The Reaction of Thiol Compounds and Chloroacetamide

2. THE REACTION BETWEEN CHLOROACETAMIDE AND CYSTEINE PEPTIDES*

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(Received 7 July 1961)

Lindley (1960) showed that the reaction between chloroacetamide and thiol compounds proceeds via the thiol anion RS^- , and hence it was possible to show for cysteine and β -mercaptoethylamine that the two ionic species $^-S \cdot R \cdot NH_3^+$ and $HS \cdot R \cdot NH_2$ occurred simultaneously in solution. The paper reports the results obtained in a similar investigation of the reaction between chloroacetamide and cysteine peptides which was undertaken to study the effect of increased separation of the charged groups and neighbouring side chains on thiol reactivity.

THEORETICAL

Lindley (1960) showed that for a thiol group with dissociation constant K uncomplicated by other groups dissociating in the same pH range, the pH dependence of the kinetics of the reaction with chloroacetamide could be described by the equation

$$k_0 = \frac{kK}{[H^+] + K}, \quad (1)$$

where k_0 is the apparent second-order rate constant related to the total concentration of thiol in all ionic forms and k is the true second-order rate constant related to the concentration of the reactive thiol anion. Inversion of (1) leads to

$$\frac{1}{k_0} = \frac{[H^+]}{kK} + \frac{1}{k}. \quad (2)$$

This form is useful since it demonstrates that a plot of $1/k_0$ versus $[H^+]$ is linear with an intercept of $1/k$ on the $1/k_0$ axis, and furthermore the ratio of the intercept to the slope is equal to K . Another alternative form of this equation (1) is

$$\frac{k - k_0}{k_0} = \frac{[H^+]}{K} \quad (3)$$

$$\text{or} \quad \log \frac{k - k_0}{k_0} = -\text{pH} + \text{p}K. \quad (4)$$

Hence a plot of $\log [(k - k_0)/k_0]$ versus pH is a straight line of slope -1 with an intercept on the pH axis equal to $\text{p}K$. The graphical form of

equation (2) can be used to obtain a value for k from results over a fairly restricted pH range, but is not a very satisfactory way of plotting data over a wide range of pH values. The graphical form of equation (4) is very suitable for this purpose with a value of k obtained from the inverse plot of equation (2) over a restricted pH range.

The case of the kinetics of reaction of amino-thiols with two groups dissociating in the same pH range was also considered by Lindley (1960). By using the general ionization scheme (Scheme 1),

and the dependent relations

$$K_1 = K_A + K_B; \quad K_1 K_2 = K_A K_C = K_B K_D;$$

and

$$1/K_2 = 1/K_C + 1/K_D$$

[this relationship was previously printed (Lindley, 1960) in error as $1/K_2 = 1/K_C = 1/K_D$], where K_1 and K_2 are the titration constants, it was shown that

$$k_0 = \frac{k_1 K_A [H^+] + k_2 K_1 K_2}{[H^+]^2 + K_1 [H^+] + K_1 K_2}, \quad (5)$$

where k_1 and k_2 are the true second-order rate constants for the ionic species $^-S \cdot R \cdot NH_3^+$ and $^-S \cdot R \cdot NH_2$ respectively. Furthermore, if K_1 and K_2 are well separated and k_2 is not inordinately large compared with k_1 , at low pH values the expression reduces to

$$k_0 \div \frac{k_1 K_A [H^+]}{[H^+]^2 + K_1 [H^+]} = \frac{k_1 K_A}{[H^+] + K_1}. \quad (6)$$

* Part 1: Lindley (1960).

