Papain- and Ficin-Catalyzed Reactions. Effect of pH on Activity and Conformation of Ficin*

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ABSTRACT: The effect of pH on the kinetic parameters, k_{cat} and K_m(app), and K_I for the competitive inhibitor, α-*N*-benzoyl-D-arginine ethyl ester, for the hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester by component G of *Ficus glabrata* latex has been determined over the pH range of 4.0–9.5.

k_{cat} was found to be dependent upon two prototropic groups with apparent p*K*'s of 4.46 and 8.37. K_m(app) and K_I were essentially identical and independent of pH over the pH range of 4.5–9.5. It is concluded from these data that: (1) there is no pH-induced conformational change in the protein which affects the activity of ficin over the pH range of 4.5–9.5, (2) the acylation rate constant, k_2, is dependent upon two prototropic groups with apparent p*K*'s of 4.74 and 8.44, and (3) the rate-controlling step in the hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester by ficin is the acylation step.

It has been shown that k_2/K_S (equivalent to k_{cat}/K_m (app)) *vs.* pH for papain- and ficin-catalyzed hydrolyses of α-*N*-benzoyl-L-arginine ethyl ester and α-*N*-benzoyl-L-argininamide are bell-shaped curves (Hammond and Gutfreund, 1959; Whitaker and Bender, 1965; Kramer and Whitaker, 1969). Such curves could be the result of: (a) the dependence of k_2 upon two prototropic groups, (b) the dependence of K_S upon two prototropic groups, or (c) the dependence of each upon one prototropic group. It has been shown that k_3 (see eq 1, where E and S are enzyme and substrate, respectively, E·S is enzyme–substrate complex, ES′ is an acyl-enzyme intermediate, and P_1 and P_2 are products formed from the substrate)

$$E + S \underset{}{\overset{K_S}{\rightleftharpoons}} E\cdot S \xrightarrow{k_2} ES' \xrightarrow[H_2O]{k_3} E + P_2 \qquad (1)$$
$$+$$
$$P_1$$

for papain-catalyzed hydrolyses of several substrates is dependent upon a single prototropic group with a p*K* near 4 (Whitaker and Bender, 1965; Brubacher and Bender, 1966; Bender and Brubacher, 1966; Williams and Whitaker, 1967). If k_2 and k_3 are the microscopic reverse of each other one would expect that k_2 would also be dependent upon a single prototropic group, a result not substantiated by calculations based on data for papain-catalyzed hydrolyses (Whitaker and Bender, 1965; Bender and Brubacher, 1966).

In view of this apparent inconsistency in k_2 and k_3 dependence upon pH and because of the recent results with chymotrypsin which indicate an alkaline pH dependence of K_S (Himoe *et al.*, 1966; Bender *et al.*, 1966b), additional data are needed on the pH dependence of K_S for the sulfhydryl proteolytic enzymes, ficin and papain. Data on the effect of pH on k_{cat} and K_m(app) for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester and the effect of pH on K_I of α-*N*-benzoyl-D-arginine ethyl ester in ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester are presented here.

Materials and Methods

Materials. Component G of *Ficus glabrata* latex was purified by a modification of the method described earlier (Sgarbieri *et al.*, 1964). The latex was activated with cysteine, then completely inactivated with an excess of sodium tetrathionate (Englund *et al.*, 1968). All solutions used in the purification contained 1×10^{-5} M sodium tetrathionate. Component G was homogeneous by polyacrylamide gel electrophoresis and by chromatography on CM-cellulose. Protein concentration of the solutions was determined at 280 mμ using E_{280} 54,000 (Williams, 1968) based on a molecular weight of 25,000 (Englund *et al.*, 1968). The reported enzyme concentrations are based on total protein, not activity.

Papain (two-times crystallized, lot PAP 6JB from Worthington Biochemical Corp.) was recrystallized as the mercuripapain derivative as described by Whitaker and Pérez-Villaseñor (1968). Protein concentration of papain solutions was determined at 280 mμ using E_{280} 51,100 (Bender *et al.*, 1966a).

