show exceedingly poor agreement with the experimental results with an average deviation of 37% for the direct refolding data in Table I and an average deviation of 18% for the fluorescence refolding assay results in Table II.

SUMMARY

In this study, rapid HPLC gel filtration has been used to separate the unfolded and refolded forms of RNase subsequent to a short $(NH_4)_2SO_4$ pulse, using column conditions which slow down the I_N to N reaction as well as the refolding steps. Using this technique, it was possible to obtain fractionated RNase samples which are largely depleted of unfolded forms (ca. 7%) but which still have I_N strongly populated (ca. 33%) and then to compare these samples with others which have not been depleted of unfolded forms. The results of these studies clearly lead to two qualitative conclusions: (1) Samples depleted of unfolded forms but containing large amounts of I_N show only a small amplitude for fluorescence refolding. Therefore, the major part of the fluorescence change during slow refolding occurs prior to the I_N to N step, i.e., either during isomerization in the unfolded forms or during the refolding step itself. (2) Samples containing large amounts of I_N but depleted of unfolded forms show a fluorescence unfolding amplitude very similar to native RNase. It follows that I_N must therefore have the same cis isomer for proline-93 as does native RNase if this unfolding assay truly measures only the isomerization of proline-93, as thought.

At the quantitative level, all of the results were shown to be consistent with our earlier model and inconsistent with an alternative model which assumes that the entire slow phase is due to isomerization of proline-93 and that I_N contains the incorrect isomer for proline-93.

Registry No. RNase, 9001-99-4.

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Experimental Charge Measurement at Leaving Oxygen in the Bovine Ribonuclease A Catalyzed Cyclization of Uridine 3′-Phosphate Aryl Esters†

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ABSTRACT: The title esters are demonstrated to be specific substrates of bovine pancreatic ribonuclease A (EC 3.1.27.5). The Brønsted dependence of k_{cat}/K_m at pH 7.50 for the enzyme-catalyzed cyclization versus the pK_a of the leaving phenol exhibits two regression lines of almost identical slope for respectively 2-chlorophenols and 2,6-unsubstituted phenols: $\log k_{cat}/K_m = -0.20pK_a^{ArOH} + 5.47$ ($n = 5$, $r = 0.957$); $\log k_{cat}/K_m = -0.17pK_a^{ArOH} + 5.79$ ($n = 4$, $r = 0.965$). Comparison of the Brønsted β_{lg}'s with that for the standard reaction where imidazole catalyzes the cyclization ($\beta_{lg} = -0.59$) indicates considerably less development of negative charge on the leaving oxygen in the enzyme case, providing experimental evidence for the hypothesis that electrophilic assistance is involved in catalysis. The existence of two essentially parallel Brønsted correlations is not reflected in the standard reaction of substrate with imidazole. Modeling studies indicate that the phenyl ring of the substrate can take up a range of positions away from the active site; the presence of ortho chloro substituents considerably restricts the motion of the phenyl leaving group.

Bovine pancreatic ribonuclease A (EC 3.1.27.5) has been studied intensively with regard to its structure and the structure of enzyme–effector complexes [see, for example, Howlin et al. (1987) and Richards and Wyckoff (1971)]. The currently accepted mechanism (Figure 1) (Fersht, 1985) involves formation and decomposition of the cyclic 2′,3′-nucleotide by closely similar processes. Formation of the intermediate is thought to involve histidine-119 has an acceptor acid for the leaving 5′-hydroxyl group of the adjacent nucleotide; histidine-12 could function as a base to remove the proton of the 2′-hydroxyl group, thus assisting its intramolecular attack on the phosphodiester. The mechanism involving acid–base catalysis was inferred from the pH dependence of the kinetics (Deavin et al., 1966).

