The contribution of noncatalytic phosphate-binding subsites to the mechanism of bovine pancreatic ribonuclease A

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Abstract. The enzymatic catalysis of polymeric substrates such as proteins, polysaccharides or nucleic acids requires precise alignment between the enzyme and the substrate regions flanking the region occupying the active site. In the case of ribonucleases, enzyme-substrate binding may be directed by electrostatic interactions between the phosphate groups of the RNA molecule and basic amino acid residues on the enzyme. Specific interactions between the nitrogenated bases and particular amino acids in the active site or adjacent positions may also take place. The substrate-binding subsites of ribonuclease A have been characterized by structural and kinetic studies. In addition to the active site (p₁), the role of other noncatalytic phosphate-binding subsites in the correct alignment of the polymeric substrate has been proposed. p₂ and p₀ have been described as phosphate-binding subsites that bind the phosphate group adjacent to the 3' side and 5' side, respectively, of the phosphate in the active site. In both cases, basic amino acids (Lys-7 and Arg-10 in p₂, and Lys-66 in p₀) are involved in binding. However, these binding sites play different roles in the catalytic process of ribonuclease A. The electrostatic interactions in p₂ are important both in catalysis and in the endonuclease activity of the enzyme, whilst the p₀ electrostatic interaction contributes only to binding of the RNA.

Key words. Ribonuclease; binding sites; RNA; catalysis; enzyme kinetics.

Introduction

Formation of the enzyme-substrate complex is a basic step in enzymatic catalysis. In the case of enzymes that catalyse reactions with low molecular mass substrates, binding takes place only at the active site of the enzyme. However, in the case of enzymes acting on polymeric substrates, the interaction may be more complex due to the need for a correct alignment between the enzyme and the substrate regions flanking that which actually occupies the active site. This is a general feature seen in all enzymes that catalyse the breakdown of polymeric substrates, such as proteins, polysaccharides and nucleic acids.

In the case of proteinases the noncatalytic binding subsites were designated as S₁, S₂, S₃ and so on, and S'₁, S'₂, S'₃ and so on (amino-terminal and carboxy-terminal residues next to the scissile peptide bond, respectively). Lysozyme is an example of a glycosidase that catalyses the hydrolysis of a complex polysaccharide. The binding of its substrate takes place in a well-defined deep cleft in which six binding subsites have been defined (A, B, C, D, E and F). Cleavage takes place in the glycosidic bond between the residues that bind to subsites D and E [1].
Enzyme-substrate binding for nucleic acids may be very different because of the substrate structure (single- or double-stranded) and the specificity of the enzyme. The binding of DNase I, a nuclease that acts on double-stranded DNA but with a low specificity for base sequence has been analysed by X-ray crystallography. From the DNase I-d(GGTATACC)_2 complex it has been shown that DNase I binds in the minor groove of a right-handed DNA duplex, and to the phosphate backbones on either side over 5 bp resulting in a widening of the minor groove [2]. On the other hand, more specific enzymes such as type II restriction enzymes need the specific recognition of the substrate in the regions of the DNA near to the scissile bonds. Only in a few of these enzymes, notably Eco RI and Eco RV, has the substrate binding been analysed: each of these enzymes follows a quite different mechanism for DNA recognition and/or cleavage [3].

In the case of RNases and RNA, enzyme-substrate binding may be directed by electrostatic interactions between the phosphate groups of the substrate and basic amino acid residues (Lys and Arg) of the protein structure as well as by specific interactions between nitrogenated bases and amino acids in the active site or adjacent positions. Bovine pancreatic RNase A-binding subsites are the best characterised from the point of view of both structure and kinetics, but binding subsites in other RNases of the same superfamily such as onconase and eosinophil-associated RNases or microbial RNases such as barnase have also been analysed. According to the nomenclature for the RNase A-binding subsites [4], B1R1 corresponds to the enzyme region which binds the nucleoside moiety that is linked through the 3' position to the phosphate group located at the phosphate-binding site p1 in the active centre. B2R2 is the enzyme region which binds the nucleoside moiety that is linked through the 5' position to the phosphate at p1. p0 and p2 are additional noncatalytic phosphate-binding subsites (fig. 1).

Barnase

Barnase is an extracellular enzyme secreted by Bacillus amylobacteriacei that shows RNase activity. From the catalytic point of view, the enzyme reaction is very similar to that of RNase A: a transphosphorylation from the 5' position of a nucleotide to the 2' position of the adjacent nucleotide takes place, and a 2',3'-cyclic phosphate product is formed. In an aqueous environment a hydrolysis reaction that cleaves the 2'-phosphate bond forming a nucleoside 3'-phosphate eventually occurs. The proposed mechanism is similar to the acid-base mechanism described for RNase A, although barnase uses a histidine (His-102) and a glutamate (Glu-73) residues instead of two histidines (His-12 and -119) as in the case of RNase A [5]. Barnase is specific for phosphodiester bonds that have a guanine in the 3' position (GpN) although the catalytic efficiency increases with the substrate length, and the presence of a phosphate group on the 3' position of the second nucleotide increases the rate of the transphosphorylation reaction. This effect is more pronounced when a third cytidine nucleotide is present (GpUpC or GpApC). It has been suggested that the third base modifies the position of the second phosphate, lowering the tran-sition-state energy [6]. X-ray crystallography of the complex of barnase and the oligodeoxynucleotide d(CGAC) [7] indicates that the guanosine plays a major role in the binding of the tetranucleotide and that the adenine base also contributes to the binding. However, most of the interaction energy is provided by the negative charge on the 3'-phosphate of the guanosine nucleotide and, to a lesser extent, by that of the adenosine nucleotide interacting with basic groups of the enzyme.

Onconase

Onconase has a lower specific activity towards common substrates in relation to pancreatic RNase A. Onconase activity is higher for RNA and polyuridylic acid [poly(U)] than for polycytidylic acid [poly(C)], while pancreatic RNases prefer poly(C). Substrate preference for uridine in the B1 site and guanosine in the B2 site [8], as deduced from activity assays with dinucleotides, is shared with other frog RNases studied [9, 10]. A computer model of onconase with d(UpG) involves Lys-33 in uridine B1 specificity (position of Val-43 in RNase A) and Ser-54, Asn-56 and Glu-91 (positions 65, 72 and 111 in RNase A) in the B2 binding [11].