α-*N*-Benzoyl-L-arginine ethyl ester hydrochloride (lot no. S5047), α-*N*-benzoyl-D-arginine ethyl ester hydrochloride (lot no. L-1984), acetylglycine (lot no. 2543), and benzoylglycinamide (lot no. 4165) were from Mann Research Laboratories. α-*N*-Benzoyl-L-arginine (lot no. 3339), guanidoacetic acid (lot no. 4567), and argininic acid (lot no. 4383) were from Nutritional Biochemical Co. Benzamidine hydrochloride (lot no. 11069) was from K & K Laboratories. Guanidine hydrochloride was from Eastman. Benzoylglycine (mp 186–187°, lit. mp (Ingersoll and Babcock, 1943) 186–187°) and benzyloxy-

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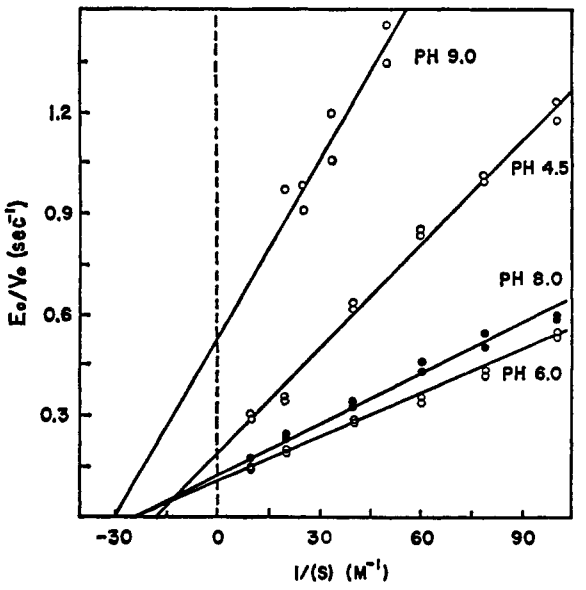

FIGURE 1: Lineweaver–Burk plots showing the effect of substrate concentration and pH on the velocity of hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester by ficin at 35.0°. Reaction conditions were those described in Table II.

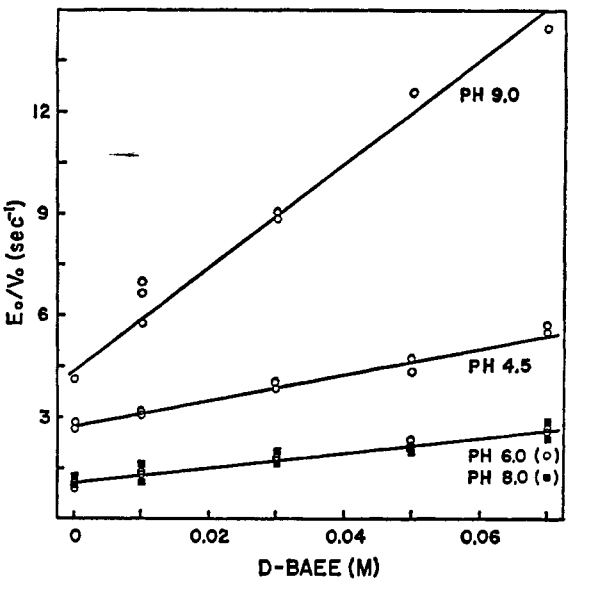

FIGURE 2: Plots showing the effect of inhibitor concentration (α-*N*-benzoyl-D-arginine ethyl ester) and pH on the velocity of hydrolysis of 0.005 M α-*N*-benzoyl-L-arginine ethyl ester by ficin. The conditions were those described in Table II.

carbonylglycine (mp 120–121, lit. mp (Carter *et al.*, 1955) 120°) were synthesized in this laboratory. Cysteine hydrochloride and Versene were from Eastman. All other compounds were reagent grade and deionized water was used throughout these experiments.