We present here a study of the kinetics of the ribonuclease A catalyzed reaction of the cyclization of uridine 3′-(aryl phosphate) substrates (I). The sensitivity of k_{cat}/K_m to leaving group substituent gives rise to a Brønsted β_{lg} coefficient that may be used to determine the change in charge at the leaving oxygen atom from ground state to the transition state of the

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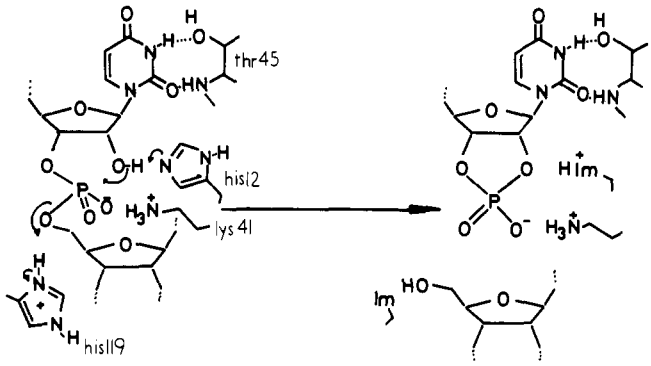

FIGURE 1: Mechanism of ribonucleotide fission to give nucleoside 2′,3′-cyclic monophosphate ester (Im = imidazolyl side chain and HIm^+ = conjugate acid of imidazolyl side chain).

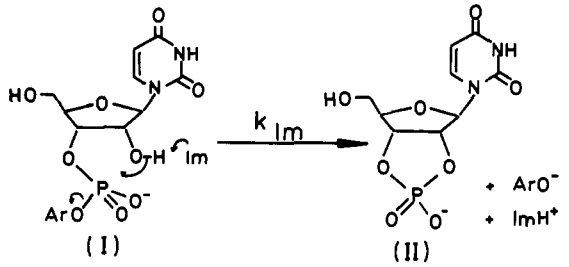

FIGURE 2: Calibration reaction for the ribonuclease A catalysis.

rate-limiting step in the intramolecular phosphorylation reaction (Williams, 1984; Thea & Williams, 1986). Comparison of the change in charge with that in the model system (Figure 2; Davis et al., 1988) will enable us to probe the significance of polar interactions between the enzyme and the leaving oxygen atom in the catalytic reaction.

The success of an approach to determine the polar effect in enzyme–substrate reactions depends on any nonelectronic interaction of enzyme with leaving group having either a constant or negligible energy independent of the substituents; this is potentially so with ribonuclease. This study indicates that there are some minor energies of interaction between leaving group and enzyme that do not affect the conclusions to be drawn.

Experimental Procedures

Materials. Bovine pancreatic ribonuclease A was obtained from Sigma (Type IIA); the samples had between 81 and 116 Kunitz units/mg of protein. Cytidine 2′,3′-cyclic monophosphate sodium salt was obtained from Sigma. 2′,5′-Bis-(tetrahydropyranyl)uridine 3′-(aryl phosphate)s were obtained from a previous study (Davis et al., 1988). Buffer materials were either of analytical reagent grade or were recrystallized or redistilled from bench grade products. Water used throughout this investigation was doubly distilled from an all-glass apparatus.

Methods. Enzyme was assayed routinely with cytidine 2′,3′-cyclic monophosphate according to the method of Crook et al. (1960). Protein concentration of the enzyme was determined by weight and from the absorbances at 280 and 260 nm (Warburg & Christian, 1941).

A typical run involved the addition of a stock solution of the enzyme to the substrate dissolved in buffer (2.5 mL) at the appropriate pH. The absorbance was then measured as a function of time with a Perkin-Elmer Lambda 5 spectrophotometer; pseudo-first-order rate constants were obtained from plots of log $(A_t - A_\infty)$ versus time. The pH was measured at the end of each kinetic run with a Radiometer pH-meter PHM 26 calibrated to ±0.02 pH unit with EIL standard buffer powders. Identities of the buffers employed in the reactions are given in the table. Michaelis–Menten parameters were determined from initial rates as previously described (Hall & Williams, 1986). The bistetrahydropyranyl esters were deprotected prior to use by the following procedure. A stock solution of the protected ester (2 mL, 10 mM) was prepared in water to which was added wet, swelled, strong acid ion exchange resin (Amberlyst 15, 50 mg); the resin was purified before use by several acid–base changes, washing thoroughly with analytical grade MeOH and sedimented until light particulate foreign matter had been removed. The reaction mixture was stirred for 30 min and the resin then removed by filtration and the solution used directly. The pH of the solution stock was about 2.5, and the solution was stable over a few days when kept at 4 °C.

