very well if $w/kT = 2$, as can be seen in Fig. 3 where it is superimposed on the rate data.

Discussion of Model G.—The side-chain carboxyls in the random coil are about 10 Å. apart and an interaction energy of $2kT$ is quite reasonable. The titration curve of a helical region is different from that of a random coil region whereas we used the average titration curve in making the calculations. As soon as the helix–coil transition is complete, we will have no error. Error from this source, therefore, will contribute only below pH 5. Another source of error is higher order interactions. This cannot be easily corrected without going to machine calculations. At high degrees of ionization it can be approximated by assuming that the corrected value for the pairwise nearest-neighbor interaction energy is somewhat less than the value for w necessary to fit the data.

If the experimental data are corrected to maximum velocities, the shape of the curve will be changed very little, at least in the pH range of 4–7. Due to ionizable groups involved in the catalytic site, the activity of the enzymes will change with pH. However, from pH 5 to 7 the correction factor for papain, ficin, and chymotrypsin will amount to little more than the experimental error. The pH dependence of subtilisin and elastase is not known well enough to allow a similar estimate.

Assuming the model correctly explains the dominant features in the pH dependence of the rate, dividing the rate at any pH by the corresponding value of F_G will give the rate at which the enzyme hydrolyzes a bond with adjacent side chains uncharged in a random coil region. These rates, corrected to V_m through use of the Michaelis constants and expressed as first-order rate constants, are given in Table III.

Changing w should produce a large effect on the rate above pH 5. The interaction energy can be altered by changing the ionic strength and should provide a good check on the validity of this interpretation of the mechanism.

The pH Dependence of the Rate Catalyzed by Pepsin.—The data for the pepsin-catalyzed reaction are scanty and are included mostly to point out the difference between it and the other endopeptidases. Pepsin is a poor catalyst and at pH 4.6 the rate is the same as that for chymotrypsin. Polyglutamate is difficult to work with below pH 4.5 unless one goes to much lower concentrations, where the rates become difficult to measure. A further complication results from the poor catalytic ability. Enzyme concentrations comparable to the substrate concentration must be used in order to obtain measurable rates, thus making the steady-state assumption questionable. This apparently leads to the observed results of reduced rates at a given pH becoming dependent on the substrate to enzyme ratio. Bull and Currie[19] report pepsin has an ionizable group with a pH of 2.1 involved in the catalytic site. Above pH 3, then, the log of the enzyme activity should vary linearly with pH. The slope of the curve in Fig. 2 is much greater than this. It has been suggested that pepsin acts on the helical form of polyglutamate.[3] Our data support this but cover too narrow a pH range to make possible knowledgeable statements.

TABLE III

RATE CONSTANT FOR HYDROLYSIS OF A PEPTIDE BOND WITH ADJACENT SIDE CHAINS UNCHARGED IN A RANDOM COIL REGION OF PGA, ASSUMING MODEL G

Enzyme	Rate constant, sec.$^{-1}$
α-Chymotrypsin	0.01
Elastase	3.0
Ficin	250
Papain	40
Subtilisin	40

Acknowledgments.—We wish to thank Mr. James Monroe for help in carrying out some of the preliminary experiments and to Mr. Robert Nylund for supplying the hypochromicity curve.

(19) H. B. Bull and B. T. Currie, *J. Am. Chem. Soc.*, **71**, 2758 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF IOWA, IOWA CITY, IOWA]

Degradation of Synthetic Polypeptides. III. Degradation of Poly-α,L-lysine by Proteolytic Enzymes in 0.20 *M* Sodium Chloride[1]

BY WILMER G. MILLER

RECEIVED MARCH 16, 1964

The initial action of carboxypeptidase A and B, α-, β-, γ-, and Δ-chymotrypsin, elastase, ficin, leucine aminopeptidase, papain, pepsin, subtilisin, and trypsin on high molecular weight poly-α,L-lysine in 0.20 *M* NaCl at 25° has been investigated. The endopeptidases chymotrypsin, elastase, ficin, papain, and subtilisin, after correcting to constant enzymic activity, exhibit the same pH dependence of the rate of hydrolysis. Analogous to the action of these enzymes on polyglutamic acid, the pH dependence is quantitatively predicted assuming enzymic attack of lysyl–lysyl bonds with adjacent side chains uncharged in random coil regions of the molecule. Trypsin is shown to be insensitive to the specific charge state of the polylysine side chains. Below pH 9 the rate is quantitatively accounted for, assuming trypsin has an ionizable group with a pK of 6.6 associated with its catalytic activity. Of the exopeptidases, carboxypeptidase B and leucine aminopeptidase showed catalytic activity.

