMARY

1. A study of the kinetics of the reaction between mercaptoacetic acid and chloroacetamide shows that reaction proceeds via the \( RS^- \) form.
2. A similar study for \( \beta \)-mercaptoethylamine and cysteine shows conclusively that, when the reactivity of sulphhydryl group is considered, the assignment of \( pK \) values given by Cohn & Edsall (1943) is incorrect.
3. The kinetic data for \( \beta \)-mercaptoethylamine and cysteine do not permit differentiation between
the ionization schemes proposed by Calvin and Edsall.

4. Kinetic data permit accurate estimates of titration pK values to be made.

5. The problem of the determination of intrinsic dissociation constants is discussed with reference to cysteine.

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REFERENCES


Studies on the Particulate Components of Rat Mammary Gland

5. COMPARISON OF LARGE PARTICLES FROM LIVER AND MAMMARY GLAND*

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It is generally considered that the mitochondrial fraction of tissue suspensions contains the major part of the oxidative-enzyme systems of the disrupted cells. In liver tissue the mitochondrial fraction can be separated by differential centrifuging in 0.25 M sucrose for approximately 100,000 g min. Similar fractions can be obtained, under more or less identical conditions, from a variety of other tissues. This paper emphasizes that large variations can occur between certain tissues in the sedimentation properties of the particles containing such oxidative enzymes as succinic dehydrogenase and cytochrome oxidase, and that the results obtained for sedimentation patterns in liver tissue are not always applicable to other tissues such as mammary gland.

Comparative studies on the mitochondrial fraction from various tissues, contrasting sedimentation properties with enzymic distributions, have seldom if ever been made. Cytological investigations (Weatherford, 1929; Dempsey, Bunting & Wislocki, 1947) and biochemical studies (Greenbaum & Slater, 1957c) on rat mammary gland have indicated a change in size of mitochondrial particles over the lactation cycle. Other reports (Dmochowski & Strickland, 1953; Tuba, Orr & Wiberg, 1955) have suggested a size difference between the mitochondria from liver and mammary gland. It was therefore of interest to centrifuge liver and mammary-gland suspensions under identical conditions, tissues from animals at various stages of the lactation cycle being used, to check for: (a) possible variations in the size of mammary-gland mitochondria over the lactation cycle; (b) possible variations in the size of liver mitochondria over the lactation cycle; (c) possible differences between liver and mammary-gland mitochondria.

METHODS

The animals used were hooded Norwegian adult female rats (body wt. 180–200 g.) of the Medical Research Council strain. The litters of lactating animals were restricted to 6–11 young. Three stages of the lactation cycle were studied: late pregnancy (18–19 days of pregnancy), 3 days lactating and 18 days lactating. Animals were killed by cervical dislocation and the livers and mammary glands were quickly removed and placed in ice-cold aqueous 0.25 M sucrose. Livers and mammary glands were homogenized for the same time by the method described by Greenbaum & Slater (1957a); after straining through muslin the suspensions were briefly rehomogenized with a plastic pestle (diam. difference, 0.005 in.). Tissue suspensions so prepared were finally diluted (1:5) with ice-cold aqueous 0.25 M sucrose.

The tissue suspensions of liver and mammary gland from the same animal were centrifuged simultaneously in a MSE Angle 13 centrifuge by the procedure shown in Fig. 1.