At high pH values, on the other hand, the expression reduces to

$$k_0 \doteq \frac{k_2 K_1 K_2}{K_1 [H^+] + K_1 K_2} = \frac{k_2 K_2}{[H^+] + K_2} \quad (7)$$

Both equation (6) and (7) are of the same form as equation (1), and by the inverse plot it is thus possible to determine $k_1 K_A$, K_1 , k_2 and K_2 . These relationships have been discussed and utilized by Lindley (1960), but the case where K_1 and K_2 do not differ greatly in magnitude has not been considered since it was irrelevant to the earlier work. A plot of k_0 versus pH has obvious analogies with a titration curve, and it is well known that the shape of a titration curve involving two dissociating groups on the same molecule is dependent on the ratio of the two titration dissociation constants. In fact, when the ratio $K_1/K_2 = 4$ the titration curve becomes identical with that of a single group with a dissociation constant of $0.5K_1$ or $2K_2$. (It is perhaps worth interpolating here that this is true only for cases involving the two alternate ionization pathways mentioned earlier: for a mixture of two independent compounds with similar dissociation constants the titration curve only becomes equivalent to that of a single group when the two dissociation constants are equal.) It is obviously important to establish if the plot of k_0 versus pH also reaches a stage where it becomes indistinguishable from a simple titration curve as K_1 approaches K_2 and if so what factors, if any, are involved in addition to K_1 and K_2 .

Equation (5) can be readily transformed to the form

$$\frac{k_2 - k_0}{k_0} = \frac{[H^+] (k_2 [H^+] + k_2 K_1 - k_1 K_A)}{k_1 K_A [H^+] + k_2 K_1 K_2} \quad (8)$$

The condition that this will assume the same form as that given by a simple thiol of dissociation constant K^1 is

$$\frac{[H^+] (k_2 [H^+] + k_2 K_1 - k_1 K_A)}{k_1 K_A [H^+] + k_2 K_1 K_2} = \frac{[H^+]}{K^1} \quad (9)$$

For this to be true there must be a common factor relating the numerator and denominator of the left-hand side of equation (9) and hence

$$\frac{1}{K^1} = \frac{k_2}{k_1 K_A} = \frac{k_2 K_1 - k_1 K_A}{k_2 K_1 K_2}; \quad (10)$$

by simple algebra this gives

$$k_1 K_A = \frac{k_2 K_1}{2} \left[1 \pm \sqrt{1 - \frac{4K_2}{K_1}} \right] \quad (11)$$

Thus if $K_1 < 4K_2$ the roots are imaginary. For the condition $K_1 = 4K_2$, and with the further implied condition that $k_1 = k_2$, a single value of

$$K^1 = 2K_2 = 0.5K_1$$

exists. This corresponds precisely to the titration case mentioned earlier. When $K_1 > 4K_2$ two roots exist, i.e.

$$K^1 = \frac{K_1}{2} \left[1 \pm \sqrt{1 - \frac{4K_2}{K_1}} \right]$$

This expression has the form

$$pK^1 = pK_1 + \delta pK \quad \text{or} \quad pK^1 = pK_2 - \delta pK$$

and the term δpK is a function of K_1/K_2 . The term δpK has a maximum value of 0.30 when $K_1/K_2 = 4$, and rapidly decreases as K_1/K_2 or $pK_2 - pK_1$ increases.

The conditions that a plot of k_0 versus pH for a compound with two different groups dissociating in the same pH region should simulate exactly a titration curve for a simple group are therefore extremely restrictive. As we have seen, even for the case where $K_1 = 4K_2$ and the titration curve itself becomes indistinguishable from that of a group having $pK = 2K_2 = 0.5K_1$ the kinetic plot would show deviations from a simple titration curve for all cases except when $k_1 = k_2$.