Introduction

The hydrolysis of poly-α,L-lysine by tryspin has been extensively studied by Waley and Watson at pH 7.6.[2] Several less quantitative studies have been carried out,[3–5] mostly concerning hydrolysis products, though pH

(1) Presented in part at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 8–13, 1963. This investigation was supported in part by Public Health Service Research Grant GM-08409.

(2) S. G. Waley and J. Watson, *Biochem. J.*, **55**, 328 (1953).

(3) (a) E. Katchalski, I. Grossfeld, and M. Frankel, *J. Am. Chem. Soc.*, **70**, 2094 (1948); (b) J. A. Gladner and J. E. Folk, *J. Biol. Chem.*, **231**, 393 (1958).

(4) E. Katchalski, Y. Levin, H. Neumann, E. Riesel, and N. Sharon, *Bull. Res. Council Israel*, **10A**, 159 (1961).

(5) H. Neumann, N. Sharon, and E. Katchalski, *Nature*, **195**, 1002 (1962).

dependence of the rate has received some attention. In this study the pH dependence of the initial rate of hydrolysis of long-chain molecules was determined and compared to analogous studies on the negatively charged polyglutamate.[6]

Polylysine is similar to polyglutamate in that the molecules are in a helical conformation when predominantly uncharged and go into a random coil conformation as the side chains are titrated and become predominantly charged.[7] The helix–coil transition is sensitive to electrolyte concentration. Polylysine and polyglutamate do not, however, show analogous effects from changes in electrolyte concentration.[7-9] In 0.20 *M* NaCl the helix–coil transition occurs about pH 10 and the apparent p*K* is also near 10. The effect of charge state as well as conformation on the rate of hydrolysis must be considered.

Experimental

Poly-α,L-lysine was purchased from Pilot Chemicals, Inc., as the hydrobromide and had a weight average molecular weight of about 60,000. Chromatography on CM cellulose indicated the presence of detectable amounts of monomer. No attempt was made to remove either monomer or the bromide. The polymer was soluble to a concentration of 8 g./l. below pH 10.2. Solutions with a polymer concentration of 8 g./l. were metastable above this pH and formed copious precipitates after 1–4 days standing.

The enzymes used in the investigation, their source, and state of purity were the same as in the preceding paper. This also applies to preparation of the enzyme solutions and determination of their concentration. Enzyme concentration ranged from 1×10^{-10} to 7.5×10^{-5} mole/l.

All solutions were 0.200 *M* in NaCl, 0.03–0.04 *M* in bromide and sometimes 5 m*M* in cysteine (ficin and papain) or 2 m*M* $MnCl_2$, 5 m*M* $MgCl_2$ (leucine aminopeptidase). In approximately half of the runs 10 m*M* tris(hydroxymethyl)aminomethane was used as buffer with no detectable change in rate. Substrate concentrations were in the range 6–8 g./l. with a wider range used when determining Michaelis constants. All kinetic measurements were made at 25 ± 0.03° in Ostwald viscometers. Rates were calculated from the viscosity measurements by a method described previously.[10] In making the calculations it was assumed that the Staudinger constant (a) was the same as for polyglutamic acid. If this assumption is in error by as much as 0.2, the rates as calculated would be in error by less than a factor of two.

Chromatographic analyses of partially degraded polypeptide were carried out by gradient elution from CM cellulose. The procedure was similar to that of Stewart and Stahmann.[11] A Technicon AutoAnalyzer was used for peptide detection.

Optical rotations were measured with a Rudolph Model 200 high precision polarimeter, using a zirconium arc lamp and a 40-cm. cell. Polylysine concentrations of 1 g./l. and 5 g./l. were investigated.