Molecular graphics studies were carried out with the University's VAX 8800 system and the computer program

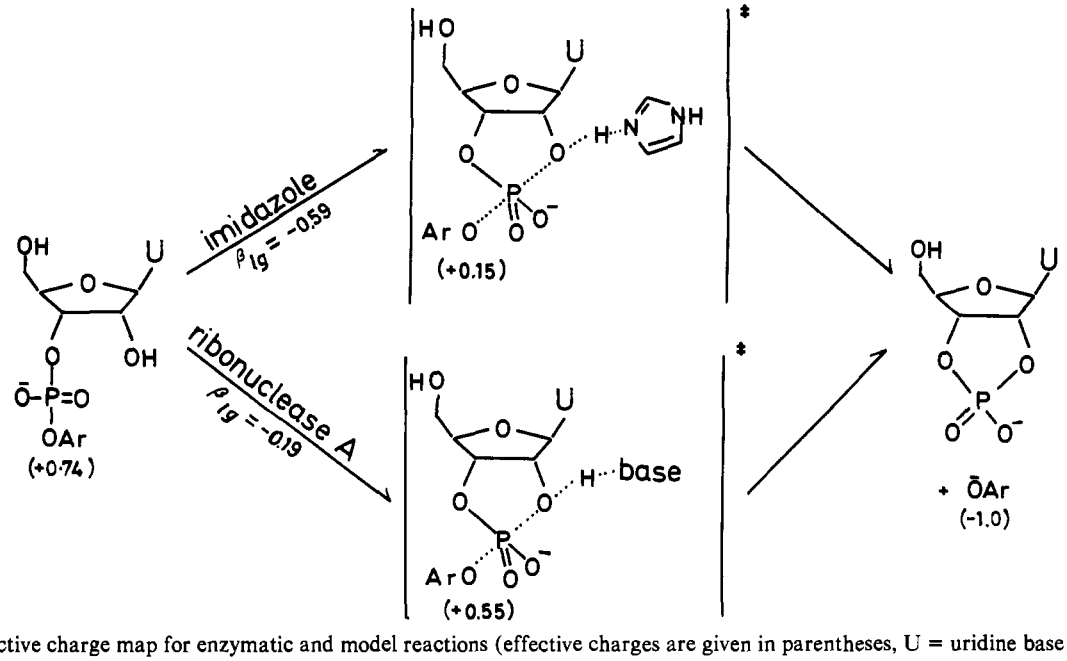

FIGURE 3: Effective charge map for enzymatic and model reactions (effective charges are given in parentheses, U = uridine base as in Figures 1 and 2).

Table I: Kinetic Parameters for the Cyclization of Substituted Uridine 3′-(Phenyl Phosphate)s by Bovine Pancreatic Ribonuclease A[a]

substituent[b]	pK_a^{ArOH}	k_{cat}/K_m[c] (M^{-1} s^{-1})	N[d]	10^6[E][e] (M)	λ[f] (nm)
parent	9.95	2400	4	0.4–3	230
4-Cl	9.38	3900	3	1–3	230
2-Cl	8.48	20000	4	0.5–3	241
3-NO_2	8.35	7400	4	0.5–3	235
3,5-Cl_2	8.18	5600	4	0.5–3	241
2,5-Cl_2	7.51	41000	4	0.5–3	240
4-NO_2	7.14	9600[g]	4	0.5–3	400
2,4,5-Cl_3	6.72	46000	4	0.5–3	240
2-Cl, 4-NO_2	5.45	70000	6	0.5–3	400

[a] Conditions: 25 °C, pH 7.50, ionic strength made up to 0.25 M with Na_2SO_4, buffer = tris(hydroxymethyl)aminomethane at 1 M. [b] Substrate concentration kept at 0.1 mM for each case. [c] Rate constants have confidence limit of ±8% of the listed value. [d] Number of data points not including duplicates. [e] Range of enzyme concentration obtained from the weight per milliliter and molecular weight; the routine assays with cytidine 2′,3′-cyclic phosphate gave k_{cat}/K_m = 816 M^{-1} s^{-1} at pH 7.50, which may be compared with the value 850 M^{-1} s^{-1} at this pH (Herries et al., 1962). Enzyme molarity was obtained from protein concentration and M_r 13683 (Campbell & Petsko, 1987). [f] Wavelength for kinetic study. [g] The value of K_m at pH 6.5 = 1.1 ± 0.03 mM and k_{cat}/K_m = 8390 ± 20 M^{-1} s^{-1}. Number of substrate concentrations was 5 in the range 0.2–0.8 mM; buffer was imidazole at 0.1 M.