However, a theoretical mathematical proof of non-identity does not necessarily imply that differences can be detected in practice. It is difficult to distinguish the pH dependence curves of some of the peptides with a low ratio for K_1/K_2 from curves that would have been given by a simple thiol with pK intermediate between pK_1 and pK_2 . Some theoretical curves showing the dependence of k_0 on pH are plotted from equation (5) and illustrate the effect of altering various parameters. In Fig. 1 K_1 , $k_1 K_A$ and k_2 have been kept constant and the family of curves shows the effect of varying the ratio K_1/K_2 from 4 to 100. Values for $k_1 K_A$ and k_2 were chosen so that the case $K_1/K_2 = 4$ corresponds to a simple titration curve and the whole family of curves is analogous to those illustrating the effect of increasing separation of pK_1 and pK_2 on titration curves. Fig. 2 shows the effect of variation in the ratio of $(k_1 K_A)/k_2$ on the pH dependence of the observed rate constant when $pK_1 = 7.7$ and $pK_2 = 9.7$. It can be seen that the shape and position of the curve are most sensitive to changes in $(k_1 K_A)/k_2$ when the value of this is close to K_1 . Curves for other cases where pK_2 approaches pK_1 more closely show a similar displacement of the curve along the pH axis accompanied by a steepening of the slope without any marked shape change. In these cases, too, small changes in $(k_1 K_A)/k_2$ produce their maximum effect when the value of $(k_1 K_A)/k_2$ is approximately equal to K_1 . Fig. 3 is similar to Fig. 1 except that in this case $k_1 K_A > k_2 K_1$ and since K_1 must be greater than K_A this implies $k_1 > k_2$. In this family of curves k_0 passes through a series of maxima and approaches k_2 asymptotically at high pH values.

One other theoretical aspect which has been considered is the effect on the pH dependence of the kinetics of one particular ionic species being in equilibrium with some tautomeric form, e.g. an H-bonded form. However, although such a situ-

ation introduces an apparent displacement of the pK of the reacting group with respect to its intrinsic pK by a factor dependent on the magnitude of the equilibrium constant between the tautomeric forms, further consideration shows that the titration pK would be affected in precisely the same fashion. It follows therefore that comparison of kinetic and equilibrium data do not permit the detection of simple tautomeric equilibria.

EXPERIMENTAL

Materials. The L-cysteinyl-L-asparagine was a specimen prepared by Leach & Lindley (1954a). The sample had been kept in a specimen tube since 1952 but showed no evidence of oxidation in this time. The glutathione was a gift from The Distillers Co. Ltd. and the remaining peptides were prepared by Dr W. E. Savidge in this Laboratory.

Apparatus and technique. The apparatus and technique were reported by Lindley (1960). Both kinetic and titration data were obtained at 30° in the presence of 0.1 M-KCl. The usual experimental procedure was to weigh 3–10 m-moles of the peptide into the reaction vessel of the pH-stat assembly, where it was dissolved in 10 ml. of 0.1 M-KCl and titrated to the required pH with 0.2 N-KOH. To this was added 10 ml. of a chloroacetamide solution in 0.1 M-KCl at 30°. For all pH values greater than 9 the pH of the chloroacetamide solution was adjusted before mixing. The concentration of the chloroacetamide solution was used as a convenient variable to adjust the reaction rate to experimental conditions. Thus at low pH values it was present in up to 50-fold excess, whereas at higher pH values a molar ratio of chloroacetamide to peptide of approximately 2:1 was used. The end point of the reaction was found by adding a few crystals of iodoacetamide, which took the reaction to completion very quickly. Velocity constants were calculated from the plot of alkali uptake versus time by using the integrated form of the equation for a second-order reaction.

Fig. 1. Theoretical curves showing the pH dependence of experimental second-order rate constant k_0 plotted from the rate expression

$$k_0 = \frac{k_1 K_A [H^+] + k_2 K_1 K_2}{[H^+]^2 + K_1 [H^+] + K_1 K_2}$$

The following values were assumed: pK_1 7.7, k_2 0.2, $(k_1 K_A)/k_2$ 10^{-8} . Curves A–E are shown for pK_2 8.3, 8.5, 8.7, 9.0 and 9.7 respectively.

Fig. 2. Theoretical curves for the rate expression for k_0 given in the legend to Fig. 1, assuming the parameters pK_1 7.7, pK_2 9.7, k_2 0.2 and showing the effect of variation in $(k_1 K_A)/k_2$ at 10^{-8} (A), 10^{-9} (B) and 10^{-10} (C).

