ARRANGEMENT OF THE CHARGED GROUPS IN THE THREE DIMENSIONAL STRUCTURE OF PEPSIN

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1. Introduction

The presence of internal hydrophobic cores is a common property of all globular proteins. At the same time charged groups or ion pairs which these groups can form are mainly located on the surfaces of such molecules. However in some cases ion pairs have been found in the hydrophobic interior where the charge interactions are enhanced, as compared to those for groups on the surfaces, due to their hydrophobic environment. Such ion pairs can therefore play a special role in the stabilization of structural elements. The other important role of some internal ion pairs is concerned with their involvement in the system of interactions in the active sites of several enzymes [1]. Therefore the observation of such pairs by X-ray crystallographic methods is an important step for structural studies. Thus the observation of the ion pairs ASP-102–His-57 and Asp-194–NH_3^+-terminus in chymotrypsin was very important for the elucidation of the most significant interactions in the active site of serine proteases and the mechanism of their zymogen activations [2].

During X-ray crystallographic studies of porcine pepsin we have found important charge interactions in the three-dimensional structure of the enzyme. Some of them concern the groups which are near the active site, some of them induce the decrease of enzyme activity during modification. The observed charge interactions of the N-terminus are important for the understanding of the porcine pepsin activation process. The data provide the explanation of the lowest isoelectric point among proteins of porcine pepsin.

2. Analysis of the arrangement of charged groups in porcine pepsin molecules, as revealed by the electron density maps

We are working with the electron density maps of monoclinic pepsin crystals [3,4] at 3–2.7 Å resolution. The work with the maps is at the stage when the analysis of side chain interactions has been done.

During these studies the following charge interactions in the three-dimensional structure of the enzyme were found.

The ion pair Asp-11–Arg-308, which is located in the bottom of the cleft between domains of the molecule [4]. Arg-308 is the end group of a one-turn helix near the active Asp-215. Electron density for the side chain Arg-308 corresponds to its extended configuration; density falls nearby the 8–12 loop close to the easily visible side chain of Asp-11. An ion pair of the same type, Lys-308–Asp-11, was found in penicillopepsin, and its hypothetical role in the catalytic action of the enzyme was discussed [5].

The second ionic pair was found in the N-terminal domain, in the region unaccessible to interaction with the solvent. This pair is formed between the charged NH_3^+-terminus and the carboxyl group of Glu-4 (fig.1). In contrast to mold acid proteases the N-terminal β-strand of pepsin changes direction at the α-carbon atom of Gly-2 and the Ile-1 residue comes of from the surface into the interior of the N-terminal domain. The side chains of the N-terminal β-strand: of Asp-3, Pro-5 and Glu-7 are oriented into the solvent, while those of Glu-4 and Leu-6 are turned towards the inside of the molecule in such way that the carboxyl group of Glu-4 comes near the NH_3^+ group of the N-terminus. The orientation of this NH_3^+ group is fixed by the well visible orientation of the Ile-1 side

chain and the position of the Ile-1 carbonyl oxygen. Near this ion pair Glu-4—NH_3^+-terminus the following hydrophobic groups are located: Ile-1; Ile-18; Val-165; Leu-166; Phe-31; Val-91. From the other side of this ion pair the phenolic ring of Tyr-14 is arranged with the probable formation of a hydrogen bond between its hydroxyl group and the carboxyl group of Glu-4. The peptide group 30–31 is also not far from this carboxyl. This group belongs to the B_1 loop [4], containing the active aspartic acid 32 residue. The Phe-31 NH group is not involved in hydrogen bonding within the sheet and is oriented towards Glu-4. The distance of this NH group from the Glu-4 carboxyl seems to be slightly large for the formation of a hydrogen bond.

All other basic groups of porcine pepsin (His-53, Lys-320, Arg-316) are located on the surface of the molecule. The Arg-316 side chain lies along the surface near the segment 122–132, protecting the active site from the solvent. Near the Arg-316 side chain two aspartic residues are located (Asp-149, Asp-142). As the electron density in this region is not well enough resolved, the formation of an ion pair for this basic group remains to be proved by higher resolution studies, as well as for His-53 which is not far from the Asp-26 residue. The Lys 320 residue seems not to be involved in the formation of ion pairs with any acid group of the protein.