MACROMODEL, which was purchased from Professor W. C. Still (Chemistry Department, Columbia University). Enzyme coordinates were purchased from the Brookhaven Data Base; the combined X-ray and neutron diffraction data of Wlodawer et al. (1983) for the ribonuclease A–uridine vanadate were employed to estimate the probable binding position of the uridine-3′-phosphate portion of the aryl ester substrates. Energy calculations to determine the favored conformations of the substrate bonds in the complex (other than those already fixed) were done with the MULTICONFORMER submode of the program, which is based on the "Eprox" interaction method of Pattabiraman et al. (1985).

Curve fitting for the bell-shaped pH dependence of k_{cat}/K_m was carried out with a BBC microcomputer and a BASIC version of the grid search program (Williams, 1969).

Results

Ribonuclease A samples had activity against the hydrolysis of cytidine 2′,3′-cyclic monophosphate close to that found by Herries et al. (1962). Under conditions where [S] < K_m the aryl ester fission obeyed excellent pseudo-first-order kinetics up to 90% of the total reaction. The initial absorbance change, from which kinetics are deduced, was in many cases followed by a slower change due to the catalyzed hydrolysis of the uridine 2′,3′-monophosphate intermediate. Substrate concentrations were kept low in the majority of cases, and the rate law (eq 1) was obeyed. Values of k_{cat}/K_m were obtained by

$$\text{rate} = k'[S] = k_{cat}/K_m[E][S] \quad (1)$$

plotting k' against enzyme concentration. That [S] < K_m was tested in each case by varying the overall substrate concentration and showing that the kinetics remained first order and had the same value of k'. Product inhibition was demonstrated to be negligible at all the aryl ester concentrations employed under first-order conditions by carrying out a hydrolysis to completion in the presence of the enzyme and then injecting a further quantity of substrate; up to 0.4 mM product concentration gives no observable difference between k' for the first and second kinetic runs. The derived values of k_{cat}/K_m for the substrates are given in Table I with the conditions of their measurement; their dependence on the pK_a of the leaving phenol is illustrated in Figure 4; essentially two correlations are observed, and these are governed by eq 2 for phenol leaving

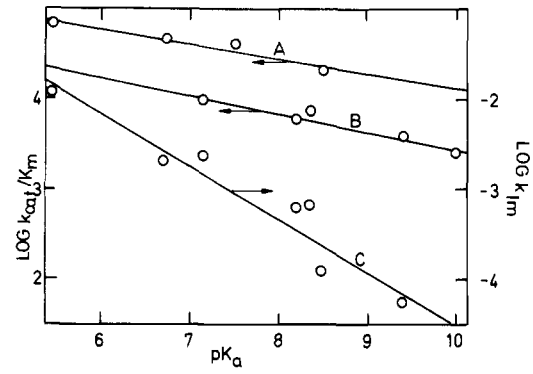

FIGURE 4: Brønsted dependencies against pK_a of the leaving phenol for the cyclization of substituted phenyl uridine 3′-(phenyl phosphate)s: (A) Reaction of 2-chloro-substituted esters catalyzed by ribonuclease A; (B) enzyme-catalyzed cyclization of phenyl ester substrates with free 2,6-positions; (C) imidazole-catalyzed cyclization. Data for (A) and (B) from the table, and lines are calculated respectively from eq 2 and 3; line and data for (C) are from Davis et al. (1988).

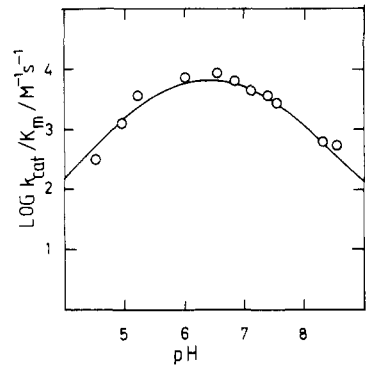

FIGURE 5: Dependence on pH of k_{cat}/K_m for uridine 3′-(4-chlorophenyl phosphate). Conditions are as in the table, and the buffers employed were acetate (pH 4–6), imidazole (pH 6–7.4), and tris(hydroxymethyl)aminomethane (pH 7.4–9). Line is calculated from the pK_a values 5.8 and 7.2 for the acid and base limbs of the pH profile and a limiting value for k_{cat}/K_m of 10^4 M^{-1} s^{-1}. No background cyclization due to buffer catalysis was observed with this ester in the pH range studied.