Hypochromism was determined at 201 mμ on a Cary 14 spectrophotometer using Beckman far-ultraviolet short path cells with Teflon spacers. The cells had a path length of 0.0379 cm. as determined by interference fringe measurements. Bromide ion absorbs around 200 mμ much more than chloride ion. Rather than remove the bromide by dialysis, enough sodium bromide was added to the reference cell to approximately equal the bromide concentration in the polymer solution. As the matching of bromide was only approximate, the low pH absorption coefficient of polylysine as determined by Tinoco[12] was assumed and absorption coefficients at other pH are relative to this. Above pH 10.5 corrections for hydroxyl ion absorption were made. Polylysine concentrations of 1 g./l. and 5 g./l. were investigated.

(6) W. G. Miller, *J. Am. Chem. Soc.*, **86**, 3913 (1964).

(7) J. Applequist and P. Doty, "Polyamino Acids, Polypeptides and Proteins," M. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, Chapter 14.

(8) M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **80**, 2387 (1958).

(9) A. Wada, *Mol. Phys.*, **3**, 409 (1960).

(10) W. G. Miller, *J. Am. Chem. Soc.*, **83**, 259 (1961).

(11) J. Stewart and M. Stahmann, "Polyamino Acids, Polypeptides and Proteins," M. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, Chapter 9.

(12) I. Tinoco, A. Halpern, and W. Simpson, *ibid.*, Chapter 13.

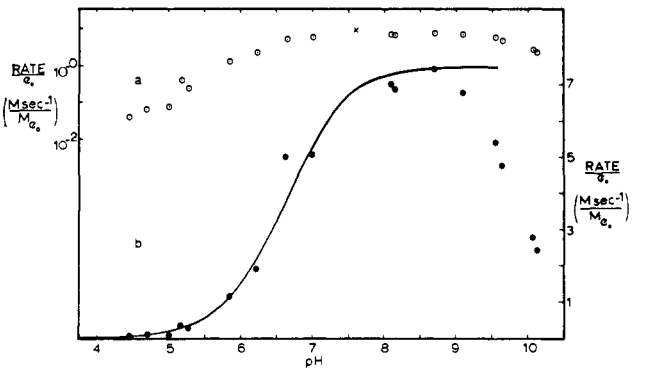

Fig. 1.—Hydrolysis of polylysine by trypsin: (a), rates plotted on a logarithmic scale; (b), rates on a linear scale. Solid line calculated from eq. 1 with $pK_a = 6.6$; (×), datum from Waley and Watson.

The titration curve of a 1.5 g./l. polylysine HBr solution was obtained using a Cary Model 31 vibrating reed electrometer.

Results

Rate Law.—As with polyglutamate hydrolysis,[6] the data for polylysine hydrolysis by endopeptidases followed the Michaelis–Menten rate law. The rates were first order in enzyme concentration. The precision of the data and the problems encountered were completely analogous to polyglutamate hydrolysis.

Exopeptidases.—Carboxypeptidase A(DFP), concentration 2×10^{-5} *M*, gave no detectable hydrolysis at 5.6, 7.2, 7.7, or 9.5. An insoluble complex slowly formed in these solutions and gave rise to slight viscosity changes. Carboxypeptidase B, however, caused readily detectable hydrolysis at pH 7.9, 8.5, 8.8, and 9.3. Chromatographic analysis showed the monomer to be the hydrolysis product. At an enzyme concentration of 2×10^{-6} *M* the specific viscosity decreased to half of its initial value in 1–2 hr., depending upon the pH. These results are in agreement with those of Gladner and Folk[3] but extend over a wider pH range.

Leucine aminopeptidase, concentration 2×10^{-7} *M*, hydrolyzed monomer from polylysine at pH 4.3, 6.9, 7.6, 8.0, and 9.7. The viscosity decreased much more rapidly at pH 4.3 and 9.7 than at the other pH studied. The significance of this is not understood. Above pH 9.5 a yellow precipitate sometimes formed. The precipitate is most likely a complex between polylysine and one of the cations (Mg^{2+} or Mn^{2+}) added for activation of the enzyme. The precipitate may also involve the enzyme, a ternary complex being formed from the enzyme, the substrate, and the metal ion.[13]