Fig. 3. Similar to Fig. 1 except that $(k_1 K_A)/k_2 = 10^{-7}$. Note the change in the scale of k_0 axis. Curves A–C are shown for pK_2 8.3, 8.7 and 9.7 respectively.

RESULTS

L-Cysteinyl-L-asparagine. Titration of this peptide gave pK_1 6.75 and pK_2 9.32 at 30° and I 0.1, with a purity of 100%. Leach & Lindley (1954*b*) give pK values of 7.05 and 9.07 at 18° and zero ionic strength.

Fig. 4 shows the dependence of the apparent second-order rate constant on pH for this peptide. The curve has obvious similarities to a titration curve but has a pronounced tail. Plots of the reciprocal of the apparent rate constant against $[H^+]$ at low (Fig. 5) and high (Fig. 6) pH values enable the following values to be obtained: pK_1 6.72, pK_2 9.39, k_1K_A 0.25×10^{-8} l.mol.⁻¹ sec.⁻¹ and k_2 0.263 l.mol.⁻¹ sec.⁻¹. The agreement between the pK values obtained in this way and the titration values is satisfactory and it is possible to obtain the maximum information from the kinetic data: in the earlier investigations on cysteine and β -mercaptoethylamine k_2 was obtained by making use of the experimental value of pK_2 obtained from titration data.

Glycyl-L-cysteine. The purity of the sample of this peptide was 95.5%, as estimated by the technique of Benesch & Benesch (1957); titration gave 8.01 and 9.58 for the two pK values.

Fig. 7 shows the plot of the observed second-order rate constant versus pH and by purely visual inspection it would appear that there is a close

Fig. 4. pH dependence of the observed second-order rate constant (k_0) for the reaction between chloroacetamide and L-cysteinyl-L-asparagine at 30° and I 0.1.

similarity to a simple titration curve. However, a plot of $1/k_0$ versus $[H^+]$ for pH 7–8 is not linear and hence there is some deviation from the theoretical curve to be expected from a single dissociation constant. The results do not therefore permit the direct determination of k_1K_A and pK_1 . A similar plot of $1/k_0$ versus $[H^+]$ for pH 9–10 gives a reasonable straight line, from which estimates of k_2 0.322 l.mol.⁻¹ sec.⁻¹ and pK_2 9.46 can be obtained. The agreement between this latter value and that obtained by titration is satisfactory, although not so close as has been obtained in other cases. As a check on the results and also in order to obtain an estimate of k_1K_A , values for k_0 , $[H^+]$, K_1 and K_2 have been substituted in equation (5). This leads to a series of simultaneous equations involving k_1K_A and k_2 which can be solved by taking pairs. Values obtained in this way

Fig. 5. Plot of the reciprocal of the apparent rate constant $1/k_0$ against $[H^+]$ for the reaction between chloroacetamide and L-cysteinyl-L-asparagine (pH 6–7).

Fig. 6. As Fig. 5 except that results refer to the range pH 9–10.

were $k_1 K_A$ 1.85×10^{-10} l.mol.⁻¹ sec.⁻¹ and k_2 0.348 l.mol.⁻¹ sec.⁻¹.

Glutathione. Assay of this compound indicated 100% purity and gave titration pK values of 3.61, 8.65 and 9.52 at 30° and I 0.1. Previously recorded values are 3.53, 8.66 and 9.62 at 20° (Pirie & Pinhey, 1929), 3.59, 8.75 and 9.65 at 25° and I 0.15 (Li, Gawren & Bascaus, 1954); Martin & Edsall (1958) obtained 8.64 and 9.52 at 25° and I 0.16.

Fig. 8 shows the pH dependence of the observed second-order rate constant and it can be seen that the points fall on a smooth curve very similar to that expected from a single dissociation constant. When $1/k_0$ is plotted against $[H^+]$ a value of 0.259 l.mol.⁻¹ sec.⁻¹ for k_2 was obtained and, as shown in Fig. 9, when this value of k_2 is used to plot $\log [(k_2 - k_0)/k_0]$ versus pH the resulting plot is a good approximation to a straight line, with a slope which is close to the theoretical value of -1.0 . Both the reciprocal plot and the logarithmic plot yield a value of 8.9 for the pK which is not in very good agreement with either of the titration values.