ortho chloro:

$$\log k_{cat}/K_m = (-0.17 \pm 0.03)pK_a^{ArOH} + (5.79 \pm 0.23)\ (n = 4,\ r = 0.965) \quad (2)$$

both ortho positions free:

$$\log k_{cat}/K_m = (-0.20 \pm 0.04)pK_a^{ArOH} + (5.47 \pm 0.31)\ (n = 5,\ r = 0.957) \quad (3)$$

groups bearing an ortho chloro substituent and by eq 3 for phenol leaving groups with both ortho positions free. A full Michaelis–Menten study was carried out on the 4-nitrophenyl ester substrate, and the resulting parameters are given in the table.

The pH dependence of k_{cat}/K_m was determined for the 4-chlorophenyl ester substrate, and this is illustrated in Figure 5. The data fit eq 4 derived from the mechanism involving

$$k_{cat}/K_m = k_{cat}/K_{m(lim)}/(1 + 10^{K_2}/[H^+] + [H^+]/10^{K_1}) \quad (4)$$

a diprotonic enzyme acting in the monoprotonated form. The values of pK_1, pK_2, and $k_{cat}/K_{m(lim)}$ are respectively 5.8, 7.2,

and 1.0×10^4 M^{-1} s^{-1}. The pK_a values are higher than those determined for the enzyme-catalyzed hydrolysis of cytidine 2′,3′-cyclic phosphate (Herries et al., 1962) but are close to those of Ramsden and Laidler (1966) that were measured under similar conditions of electrolyte composition.

Molecular modeling was carried out by using coordinates for the uridine identical with those for the riboside residue in uridine vanadate–ribonuclease complex. The atoms constituting the $-PO_3-C_6H_5$ group were substituted for the vanadyl group attached to the 3′-position; standard bond lengths and angles were employed. The coordinates of the phosphorus atom were determined for its most favorable position for reaction by minimizing its distance from the 2′ oxygen. The coordinates of the leaving oxygen were then determined for the most favorable reaction position by maximizing the distance from the 2′ oxygen keeping the phosphorus at its best position. Only the dihedral angles P–O–C–C and O–P–O–Ar are then needed for the complete definition of the substrate. The three-dimensional energy diagram for variation of these angles is given in Figure 6 for the phenyl and 2-chlorophenyl leaving groups. The nonbonded energies associated with the dihedral angles are not sufficiently accurate for a full energy contour diagram because the accuracy of the coordinates is only at 2-Å resolution (Wlodawer et al., 1983); nonshaded areas represent regions of dihedral angle combinations where there is relatively free torsional movement, and shaded areas are disallowed regions with large energy values. The three-dimensional structure of the ribonuclease A–substrate complex (Figure 7) is drawn from the coordinates where the phenyl is placed so that the dihedral angles are at the point noted in Figure 6.