Endopeptidases.—There was no detectable hydrolysis by pepsin, concentration 2×10^{-5} *M*, at pH 4.6 nor at pH 3.5 by 2×10^{-4} *M* pepsin. This is consistent with previous findings at pH 2.[2]

The hydrolysis by trypsin as a function of pH is shown in Fig. 1a. On a logarithmic scale the pH dependence of the rate is very small. The rate at pH 7.6 agrees well with that found by Waley and Watson using a pH stat to measure hydrolysis.[1] The Michaelis constant (K_m) at pH 7.05 was 1.4×10^{-4} $M_{(polymer)}$, in reasonable agreement with a value of 1.8×10^{-4} *M* at pH 7.6 derived from the data of Waley and Watson by logarithmic extrapolation to 60,000 molecular weight. Chromatograms of polylysine having 3–5% of the original peptide bonds hydrolyzed indicated

(13) E. L. Smith and D. H. Spackman, *J. Biol. Chem.*, **212**, 271 (1955).

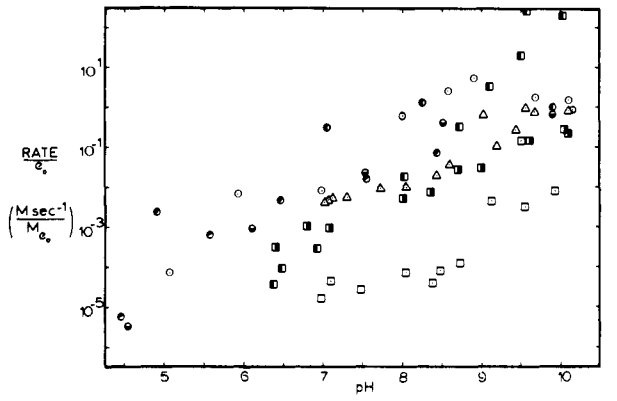

Fig. 2.—Hydrolysis of polylysine by α (○), β (◒), γ (◓), Δ (◐) chymotrypsin, elastase (□), ficin (△), papain (◨), and subtilisin (■□).

qualitatively random degradation except for discrimination near the chain ends. This is in agreement with the findings of Katchalski, *et al.*,[4] and in contrast to the observations of Waley and Watson.

Each of the other endopeptidases showed catalytic activity towards polylysine, though exhibiting a larger pH dependence of the rate than that observed with trypsin. Unlike the catalytic activity of these enzymes on polyglutamate, the pH–rate curves were not superimposable. The data for the chymotrypsins, elastase, ficin, papain, and subtilisin are shown in Fig. 2.

Discussion

Superposition of the pH–Rate Curves for the Chymotrypsins, Elastase, Ficin, Papain, and Subtilisin.—Most enzymes, when acting on small substrates, show a pH dependence of their catalytic activity which can be accounted for if a specific charge state is assumed to be the activity form of the enzyme. The concentration of catalytically active enzyme (e) relative to the total enzyme concentration (e_0) can be described frequently by

$$(e) = \frac{(e_0)}{1 + (H^+)/K_a + K_b/(H^+)} \qquad (1)$$

where (H^+) is the hydrogen ion concentration and K_a and K_b are constants commonly identified as equilibrium constants for ionizable groups connected with the catalytic site of the enzyme. The constants K_a and K_b may be different for an enzyme–substrate complex than for the free enzyme. Using this approach, the pH dependence of the maximum velocity (V_m) will be governed by K_a and K_b for the enzyme substrate complex whereas V_m/K_m will depend on K_a and K_b for the free enzyme.[14] For a number of enzymes pK_a falls in the range 4 to 6 and pK_b in the range 6 to 8.