This result shows that it is easy to be misled by application of these graphical methods if the two titration pK values are close together. In the present work the additional information available from titration curves permits the substitution of values for k_0 , $[H^+]$, K_1 and K_2 in equation (5), leading to a series of simultaneous equations which can be solved for $k_1 K_A$ and k_2 . The values found by this procedure were $k_1 K_A$ 3.37×10^{-10} l.mol.⁻¹ sec.⁻¹ and k_2 0.288 l.mol.⁻¹ sec.⁻¹. The curve drawn in Fig. 8 is actually the theoretical curve employing

these parameters whereas the points are experimental data.

In addition to the normal kinetic runs an extra determination was done for glutathione in the presence of 4M-urea and 0.1M-KCl at pH 8.0 and 30°. This gave a value of k_0 2.72×10^{-2} l.mol.⁻¹ sec.⁻¹ compared with the figure of 2.87×10^{-2} l.mol.⁻¹ sec.⁻¹ in the absence of urea. In view of the uncertainty attached to the measurement of pH in 4M-urea solutions this was regarded as indicating that urea had no effect on the kinetics.

Glycyl-L-cysteinylglycine. Titration data indicated a purity of 96% and gave pK values of 3.38, 7.82 and 9.04 for this peptide. The pH dependence of the observed second-order rate constant is shown in Fig. 10. In this case, too, graphical methods appeared to show that the data fitted the reaction with a single group having pK 8.70. However, by using the method of solving pairs of simultaneous equations it was shown that the data can be fitted to the parameters $k_1 K_A$ 5.55×10^{-10} l.mol.⁻¹ sec.⁻¹ and k_2 0.265 l.mol.⁻¹ sec.⁻¹, with the titration values for K_1 and K_2 . In Fig. 10 the curve is theoretical and the points are experimental.

Fig. 7. k_0 versus pH for the reaction between chloroacetamide and glycyl-L-cysteine (30° and I 0.1).

Fig. 8. k_0 versus pH for the reaction between glutathione and chloroacetamide (30° and I 0.1). Points are experimental; the curve is theoretical, from titration values for K_1 and K_2 and $k_1 K_A$ 3.37×10^{-10} l.mol.⁻¹ sec.⁻¹ and k_2 0.288 l.mol.⁻¹ sec.⁻¹.

Fig. 9. Plot of $\log (k_2 - k_0)/k_0$ versus pH for the reaction between glutathione and chloroacetamide with the value $0.259 \text{ l.mol.}^{-1} \text{ sec.}^{-1}$ for k_2 as found from a plot of $1/k_0$ versus $[\text{H}^+]$.

DISCUSSION

It was pointed out by Lindley (1960) that the data obtained in studies of this kind do not permit an unequivocal solution to the problem of the precise ionization pathway of amino thiols. As discussed in that paper the condition $k_1 < k_2$ is probably generally true, so that the condition $k_1 = k_2$ can be taken as limiting. This permits a minimum value of K_A (i.e. maximum value of $\text{p}K_A$) to be calculated from the experimentally determined value of $k_1 K_A$. The upper theoretical limit to K_A is K_1 (lower limit to $\text{p}K_A$), and hence from K_1 and $k_1 K_A$ we can calculate a minimum value for k_1 and hence a maximum value for the ratio k_2/k_1 . The earlier work on cysteine and β -mercaptoethylamine looked at in this way gives a maximum value of 5 for k_2/k_1 . Now k_1 is less than k_2 probably because of the presence of a positive charge (NH_3^+) on the molecule withdrawing electrons from the sulphur atom; hence this effect will be more important where the NH_3^+ and S^- groups are separated by a small number of atoms. For three of the four peptides studied in the present work this inductive effect of the NH_3^+ group will therefore be considerably less than for the previously studied compounds, cysteine and β -mercaptoethylamine. The exception is L-cysteinyl-L-asparagine, where the NH_3^+ and SH groups are separated by two carbon atoms as in cysteine and β -mercaptoethylamine. This condition that $k_2/k_1 < 5$ may therefore be used to set a probable maximum for K_A (minimum for $\text{p}K_A$).