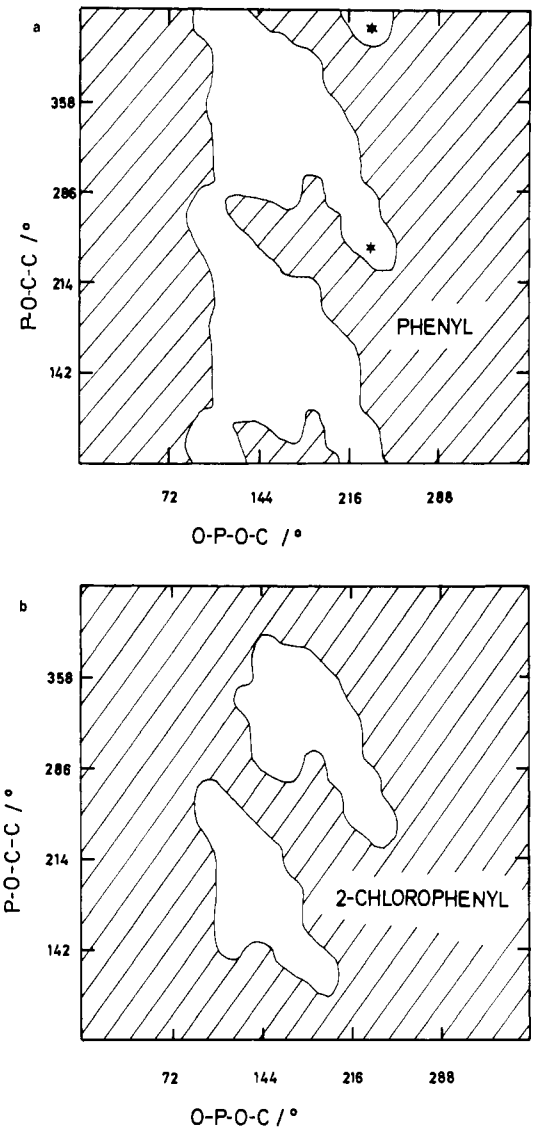

FIGURE 6: Allowed torsional angles (O–P–O–C and P–O–C–C) for phenyl (a) and 2-chlorophenyl (b) ester substrates in the modeled complex between substituted uridine 3′-(phenylphosphate) and the enzyme (see text). Shaded areas represent disallowed regions with large energy barriers. The asterisk in (a) represents the lowest energy conformation.

Discussion

Since the effective charge on the leaving oxygen atom is known in the substrate (Davis et al., 1988), the effect of polar substituents on k_{cat}/K_m enables us to calculate the effective charge in the transition state. The parameter k_{cat}/K_m is an apparent second-order rate constant; it is only a true microscopic rate constant when the rate-limiting step is the encounter between enzyme and substrate. The latter condition cannot hold because the rate constants would need to be at least 1×10^6 M^{-1} s^{-1} (Fersht, 1985). The overall reaction measured is cyclization, as evidenced by the formation and decay of absorbance in the case of several substrates. The parameter k_{cat}/K_m will therefore measure the energy between the ground state (where enzyme and substrate are free) and the transition state of the rate-limiting step (Hall & Williams, 1986; Ko & Kezdy, 1967), which is formation of the cyclic ester from the enzyme–substrate complex. The rate-limiting step in this reaction could be either (1) one of the two steps in a stepwise path involving a pentacoordinate intermediate or (2) the concerted displacement of the phenoxide ion from the ester.

Values of pK_a derived from pH profiles of k_{cat}/K_m refer to the ionization of *free* enzymes and are thus independent of substrate (Fersht, 1985); variation in k_{cat}/K_m at a given pH is therefore not due to shifts in pH–rate profile with changing substrate. The same cannot be said for k_{cat} measured at a single pH, and it is this that contributes to k_{cat}/K_m being a very useful parameter.

The present data for the cyclization reaction (eq 2 and 3) indicate that the electronic effect of the substituents in the leaving phenol group is small compared with the overall effect expected for the full cleavage of the P–OAr bond. The small charge change associated with the enzyme reaction can be compared with that ($\beta_{lg} = -0.59$) for the experimental model of ribonuclease (Davis et al., 1988); the effective charge maps of the enzymatic and model reactions are given in Figure 3. It is unlikely that the smaller negative change in effective charge on the leaving oxygen in the enzymatic reaction is due to a smaller extent of P–OAr bond fission in the transition state. Changes in extent of P–O bond fission are possible through variation of the nucleophile although increase in basicity of the base catalyst in the model (from imidazole to hydroxide ion) causes no significant alteration in β_{lg} (Davis et al., 1988).

Knowledge of the change in effective charge on the leaving oxygen atom does not afford a distinction between concerted and stepwise mechanisms for the displacement reaction. Discussion of model reactions (Davis et al., 1988) indicates that displacement at the phosphodiester is concerted, as displayed in Figures 2 and 3. Increase in positive effective charge on the leaving oxygen atom compared with that in the model could result from electrophilic interactions between the oxygen directly or with the leaving oxygen through the phosphoryl oxygens via the phosphorus atom. The direct interaction might be through general acid catalysis of leaving oxygen expulsion but since the phenoxide ion is stable enough to exist on its own