We can find no evidence to indicate that an enzyme acting on a large polymer molecule, charged or uncharged, should show pH dependence of its catalytic activity differing from that given by eq. 1. From pH 5 to 7 the enzyme activity will not vary much more than a factor of two or three, scarcely more than the error in the experimental rates. Consequently this constitutes a minor correction to polyglutamate rates, most of which are measured in this pH range. On the other hand the data for polylysine extend to pH 10 and higher, and the fraction of the catalyst molecules which are catalytically active at this pH will be considerably different from the fraction at pH 7. In order to determine the effect of substrate charge and conformation on the reaction rate, the rates given in Fig. 2 should be corrected to constant catalytic activity. This is conveniently done by multiplying each rate by the denominator of eq. 1, using appropriate values for the pK values. The pK values that are needed to make the correction depend on the value of K_m compared to the substrate concentration (S) used in determining the rates. If $K_m < S$, the rates correspond clearly to V_m and the appropriate pK values are those for the enzyme–substrate complex. If $K_m \gg S$, the rates are proportional to V_m/K_m and the necessary pK values are those for the free enzyme.

The Michaelis constants were estimated at pH 7 and 9.5 using two substrates concentrations, 1.9 and 7.5 g./l. For subtilisin, papain, and ficin $K_m \gg S$, for elastase $K_m \leqslant S$, and for α-chymotrypsin $K_m \leqslant S$ at pH 9.5 and $K_m > S$ at pH 7.0. Only for elastase and possibly for chymotrypsin is it necessary to know the pK values of the enzyme–substrate complexes. Since it is not possible to determine these from our data, pK values were taken from the literature either for enzyme–substrate complexes with small substrates or for the free enzymes. It is not known how much these values differ from those needed. From pH 7 to 10 only $K_b/(H^+)$ corrections to the enzyme activity are important. The pK_b values assumed in correcting to constant enzyme activity were as follows: chymotrypsins,[15] $pK_b = 8.0$; ficin,[16] $pK_b = 8.5$; papain,[17] $pK_b = 8.1$; elastase, pK_b estimated[18] to be 9. For subtilisin no value for pK_b could be found.

After correcting to constant enzyme activity, the pH dependence of the rate is approximately the same for each enzyme. By multiplying each rate by a scaling factor, analogous to the treatment of the polyglutamate data,[6] the pH–rate curves are superimposable. This is shown in Fig. 3, where the data for chymotrypsin, elastase, ficin, and papain have been multiplied by scaling factors such that they superimpose on the subtilisin data. Any deviation from superposition is within experimental error, except possibly the data for subtilisin.

The pH Dependence of the Rate Catalyzed by the Chymotrypsins, Elastase, Ficin, Papain, and Subtilisin.—After correcting the rate data to constant enzymic activity, any remaining pH dependence must be attributed to changes in the substrate. In going from low to high pH, polylysine changes from a charged, extended random coil molecule to the uncharged, helical state. In polyglutamate the situation is reversed. If charge and conformation play the same role in polylysine as they do in polyglutamate, Fig. 3 should be very similar to Fig. 3 (with the pH scale reversed) in the preceding paper. It is obvious that this is at least qualitatively correct. The pH dependence of the hydrolysis of polyglutamate was quantitatively predicted assuming enzymic attack at peptide bonds in random coil regions of the molecule in which both side chains adjacent to the bond were uncharged. In order to make the same calculation for polylysine its titration

(14) M. Dixon and E. Webb, "Enzymes," Academic Press, Inc., New York, N. Y., 1956, p. 133 ff.

(15) K. Laidler, *Trans. Faraday Soc.*, **51**, 550 (1955).
(16) B. R. Hammond and H. Gutfreund, *Biochem. J.*, **72**, 349 (1959).
(17) J. R. Kimmel and E. L. Smith, *Advan. Enzymol.*, **19**, 267 (1957).
(18) D. Hall and J. Czerkawski, *Biochem. J.*, **73**, 356 (1959).

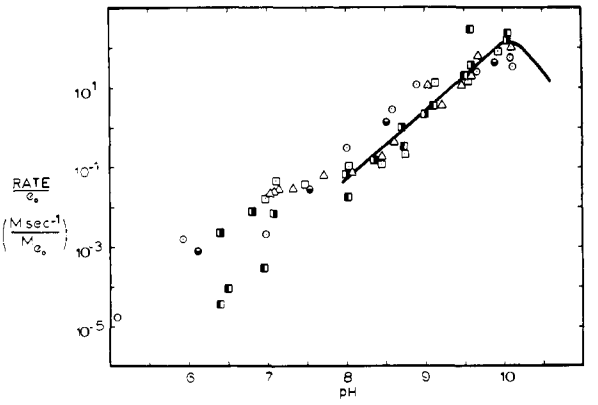

Fig. 3.—Superposition of the pH–rate curves for chymotrypsin (α and β), elastase, ficin, papain, and subtilisin acting on polylysine after correcting to constant enzyme activity and multiplying by a scaling factor. Symbols have same meaning as in Fig. 2. The solid line was calculated assuming enzymic hydrolysis of peptide bonds in random coil regions of polylysine with adjacent side chains uncharged and assuming w/kT = 2.0.