Table 1 shows the probable limits for $\text{p}K_A$ calculated in this way together with limits for $\text{p}K_B$ calculated from these values and the relationship $K_1 = K_A + K_B$. These figures suggest that for the dipeptides $\text{p}K_A > \text{p}K_B$ so that the preferred ionization pathway is

For glycyl-L-cysteinylglycine the limits are still too broad for an unequivocal conclusion to be drawn. For glutathione the limiting condition $k_2 = k_1$ yields a maximum $\text{p}K_A$ 8.93 and K_1 sets a lower limit, $\text{p}K_A$ 8.65, so that for this peptide $k_2/k_1 < 2$. Glutathione seems exceptional among the peptides studied in that $\text{p}K_A < \text{p}K_B$, though the difference may be very small.

The observation that the rate of reaction between glutathione and chloroacetamide is unchanged by the presence of 4M-urea shows that H-bonded forms of glutathione are not found to any significant extent in mildly alkaline solutions. This is in agreement with the conclusion arrived at in the theoretical section since such H-bonded tautomeric

Fig. 10. k_0 versus pH for the reaction between chloroacetamide and glycyl-L-cysteinylglycine at 30° and I 0.1. Points are experimental; the curve is theoretical, from titration values for K_1 and K_2 and calculated values 5.55×10^{-10} and $0.265 \text{ l.mol.}^{-1} \text{ sec.}^{-1}$ for $k_1 K_A$ and k_2 respectively.

Table 1. Summary of data on cysteine peptides obtained in present work

Peptide	pK_1		pK_2		$k_1 K_A$ (l.mol. ⁻¹ sec. ⁻¹)	k_2 (l.mol. ⁻¹ sec. ⁻¹)	Probable range of pK_A †	Probable range of pK_{AS}
	From titration data	From kinetic data	From titration data	From kinetic data				
L-Cysteinyl-L-asparagine	6.75	6.72	9.32	9.39	0.25×10^{-8} *	0.263*	7.3-8.0	6.78-6.89
Glycyl-L-cysteine	8.01	—	9.68	9.46	1.85×10^{-10} †	0.322*	8.6-9.3	8.03-8.14
Glutathione	8.65	—	9.52	(8.9)	3.37×10^{-10} †	0.298†	8.65-8.93¶	8.97-9.52
Glycyl-L-cysteinylglycine	7.82	—	9.04	(8.7)	5.55×10^{-10} †	0.265†	8.0-8.7	7.9-8.3

* Directly from kinetic data.
† On supposition that $1 < k_2/k_1 < 5$ (see Discussion).
‡ Known to be in error (see text).
§ From data from previous column and relationship $K_1 = K_A + K_B$.
¶ Lower limit set by pK_1 .

forms would be expected to lead to abnormal titration constants. The results obtained by Benesch, Benesch & Rogers (1954), who explained them on the basis of hydrogen bonds involving the S^- anion, therefore require re-examination. Their conclusions were based on the intensity of colour given by nitroprusside and thiols in the presence and absence of urea. The reaction is complex and involves the resultant of colour-producing and colour-destroying reactions, so that other explanations of their results can be envisaged.

The finding that in practice the pH dependence of the kinetics of reaction of chloroacetamide with glutathione which has titration pK values 8.65 and 9.52 can be closely approximated by a simple thiol of pK 8.90 suggests that care is necessary in the interpretation of the pH dependence of enzyme kinetics. Thus not only may the derived pK values be in error, but their interpretation, especially with regard to such factors as temperature dependence, may be of doubtful significance.