Methods. The reactions were performed in a Radiometer titrator (Titrator 11, Titragraph SBR 2C, pH meter 25, buret SBU1a) thermostated at 35.0°. The reaction vessel contained, in addition to the appropriate amounts of substrate, enzyme, and inhibitor, 0.01 M each of acetate, phosphate, and borate, 0.001 M Versene, 0.005 M cysteine, and sufficient potassium chloride to give a total ionic strength of 0.30. The total reaction volume was 3.0 ml and the pH was maintained constant by addition of standardized NaOH (0.04 N). All determinations were done at least in duplicate. The initial velocities were calculated from the initial slopes of the reaction progress curves. At pH 5 and below the data were corrected for incomplete ionization of the product using a pK for α-*N*-benzoyl-L-arginine of 3.24 (Whitaker and Bender, 1965). At pH 8.5 and above the data were corrected for nonenzymatic hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester. The initial reaction rate data were plotted as E_0/V_0 *vs.* $1/S_0$ according to eq 2 (see Figure 1).

$$E_0/V_0 = (1/k_{cat}) + (K_m(app)/k_{cat})(1/S_0) \quad (2)$$

The data were analyzed by means of an IBM 7040 computer program according to the method of least squares.

K_I for the competitive inhibitor, α-*N*-benzoyl-D-arginine ethyl ester, was calculated by two methods. Method 1 was according to eq 3 (see Figure 2) and used an appropriate computer program. The second method used eq 4. In eq 3 and 4 V_0 and V_0' are the observed

$$E_0/V_0 = (I)\{K_m(app)/[k_{cat}\,K_I(S)]\} + [K_m(app)/k_{cat}(S)] + 1/k_{cat} \quad (3)$$

$$V_0/V_0' = [K_m(app)(1 + \frac{(I)}{K_I} + (S)]/(K_m(app) + (S)) \quad (4)$$

rates in the absence and presence of competitive inhibitor, respectively, (I) is competitive inhibitor concentration, and K_I is the dissociation constant of the enzyme–inhibitor complex. Equation 4 has some obvious advantages over eq 3 for the calculation of K_I.

Results and Discussion

Inhibition of Papain. Since much more physicochemical and kinetic data are available for papain-catalyzed reactions, it would be appropriate to use papain in determining whether there are any pH-induced conformational changes which affect enzymatic activity. Unfortunately, we were unable to find a good competitive inhibitor of papain. Several compounds that might be expected to be competitive inhibitors of papain-catalyzed hydrolyses were tried. The results are given in Table I. With the exception of benzyloxycarbonylglycine and α-*N*-benzoyl-L-arginine there was no effect on activity or there was an enhancement of activity under the conditions used. The presence of the positively charged compounds, guanidine and benzamidine, enhanced the hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester. These data would indicate that, unlike the situation with trypsin, the positive guanidinium group of α-*N*-benzoyl-L-arginine ethyl ester does not play an important role in binding the substrate to the enzyme. The fact that benzoyl-L-citrulline methyl ester has essentially the same K_m(app) as α-*N*-benzoyl-L-arginine ethyl

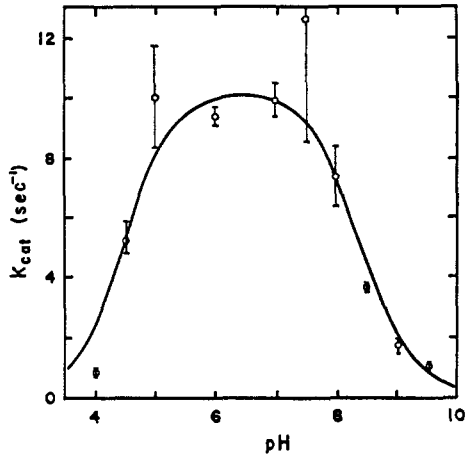

FIGURE 3: Effect of pH on k_{cat} for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester. The conditions were those described in Table II. The dashed line is a theoretical one calculated for an apparent pK_1 and apparent pK_2 of 4.46 and 8.37 and k_{cat}(lim) of 10.3 sec^{-1}.

TABLE I: Effect of Various Compounds on Papain-Catalyzed Hydrolysis of α-L-Benzoyl-L-arginine Ethyl Ester.[a]

Compound	Concn (M)	k(inhibitor)/ k(control)
Guanidine	0.12	1.31
Benzamidine	0.04	1.55
Guanidoacetic acid	0.10	1.00
Argininic acid	0.12	1.08
Benzoylglycine	0.08	1.23
Acetylglycine	0.10	1.21
Benzyloxycarbonylglycine	0.03	0.91
α-*N*-Benzoyl-L-arginine	0.05	0.80

[a] Conditions: α-*N*-benzoyl-L-arginine ethyl ester = 0.005 M, $E_0 = 1.28 \times 10^{-6}$ M, ionic strength = 0.30, pH 7.0, and 35.0°.

ester in papain-catalyzed reactions (Cohen and Petra, 1967) further substantiates this.