curve and per cent helicity as a function of pH must be known. Data for polylysine HBr in 0.200 M NaCl necessary for calculating the theoretical curve are shown in Fig. 4. The optical rotation measurements are less precise than the hypochromicity measurements. Furthermore the optical rotation of an amino acid is somewhat dependent on the charge state of the side chains in addition to any conformational effects.[19] Although optical rotation shows effects very similar to the hypochromicity changes, hypochromicity as a measure of conformation was used in calculating the theoretical pH–rate curve.

Using a one-dimensional Ising model with nearest-neighbor interactions,[6] the titration curve to determine the fraction of the side-chain amines ionized and the solid line through the molar absorptivity data in Fig. 3 to determine per cent helicity, the concentration of peptide bonds with adjacent side chains uncharged in a random coil region of the polymer was calculated as a function of pH for various values of the pairwise electrostatic interaction energy, w. The results are shown in Fig. 5. Below pH 9.8 the slope is controlled by the shape of the titration curve. As the titration curve of random coil polylysine should be similar in shape to that of random coil polyglutamate, the slope in the low pH region (below 9.8) in Fig. 5 should be very close to the slope of the analogous plot for polyglutamate in the high pH region (above 5.3). This is observed.

The theoretical curve for $w = 2kT$ is shown in Fig. 3, superimposed on the rate data. The agreement is within the experimental precision of the rate data. The data do not extend to high enough pH to observe any decrease in rate above pH 10.2. Thus, we cannot rule out the possibility that helical regions of polylysine are hydrolyzed. The data, considering individual enzymes, do suggest a plateau has been reached around pH 10 whereas the theoretical predictions allowing no conformational effects show no plateau (dashed lines in Fig. 5). Therefore, it is reasonably safe to say that helical regions are not hydrolyzed. Although the theoretical curve for $w = 2kT$ is shown in Fig. 3, the curves for $w = 0$ and $w = 1.4kT$ fit equally well. The data must be extended to higher pH or obtained with greater precision in order to force a choice more stringent than w is of the order of kT.

(19) L. I. Katzin and E. Gulyas, *J. Am. Chem. Soc.*, **86**, 1655 (1964).

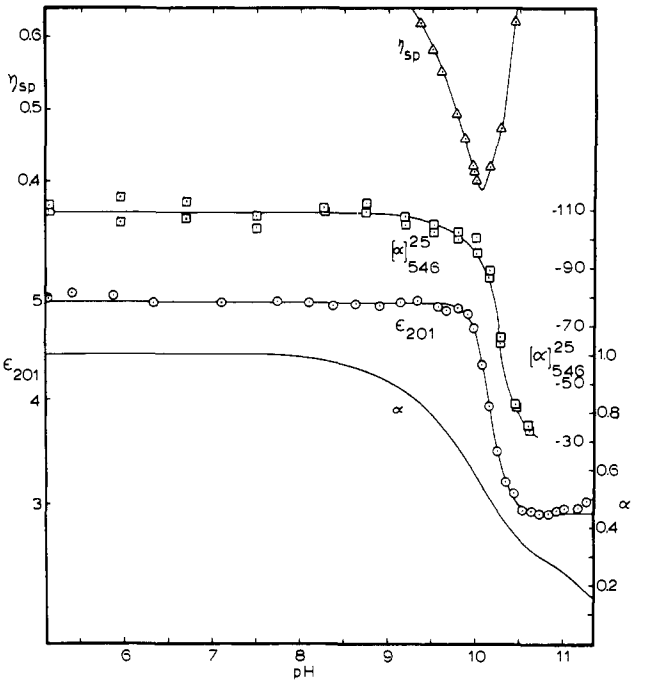

Fig. 4.—The degree of dissociation (———), molar absorption (×10⁻³) at 201 mμ (—○—), optical rotation at 546 mμ (—□—), and specific viscosity (—△—) of polylysine HBr in 0.200 M NaCl at 25°.