A further point with regard to enzyme kinetics comes from a consideration of Fig. 3. This shows obvious analogies to the pH-optimum curves obtained for some enzymes, and, if k_2 is 0, so that equation (5) reduces to

$$k_0 = \frac{k_1 K_A [H^+]}{[H^+]^2 + K_1 [H^+] + K_1 K_2}, \quad (12)$$

the curve becomes identical with the classical pH-activity curve for an enzyme. This equation could describe for example the pH dependence of an enzyme which had thiol and amino groups with overlapping pK values as active groups and was only catalytically active in the form $^-S \cdot E \cdot NH_3^+$.

Alberty & Massey (1954) have derived an expression for enzyme relating V and $[H^+]$ which is analogous to equation (12), in which K_1 replaces the K_A in the numerator. Using this equation they and other authors (Dixon & Webb, 1958) have derived methods of calculating K_1 and K_2 from the resulting quadratic expression. However, implicit in their derivation is the assumption that the addition of a proton to the enzyme-substrate complex to give the reactive species EHS proceeds by a single pathway, and only this active species is formed. The active centres of enzymes frequently involve two dissociating groups X and Y, only one of which (X) requires a bound proton for activity. If the pK values of X and Y overlap it would be possible for part of the enzyme-substrate complex to bind a proton to Y giving an inactive species. This more realistic approach is the one described by equation (12), and at first sight it would appear to differ significantly from Alberty & Massey's formula, the two expressions only becoming equivalent when $K_A = K_1$. Further consideration shows, however, that if the model

involving alternate ionization pathways is used then the significance of the observed rate constant for the breakdown of the enzyme-substrate complex is altered. In fact the observed rate constant equals the true rate constant multiplied by the factor K_A/K_1 and incorporation of this in equation (12) makes it identical with Alberty & Massey's formula. Hence their procedure for calculating K_1 and K_2 from the pH dependence of V is valid on either model of enzyme activity.

SUMMARY

1. The theory of kinetics of the reaction between chloroacetamide and amino thiols developed by Lindley (1960) has been further investigated.

2. The reaction between four cysteine peptides and chloroacetamide has been studied experimentally.

3. For three of the peptides it appears probable that $pK_{SH} > pK_{NH_3^+}$; for the fourth peptide, glutathione, $pK_{NH_3^+} > pK_{SH}$.

4. Some general aspects of the determination of pK of complex ions by kinetic methods are discussed and their relevance to some aspects of enzyme kinetics is pointed out.

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The Relationship of Structure to Activity in Plant-Growth-Regulating Compounds

2. GROWTH-REGULATING ACTIVITY OF SUBSTITUTED AMIDES OF 2,4-DICHLOROPHENOXYACETIC ACID*

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(Received 4 September 1961)

During the course of an investigation on the plant-growth-regulating activities of a number of derivatives of 2,4-dichlorophenoxyacetic acid, considerable differences in activity were shown by closely related substituted amides of this acid. Since these differences in activity could not be accounted for by known factors, the scope of the investigation was widened to gain further information on the mode of action of the amides of plant-growth-regulating acids.

There does not appear to be agreement among workers in this field as to whether the amides of auxin acids are active auxins as such or whether hydrolysis to the acid is necessary before activity is shown. In an attempt to answer this question the rates of hydrolysis of a number of these amides were determined in alkaline solution (Johnston, 1961).

* Part 1: Johnston (1961).

The auxin activities of these compounds have now been determined in order to find out whether any relationship exists between the activities of the amides and their chemical stabilities or any other chemical properties which can be related to their stabilities.

EXPERIMENTAL

The activities of the amides were determined by comparing their physiological effects on intact growing plants with that of 2,4-dichlorophenoxyacetic acid as a reference compound.

Test plants. Haricot beans were grown in normal soil in cardboard cartons approximately 2½ in. diam., two or three plants/carton. The plants were grown under standardized conditions and only healthy plants of the same size, age and stage of development were used. They were used when the first two leaves were expanding.

Test solutions. The compounds to be tested were dissolved in a mixture of methylcyclohexanone (80 parts by