The data would indicate there is a negative charge in or near the active site, however. Those compounds which resemble substrates of papain except for having an ionized carboxyl group (and absence of ester function) were ineffective as inhibitors. It has been noted previously that ionization of the carboxyl group of α-*N*-benzoyl-L-arginine causes about a 20-fold increase in K_I (Whitaker and Bender, 1965). In view of the limited solubilities of benzyloxycarbonylglycine and α-*N*-benzoyl-L-arginine, their large K_I values, and the effect of pH on the ionization of these compounds, further studies on these compounds as inhibitors were not performed.

One might expect α-*N*-benzoyl-D-arginine ethyl ester to be quite an effective inhibitor of papain. However, K_I determined for this compound at pH 7.0, 35.0°, and ionic strength of 0.30 was 0.138 M, a disappointingly large value since K_m(app) for α-*N*-benzoyl-L-arginine ethyl ester is 0.014 M at the same ionic strength (Whitaker and Bender, 1965). On the other hand, K_I for α-*N*-benzoyl-D-arginine ethyl ester in ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester at pH 7.0 was identical with K_m(app) for α-*N*-benzoyl-L-arginine ethyl ester (Table II).

Because of lack of a good competitive inhibitor (preferably neutral) for papain, we turned our attention to the effect of pH on the conformation of ficin. Ficin and papain are quite similar not only in many of their physicochemical properties (Smith and Kimmel, 1960) and the sequence of amino acids around the essential sulfhydryl group (Light *et al.*, 1964; Wong and Liener, 1964) but also in their kinetic behavior. Therefore, kinetic data obtained with one enzyme is likely to be generally applicable to the other enzyme.

Ficin-Catalyzed Hydrolysis of α-N-Benzoyl-L-arginine Ethyl Ester. The kinetic constants k_{cat}, K_m(app), and k_{cat}/K_m(app) for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester are shown in Figures 3–5 and in Table II.

The plot of k_{cat} (which is $k_2k_3/(k_2 + k_3)$ from eq 1) *vs.* pH for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester is a bell-shaped curve dependent upon two prototropic groups of apparent pK's of 4.46 and 8.37. These pK values are very similar to those found for component D of *Ficus carica* var. Kadota latex (Kramer and Whitaker, 1969) and also for papain (Whitaker and Bender, 1965) indicating that the same prototropic groups are involved in all three enzymes. An interpretation of the meaning of k_{cat} for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester will be given below.

The plot of k_{cat}/K_m(app) (equivalent to k_2/K_S) *vs.* pH for the ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester is also a bell-shaped curve which is dependent upon two prototropic groups with apparent pK values of 4.74 and 8.44. In the absence of perturbing factors such as ionization of the substrate, etc., the effect of pH on k_2/K_S permits a determination of the essential prototropic groups in the active center of the free enzyme (Peller and Alberty, 1959). The two essential groups in the active center of component G of *Ficus glabrata* latex have nearly the same pK values as the two prototropic groups involved in the active center of component D of *F. carica* var. Kadota latex (Kramer and Whitaker, 1969) and papain (Whitaker and Bender, 1965). It is reasonable to conclude that the activity of all three enzymes is dependent upon the same two prototropic groups.

The two essential prototropic groups in the active center of the free enzyme could be involved in the binding of the substrate to enzyme (thus affecting K_S), in the acylation step (reflected by k_2), or in both the binding and acylation step. One possible way of differentiating among these possibilities is to determine K_m (app) as a function of pH. It will be seen from the data of Figure 5 (and Table II) that K_m(app) is essentially