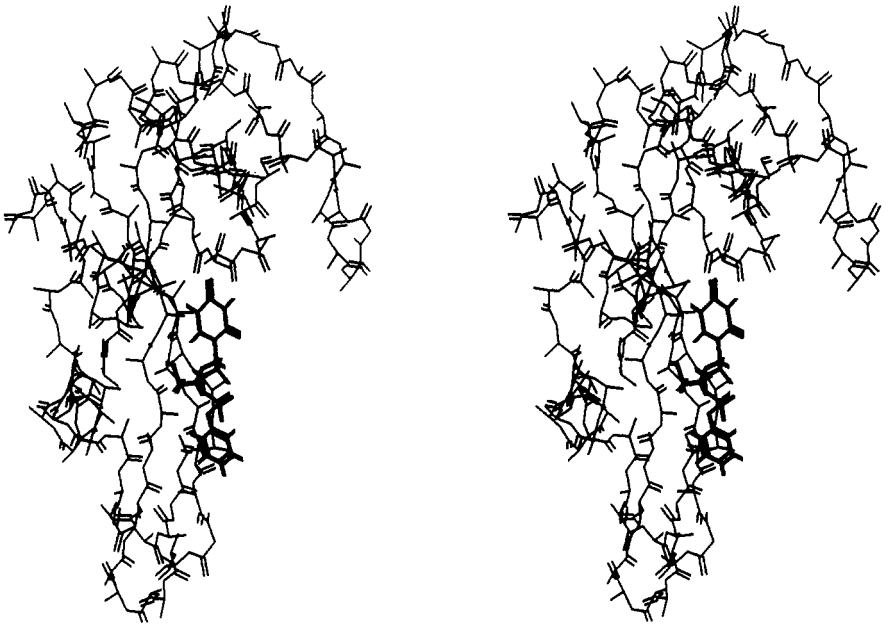

FIGURE 7: Stereo drawing of the backbone peptide chain of bovine pancreatic ribonuclease A with uridine 3′-(phenyl phosphate) (thick lines) located as described in the text with phosphorus and the leaving oxygen positioned ready for the cyclization; the phenyl group occupies the lowest energy conformation indicated in Figure 6a. Side chains are omitted for clarity, and peptide C=O bonds are represented as double bonds in the figure.

at the pH's in question, a hydrogen-bonding interaction is favored as this component.

The indirect interaction is modeled by the reaction involving protonation of the monoanion of aryl phosphate esters in water (eq 5) (Bourne & Williams, 1984).

$$\underset{(+0.74)}{H^+ + Ar\text{–}O\text{–}PO_2^-\text{–}OR} \rightleftharpoons \underset{(+0.83)}{Ar\text{–}O\text{–}PO(OH)\text{–}OR} \quad (5)$$

The direct interaction may be modeled by the hydrogen-bonding interaction in water between phenolate ions and ethylenediamine cation (eq 6) (Stahl & Jencks, 1986).

$$\underset{(-1.0)}{Ar\text{–}O^-\cdot H_2O} + RNH_3^+\cdot H_2O \overset{K_{AB}}{\rightleftharpoons} \underset{(-0.9)}{Ar\text{–}O^-\cdot H_3N^+R} \quad (6)$$

The latter model gives an upper limit to the magnitude of the effect on the phosphorylation in water since the phenolate ion represents the *final* state of the P–OAr bond fission. Either type of interaction would thus not give rise to large changes in charge. We suggest that the relatively large difference in effective charge change is as a result of electrophilic interaction at both phosphoryl and leaving oxygens. The data of this study do not differentiate between the two types of interaction.

Exclusion of water solvent from the active site could enhance the charge difference; in support of this hypothesis much larger Brønsted and Hammett selectivities are observed for non-ion-solvating media. The hydrogen-bonding equilibrium of amines with phenols has a ρ value of 1.4 in CCl_4, and the pK_a of phenols in CH_3CN has a ρ value of 4.8 (Singh et al., 1966; Clotman et al., 1970; Kolthoff & Chantooni, 1969). The values refer to different states in the same solvent, and the effects would be enhanced for changes in solvent.

Electrophilic catalysis has been postulated previously on structural grounds (Fersht, 1985; Witzel, 1964; Deavin et al., 1966) and on the reasonable basis that electrophiles must be needed in the phosphorylation mechanism; precedent for the combined action of general base and electrophile in the ribonuclease mechanism comes from model work of Breslow and Labelle (1986) and Eftink and Biltonen (1983). The present studies provide the first definitive evidence that electrophilic catalysis is important in the enzyme mechanism.