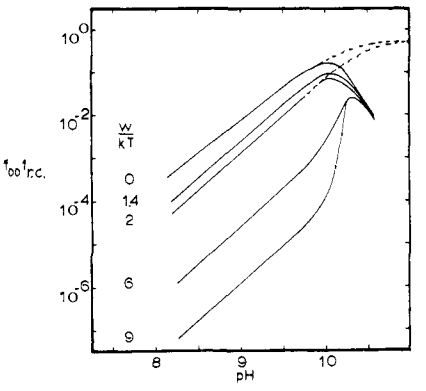

Fig. 5.—Calculated pH dependence of the rate assuming hydrolysis in random coil regions of polylysine at lysyl–lysyl bonds having adjacent side chains uncharged.

Assuming this mechanism as well as the correction to constant enzyme activity to be valid, the minimum rate at which these enzymes hydrolyze a lysyl–lysyl bond with adjacent side chains uncharged in a random coil section of polylysine may be calculated. These are shown in Table I. The rates represent a minimum for subtilisin, papain, and ficin as a reliable value for the Michaelis constant is not available.

TABLE I

RATE OF HYDROLYSIS OF A LYSYL–LYSYL BOND WITH ADJACENT SIDE CHAINS UNCHARGED IN A RANDOM COIL REGION OF POLYLYSINE

Enzyme	Rate, sec.$^{-1}$
α-Chymotrypsin	1500
Elastase	2
Ficin	>500
Papain	>300
Subtilisin	>3000

The pH Dependence of the Rate Catalyzed by Trypsin.—On a logarithmic scale the pH dependence of the trypsin-catalyzed reaction is extremely slight from pH 6 to 10, quite in contrast to the behavior of the other endopeptidases. However, on a linear scale a pH dependence much larger than the experimental uncertainty becomes evident (Fig. 1b). Studies on benzoyl-L-arginine ethyl ester[20] indicate a pK_a for the enzyme–substrate complex of 6.25 and no pK_b was evident up to pH 10. Various values for pK_a were assumed and the pH dependence predicted. As can be seen in Fig. 1b, the observed pH dependence can be predicted below pH 9 assuming $pK_a = 6.6$. Below pH 9, then, the pH dependence of the rate is controlled by the charge state of the enzyme and is independent of the charge state of the substrate. A variety of models involving the charge state of the enzyme and the charge state and conformation of the polylysine were considered in an attempt to explain the pH behavior above pH 9. Several of these predicted a decrease in rate that was qualitatively correct. None, however, stood out over the others. However, the data must be extended to higher pH and the Michaelis constants determined over the entire pH range before a meaningful interpretation can be given.

(20) H. Gutfreund, *Trans. Faraday Soc.*, **51**, 441 (1955).

Acknowledgments.—We wish to thank Mr. James Monroe for assistance in analyzing some of the data and Mr. Richard Antrim for the titration curve for polylysine.

[CONTRIBUTION FROM THE DEPARTMENT OF THERMOCHEMISTRY AND CHEMICAL KINETICS, STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIFORNIA]

On the Thermochemistry of Alkyl Polyoxides and Their Radicals[1]

BY SIDNEY W. BENSON

RECEIVED APRIL 27, 1964

From data on heats of formation of oxides and peroxides, a new value for the partial molar heat of formation of the group O–$(O)_2$ is selected. Its value is $\Delta H_f(O\text{–}(O)_2) = +19 \pm 4$ kcal./mole. Using this and other more accurate data, it is possible to deduce $\Delta H_f°$ for polyoxides RO_nR' with $n = 2, 3, 4$, etc., and R and R′ = H, alkyl, etc. Together with data on $\Delta H_f°$ for radicals, it is shown that it is unlikely that tetroxides can be produced or isolated above 80 to 100°K. The trioxides on the other hand appear to have reasonable thermal stability and recent observations by Czapski and Bielski[2a] on H_2O_3 production are in accord with these expectations. Free-radical methods will not produce very large amounts of these species. In the case of R_2O_3, where R is a tertiary radical, this is not the case and it is proposed that the recent production of a polyoxide by Milas and Djokic[2b] is very likely t-BuO_3-t-Bu rather than the claimed tetroxide.