TABLE II: Effect of pH on Ficin-Catalyzed Hydrolysis of α-*N*-Benzoyl-L-arginine Ethyl Ester and Inhibition of Ficin by α-*N*-Benzoyl-D-arginine Ethyl Ester.[a]

pH	k_{cat} (sec^{-1})	K_m(app) (M × 10^2)	k_{cat}/K_m(app) (M^{-1} sec^{-1})	K_I (M × 10^2)[b]	
				1[c]	2[d]
4.00	0.825 ± 0.049	1.20 ± 0.18	68.8	0.986	2.21 ± 0.16
4.50	5.30 ± 0.56	5.47 ± 0.75	96.9	5.20	6.82 ± 0.98
5.00	10.0 ± 1.7	7.50 ± 1.48	133.3	4.67	4.74 ± 0.35
6.00	9.40 ± 0.30	4.17 ± 0.16	226	3.92	3.36 ± 0.38
7.00	9.91 ± 0.58	3.85 ± 0.25	258	3.85	4.21 ± 0.36
7.50	12.5 ± 4.0	5.25 ± 2.01	238	4.57	4.83 ± 0.43
8.00	7.35 ± 1.00	3.63 ± 0.62	203	5.15	4.14 ± 0.86
8.50	3.62 ± 0.06	3.02 ± 0.07	120	2.72	2.00 ± 0.12
9.00	1.66 ± 0.25	2.93 ± 0.63	56.6	2.15	2.04 ± 0.40
9.50	0.940 ± 0.102	5.75 ± 0.70	16.4	3.06	3.07 ± 0.37
9.50				1.75	2.64 ± 0.80

[a] Conditions were: E_0 = 0.322–1.61 × 10^{-6} M for α-*N*-benzoyl-L-arginine ethyl ester parameters, 1.61–9.67 × 10^{-6} M for K_I determinations, temperature = 35.0°, and ionic strength = 0.30. [b] Concentration: α-*N*-benzoyl-L-arginine ethyl ester = 0.005 M, α-*N*-benzoyl-D-arginine ethyl ester = 0.0–0.07 M. [c] Calculated by computer according to eq 3. [d] Calculated according to eq 4.

independent of pH over the pH range of 4.5–9.5. There may be a decrease in K_m(app) around pH 4, a result also found with component D of *F. carica* var. Kadota latex (Kramer and Whitaker, 1969). This decrease in K_m (app) in the acid region for both ficins is in marked contrast to the results obtained with papain in this region (Whitaker and Bender, 1965).

Influence of pH on the Binding of α-N-Benzoyl-D-arginine Ethyl Ester to Ficin. One cannot conclude, *a priori*, that because K_m(app) (which is equivalent to $K_S k_3/(k_2 + k_3)$) is independent of pH over the range of 4.5–9.5 that K_S (the dissociation constant of the enzyme–substrate complex) is also pH independent over this region. The constancy of K_m(app) could be due to compensating changes in k_2 and k_3.

In the case of papain-catalyzed hydrolyses of α-*N*-benzoyl-L-arginine ethyl ester and α-*N*-benzoyl-L-arginine, it was possible to calculate K_S from the data (Whitaker and Bender, 1965) and to show that the calculated K_S was independent of pH above pH 4.5. However, the calculated values of K_S are not completely free of assumptions and possible errors of interpretation. In the case of ficin-catalyzed hydrolyses we have been unable to calculate K_S directly from the data (Kramer and Whitaker, 1969, and present report). A determination of the influence of pH on K_I of a competitive inhibitor should permit one to answer, unequivocally, whether K_S is independent of pH since the two, if the

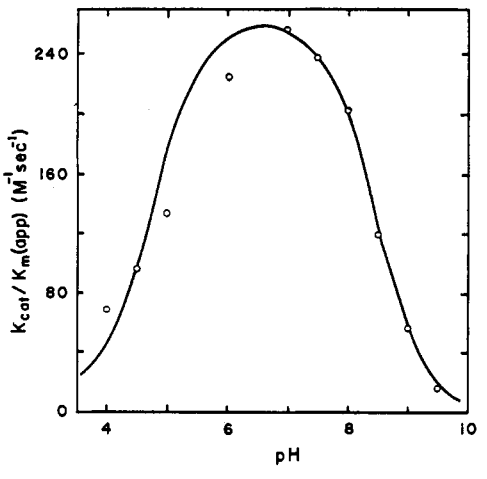

FIGURE 4: Effect of pH on k_{cat}/K_m(app) for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester. The conditions were those described in Table II. The dashed line is a theoretical one calculated for an apparent pK_1 and apparent pK_2 of 4.74 and 8.44 and (k_{cat}/K_m(app))(lim) of 264 M^{-1} sec^{-1}.

