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The Active Site and Mechanism of Action of Bovine Pancreatic Ribonuclease

7. THE CATALYTIC MECHANISM

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The previous papers in this series (Crook, Mathias & Rabin, 1960*a*, *b*; Herries, Mathias & Rabin, 1962; Findlay, Mathias & Rabin, 1962*a*, *b*; Ross, Mathias & Rabin, 1962) provide evidence that the catalytic site of ribonuclease contains two imidazole groups; one of these is required in the acid form and the other in the base form. For the hydrolysis of cytidine 2′,3′-phosphate, the shifts in the pK values of the active site on binding the substrate indicate that the acid site, but not the base site, is involved in binding the substrate. The acid site is probably hydrogen-bonded to the 2′-oxygen atom of the cyclic phosphate and this accounts for the much stronger binding of cytidine 2′-phosphate compared with that of the 3′-phosphate. There must be additional sites of enzyme–substrate binding which do not ionize over the pH range 4–8·5, since $K_{s'}$ is not identically zero. Doubtless these are interactions which define the specificity of the enzyme.

Since the site required in the base form does not interact with cytidine 2′,3′-phosphate it is possible that its function is to bind the attacking reagent, water or an alcohol. A considerable body of evidence has been presented for the existence of a site which interacts with water or an alcohol. Polyhydroxylic alcohols are favoured reagents, suggesting that the alcohol-binding site is complex. Presumably, the attacking hydroxyl group is hydrogen-bonded to the base site and the other hydroxyl groups interact with other groups of the enzyme. Water would only be bound at the base site. Evidence has been obtained for Van der Waals' interaction between the methyl group of methanol and the protein.

On the basis of these facts we propose that the enzyme–substrate complex is as shown in Fig. 1 (Findlay, Herries, Mathias, Rabin & Ross, 1961). In this representation (I) is the imidazole which binds either water or alcohol and (II) the imidazolium ion which interacts with the 2′-oxygen atom. (III) represents the sites of additional interaction with the alcohol. The specificity site is (IV) and the interaction here would seem to involve, at least in part, the pyrimidine $N_{(1)}$ (Witzel, 1960). Interaction at (II) weakens the $P-O_{(2')}$ bond and renders the phosphorus atom more susceptible to attack by nucleophilic reagents. Interaction of the attacking reagent, by hydrogen-bonding to imidazole (I), enhances the nucleophilic character of the oxygen. The transition state envisaged for the reaction is shown in Fig. 2. The curved arrows represent the direction of electron displacements, as is conventional in chemical formulations. Alternatively, this could be pictured as a movement of protons and the substituted phosphoryl group in the reverse direction to that shown for the electron displacements.

The enzyme–product complex is shown in Fig. 3, and it can be seen that the acid–base groups are now in their conjugate forms. By the principle of microscopic reversibility the reverse reaction will proceed by complete reversal of the forward process

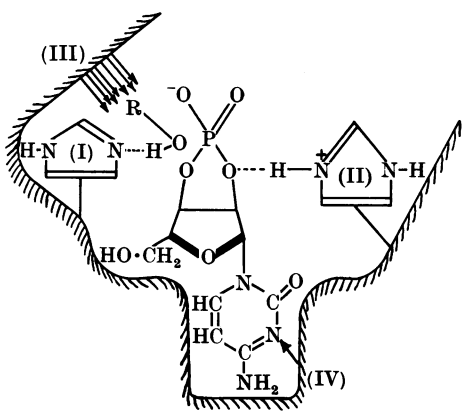

Fig. 1

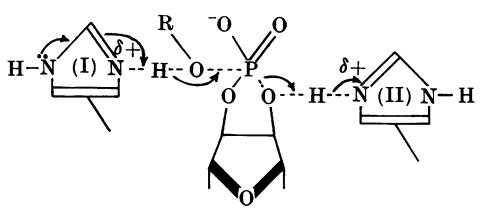

Fig. 2

(Rabin, 1958). Fig. 3 therefore represents the enzyme–substrate complex for the cyclization of an ester of cytidine 3′-phosphate. If this ester is a portion of the chain of ribonucleic acid, the enzyme–substrate complex would be as shown in Fig. 4.

At first sight it would appear legitimate to identify (I) and (II) as the histidines involved in the interaction of ribonuclease with bromo- or iodo-acetic acid. This would be consistent with the fact that the cytidine nucleotides protect the enzyme against alkylation and that their effectiveness in descending order is 2′-phosphate, 3′-phosphate, 5′-phosphate, which is identical to their order of effectiveness as inhibitors. However, sulphate and phosphate (Stein, 1960) and pyrophosphate (M. P. Lamden, A. P. Mathias & B. R. Rabin, unpublished work) protect the enzyme against alkylation but do not inhibit substrate hydrolysis under the usual conditions of assay. The function of the reactive lysine residue (Hirs, Halmann & Kycia, 1961) remains to be evaluated.

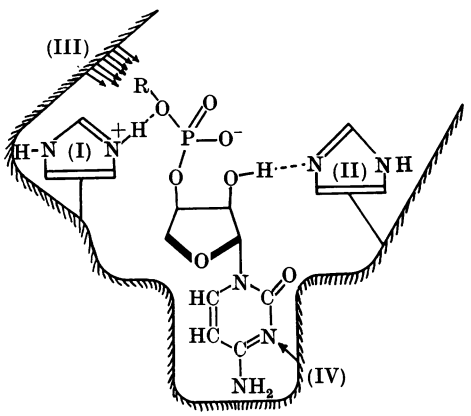

Fig. 3

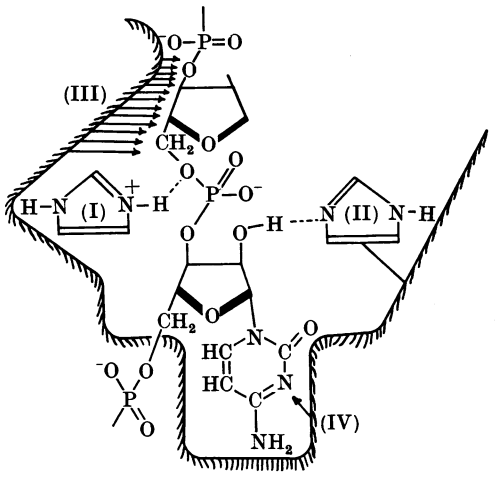

Fig. 4

SUMMARY

1. An hypothesis for the active site and mechanism of action of ribonuclease is presented. In the hydrolysis of cytidine 2′,3′-phosphate a histidine in the acid form transfers a proton to the 2′-oxygen atom of the nucleotide and a proton from water (or an alcohol) is transferred to another histidine. In the cyclization of an ester of cytidine 3′-phosphate, such as ribonucleic acid, the function of these two histidines is reversed.

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