Satoh and Inoue (1975) measured values of V_{max} for substituted guanosine 3′-(benzyl phosphate)s catalyzed by ribonuclease T_1; they observed a ρ value of 0.48 at 25 °C and pH 7.5. This value may be translated to yield a ρ_{lg} of −0.34 by using the value of ρ for the ionization of substituted benzyl alcohols (1.42) calculated from the ρ^* value found by Ballinger and Long (1960) for the ionization of substituted methanols. These results cannot strictly be compared with those of the present work because of the differences in enzyme and substrate. Moreover, the charge change calculated from the ρ_{lg} refers to the difference between two energy levels, neither of which are not "anchored" by reference to a state where the charge is known on the leaving oxygen. Thus, although the charge difference is known, the individual charges cannot be assigned.

Although it is certain that the general assumptions in the mechanism still hold, the assignment of particular groups in the active site does not readily follow from the crystallographic coordinates of the enzyme and enzyme–substrate-like inhibitors. Such coordinates are probably for conformations in local energy minima, and application of the Curtin–Hammett principle (Maskill, 1985) indicates that these are not necessarily those corresponding to the transition-state conformation. Wlodawer's results (Wlodawer et al., 1983) tend to query the usual role of histidine-12 and lysine-41 but do not exclude the former acting as general base and the latter as an electrophile. Recent work of Campbell and Petsko (1987) on the sulfate-free enzyme supports the involvement of histidine-12 as a general base. Further work will be necessary before we can assign the electrophilic component with confidence, but it is possible that both residues 119 and 41 contribute to this.

The absence of major specific substituent effects on the reactivity of the uridine 3′-(aryl phosphate)s is consistent with little interaction of the phenyl group with the protein architecture in the transition state. This is in agreement with the similarity of K_m between the 4-nitrophenyl ester (table) and the K_i and K_m for other uridine-bearing inhibitors and substrates at similar pH's. The K_m value for the cyclization reaction is close to that of the ring-opening reaction with the cyclic uridine 3′-phosphate and does not appear to depend

heavily on the leaving function, as would be expected for a strong interaction between this and the active site. Follman et al. (1967) found that K_m varies from 1.3 to 3 mM at pH 7 for uridine 3′-phosphate derivatives. The absence of the leaving function raises the K_m only slightly at pH 7 (Witzel & Barnard, 1962a,b; Gassen & Witzel, 1967; Hummel & Witzel, 1966). If we assume reasonably that the nucleotide part of the substrate occupies the same position as it does in the uridine vanadate–enzyme complex, then the positon of the phosphorus and the oxygen leaving group in the conformation prior to the transition state may be readily determined (as shown under Results). The phenyl leaving group is relatively free to move in a volume of space defined by the P–O–C–C and O–P–O–C dihedral angles. The minimum energy conformation of these dihedral angles for the free substrate is an essentially staggered one, and this (marked with an asterisk in Figure 6) is encompassed in the volume of space where no large energy interactions occur. The energy measured by k_{cat}/K_m is that from the free enzyme and free substrate to the *transition state* of the phosphorylation. The conformation of the phenyl leaving group at its minimum energy may not be that in the transition state by consideration of the Curtin–Hammett principle (Maskill, 1985); nevertheless, there is considerable space where there is little specific interaction possible between substituent and enzyme architecture.

The slightly enhanced reactivity of the 2-chlorophenyl esters is not readily explained, as the energy associated with it is very small. The computer program for the modeling would not be expected to cope with such a small energy difference, especially as the original coordinates are not known to better than 2-Å resolution. We note that there is a slight constriction of movement of the 2-chlorophenyl group compared with the parent (Figure 6) as might be expected from a sterically hindered ester.

Registry No. Ribonuclease A, 9001-99-4; uridine 3′-(phenyl phosphate), 51247-16-6; uridine 3′-(4-chlorophenyl phosphate), 115142-01-3; uridine 3′-(2-chlorophenyl phosphate), 115142-02-4; uridine 3′-(3-nitrophenyl phosphate), 115142-03-5; uridine 3′-(3,5-dichlorophenyl phosphate), 115142-04-6; uridine 3′-(2,5-dichlorophenyl phosphate), 115142-05-7; uridine 3′-(4-nitrophenyl phosphate), 115142-06-8; uridine 3′-(2,4,5-trichlorophenyl phosphate), 115142-07-9; uridine 3′-(2-chloro-4-nitrophenyl phosphate), 115142-08-0.

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