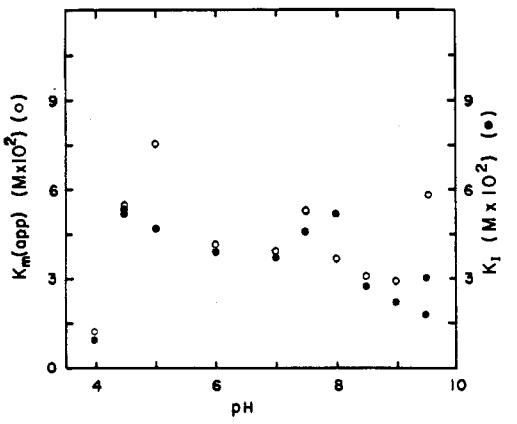

FIGURE 5: Effect of pH on K_m(app) for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester and on K_I of α-*N*-benzoyl-D-arginine ethyl ester. The conditions were as described in Table II.

substrate and inhibitor are analogs, should differ by a fixed amount over the total pH range.

K_I of the competitive inhibitor, α-*N*-benzoyl-D-arginine ethyl ester, has been determined for component G of *Ficus glabrata* latex over the pH range of 4–9.5. Analysis of the data indicated that α-*N*-benzoyl-D-arginine ethyl ester behaved purely as a competitive inhibitor. α-*N*-Benzoyl-D-arginine ethyl ester was not hydrolyzed by ficin nor did it form an irreversible acyl-enzyme derivative with ficin. The data, shown in Figure 5 and Table II, indicate that K_I is essentially independent of pH over the pH range of 4.5–9.5. It will be further noted that the values of K_I are essentially identical with those of K_m(app) determined for α-*N*-benzoyl-L-arginine ethyl ester.

We must conclude, therefore, that the pH dependence of k_2/K_S is due strictly to two essential prototropic groups in the acylation step which is controlled by the rate constant, k_2. Furthermore, and very importantly, there is no evidence for a pH-induced conformational change (as reflected in binding) over the pH range of 4.5–9.5. This is in marked contrast to the pH-controlled conformational change in α-chymotrypsin (Himoe *et al.*, 1966; Bender *et al.*, 1966b). The data clearly indicate that the acylation rate constant, k_2, is dependent upon two prototropic groups while all the available evidence (for papain) indicate that the deacylation rate constant, k_3, is dependent upon a single prototropic group (Whitaker and Bender, 1965; Brubacher and Bender, 1966; Bender and Brubacher, 1966; Williams and Whitaker, 1967). Why do k_2 and k_3 differ in this respect? All presently available data indicate that an essential sulfhydryl group is acylated by the substrate in the step controlled by k_2 (Lowe and Williams, 1964, 1965; Bender and Brubacher, 1964; Brubacher and Bender, 1966). Therefore, this essential prototropic group for acylation, with pK of approximately 8.5, is not present in the deacylation step.

Nature of k_{cat} in Ficin-Catalyzed Hydrolysis of α-N-Benzoyl-L-arginine Ethyl Ester. From the data presented here, and from other data, we conclude that the kinetic parameter, k_{cat} (which is equal to $k_2k_3/(k_2 + k_3)$ from eq 1), must be essentially equal to k_2 for ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester. Evidence that k_{cat} is approximately equal to k_2 includes the following. (1) The pH–rate profiles for k_{cat} and k_2/K_S (equivalent to k_{cat}/K_m(app)) are identical on the alkaline side (Figures 3 and 4). Since the data indicate that K_S is pH independent and that k_2 is dependent upon two prototropic groups, one in the acid region and one in the alkaline region, the identical pH–rate profile of k_{cat} and k_2/K_S indicate that $k_{cat} \simeq k_2$. (2) The similarity of the values of K_m(app) and K_I for α-*N*-benzoyl-L-arginine ethyl ester and α-*N*-benzoyl-D-arginine ethyl ester, respectively, indicate that K_m(app) and K_S cannot be greatly different. This can be so only if k_2 is predominately the rate-controlling step. (3) In the presence of saturating amounts of α-*N*-benzoyl-L-arginine ethyl ester or of α-*N*-benzoyl-D-arginine ethyl ester, the rate of reaction of chloroacetamide with the essential sulfhydryl group of component G of *F. glabrata* latex is some seven times faster than in the absence of these compounds (J. R. Whitaker, unpublished data). Reaction of chloroacetamide with the essential sulfhydryl group of ficin in the presence of saturating amounts of substrate can occur only if $k_2 \simeq k_3$ or $k_2 < k_3$.