Introduction

In a recent paper[3] it was estimated that the species H_2O_4 is thermodynamically unstable above 80°K. with respect to dissociation into $2HO_2$ radicals. The lower homolog, H_2O_3, was estimated to be sufficiently stable to be isolable at 200°K. Despite this, it was shown on kinetic grounds that attempts which had been made to produce it from gas-phase or gas-solid free-radical reactions were unlikely to succeed. Since then data have appeared[4] which further substantiated the kinetic conclusions on the unlikelihood of H_2O_3 or H_2O_4 preparation. However, some recent results on aqueous oxidation-reduction systems suggest the formation of H_2O_3 and HO_3^- *via* alternate routes. In addition some experiments[2b] on the alkyl derivatives of peroxides have given direct support to the reality of the compounds R_2O_3 or R_2O_4. In the light of these findings it was felt worthwhile reviewing the thermochemical data on the hydrogen polyoxides and extending the methods of analysis to the alkyl compounds, their radicals, and derivatives.

Thermodynamic Estimates.—The basis for the method of estimation of the heats of formation of the polyoxides derives from the principle of additivity of group properties as described by Benson and Buss[5] in conjunction with the less accurate principle of bond additivities. The former was found to give values of $\Delta H_f°$ to within ±1 kcal. in about 1000 cases, and the polyoxides should fit the scheme quite well. The problem is one of establishing the partial group value of $\Delta H_f°$ for the group O–$(O)_2$, *i.e.*, an oxygen atom bonded to two neighboring O atoms. In the preceding paper,[3] this value was estimated from bond energies. Such estimates can be made from the values of $\Delta H_f°(H_2O)$ and $\Delta H_f°(H_2O_2)$ which would yield the value $\Delta H_f°(O\text{–}(O_2)) = +25$ kcal. with a maximum expected error of ±6 kcal. based on a number of similar cases. If, however, the estimates are made from the data on $\Delta H_f°(CH_3OCH_3)$ and $\Delta H_f°(CH_3OOCH_3)$,[6] then we estimate $\Delta H_f°(O\text{–}(O)_2) = +13$ kcal. with similar error limits. The mean value of $\Delta H_f°(O\text{–}(O)_2) = +19 \pm 4$ kcal. seems a reasonable compromise of both these estimates and is to be preferred on purely empirical grounds.

This new group value of $\Delta H_f°[O\text{–}(O)_2]$ then yields, together with the other appropriate group values,[5] the estimated heats of formation shown in Table I. To calculate bond dissociation energies for the compounds listed we use the $\Delta H_f°$ for values the radicals shown in Table II.

Polyoxide Stability. Tetroxides.—The values listed for $\Delta H_f°$ of H_2O_3 and H_2O_4 are lower by about 2 and 4 kcal., respectively, than the ones suggested earlier, but

(1) This development was partly supported at Stanford Research Institute by a general project on "Reaction of Organic Compounds with Oxygen."

(2) (a) G. Czapski and B. H. J. Bielski, *J. Phys. Chem.*, **67**, 2180 (1963); (b) N. A. Milas and S. M. Djokic, *Chem. Ind.* (London), 405 (1962).

(3) S. W. Benson, *J. Chem. Phys.*, **33**, 306 (1960).

(4) P. A. Giguère, *ICSU Rev.*, **4**, 172 (1962).

(5) S. W. Benson and J. H. Buss, *J. Chem. Phys.*, **29**, 546 (1958). Following the nomenclature adopted in this article $\Delta H_f°(O\text{–}(O)_2)$ represents the partial molar group contribution to the total heat of formation of a molecule containing the group O–$(O)_2$, an oxygen atom joined to two neighboring O atoms. As an example, H_2O_2 contains only two identical groups O–(H)(O), while H_2O_3 contains the same two O–(H)(O) groups plus the O–$(O)_2$ group.

(6) See compilation in S. W. Benson, *ibid.*, **40**, 1007 (1964).