The postulation that k_2 is the rate-determining step in ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester is in contrast to the data for papain-catalyzed hydrolysis of this substrate (Whitaker and Bender, 1965). In the case of papain k_2(lim) was found to be 64.9 sec^{-1} while k_3(lim) was 20.2 sec^{-1}.

If the assumption that the rate-controlling step in the ficin-catalyzed hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester is correct, then one can determine k_2(lim) both from the data on k_{cat} (equivalent to k_2) and from the data on k_{cat}/K_m(app). Such determinations indicate k_2(lim) to be 10.3 sec^{-1} (from k_{cat}) and 10.6 sec^{-1} (from k_{cat}/K_m (app)) under the conditions of these experiments.

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Some Properties of Transfer Ribonucleic Acids from 5-Fluorouracil-Treated *Escherichia coli**

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ABSTRACT: The composition and some of the functions of transfer ribonucleic acid from 5-fluorouracil-treated *Escherichia coli* have been examined. The analog can replace as much as 70% of the uracil in transfer ribonucleic acid. This substitution has little effect on the proportions of the other major nucleotide components: the purine to pyrimidine ratio remains unchanged. Transfer ribonucleic acid from 5-fluorouracil-inhibited cells is, however, deficient in several minor base components. The pseudouridylic acid content decreases from 2.7 to 0.99 mole %; this decrease is proportional to the extent of analog incorporation. The level of ribothymidylate in this ribonucleic acid is also reduced. Despite these changes in nucleotide composition the capacity of 5-fluorouracil-containing transfer ribonucleic acid to accept amino acids remains essentially the same as that of normal transfer ribonucleic acid. The mixture of transfer ribonucleic acid isolated from cells exposed to the analog can be partially separated into normal and 5-fluorouracil-containing components by chromatography on columns of methylated albumin silicic acid, with resolution increasing as the pH of the eluting buffer is raised from 5.2 to 8.0.

The 5S ribosomal ribonucleic acid of 5-fluorouracil-treated cells also contains the analog; 35% of the uracil is replaced by 5-fluorouracil. This ribonucleic acid remains associated with the ribosome and does not contaminate the transfer ribonucleic acid fraction.

The structural and functional changes brought about by the introduction of a base analog into RNA can provide important insights into the active centers of the molecule. It has been known for some time that FU[1] will replace uracil in bacterial RNA (Horowitz and Chargaff, 1959) as well as in the RNA of higher organisms and viruses (reviewed by Heidelberger, 1965). Previous investigations have examined the inhibition of ribosome formation by the analog (see review by Osawa, 1965; Andoh and Chargaff, 1965; Hills and Horowitz, 1966) and the effects of FU incorporation into mRNA on protein synthesis (Heidelberger, 1965; Horowitz and Kohlmeier, 1967). Few studies, however, have concerned themselves with the effects of FU on tRNA. The results reported here deal with this aspect of FU action and describe the effects of the fluoropyrimidine on some of the properties and functions of the tRNA from *Escherichia coli*. It was found that FU can replace up to 70% of the uracil in tRNA and that this RNA was deficient in at least two minor pyrimidine constituents, pseudouridylic and ribothymidylic acids. Despite these changes FU-tRNA was readily able to accept amino acids, in most cases at least as efficiently as normal tRNA. FU was also incorporated into the low molecular weight (5S) rRNA; this remained associated with ribosomes. A preliminary account of these findings has appeared (Horowitz and Huntington, 1967) and Lowrie and Bergquist (1968) have since described similar observations.

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[1] Abbreviations used are: FU, 5-fluorouracil; FU-tRNA, tRNA isolated from 5-fluorouracil-treated *E. coli;* MAK, methylated bovine serum albumin adsorbed on kieselguhr; MASA, methylated bovine serum albumin adsorbed on silicic acid.