The negatively charged group of the serine-phosphate residue 68 is located on the surface and only forms contacts with the solvent. The other negatively charged group of porcine pepsin at low pH is Asp-32, the main group of the active site which has an abnormally low pK [6]. The atomic arrangement in the active site of the enzyme will be the subject of another publication.

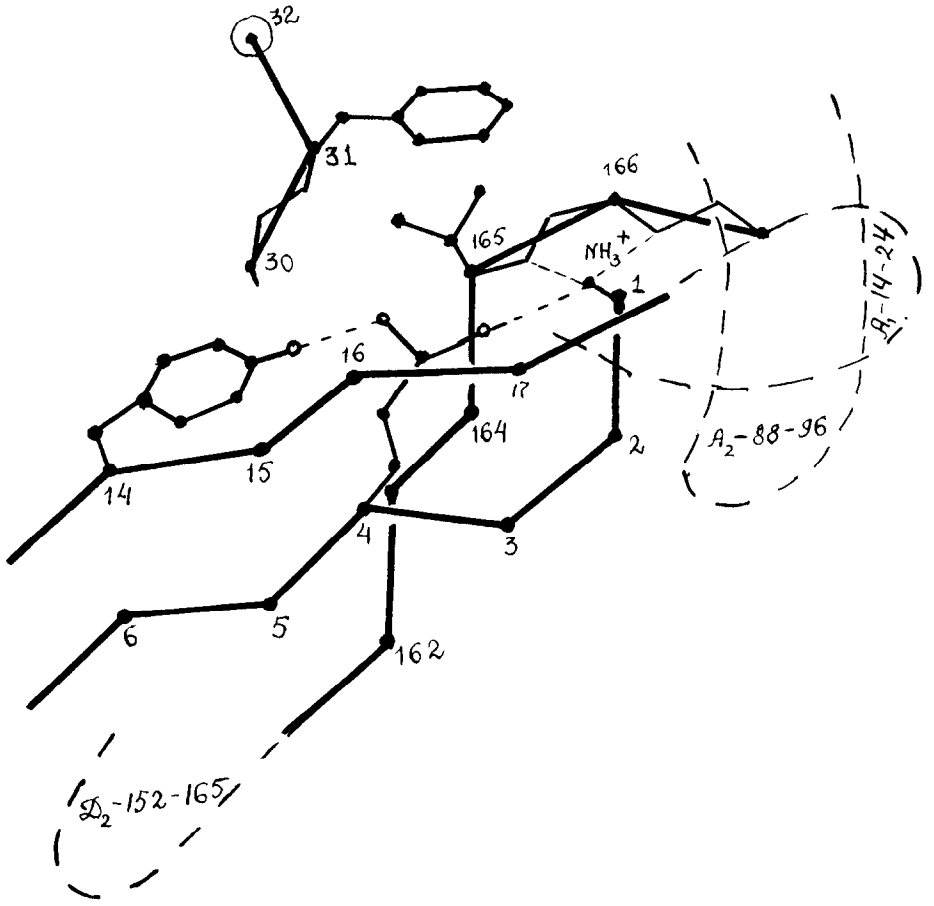

Fig.1. Scheme showing the contacts near the N-terminus of the porcine pepsin molecule.

3. Discussion

The monoclinic pepsin crystals we studied were obtained from water–ethanol solutions at pH 2. At this pH acid groups have to be neutralized, while basic groups have to be charged. Porcine pepsin has only 4 basic amino acid residues, 2 or probably 3 of them are involved in the formation of ion pairs. This property and the existence of the large number of acid groups, as well as the presence of the negative charge at serine-phosphate and at Asp-32 with an abnormally low pK, can explain the very low isoelectric point of porcine pepsin as compared to other proteins. The additional positive charge, which the NH_3^+-terminus must have, is neutralized by the formation of an internal ion pair with Glu-4. Some points concerning this ion pair will be discussed.

Due to the formation of this ion pair, the N-terminus of porcine pepsin is arranged in the hydrophobic core of a molecule. This is not a common feature of pepsin and penicillopepsin. The N-terminal β-strand of mold carboxyl proteases seems to be completely extended, and has 4 additional residues at the N-terminus as compared with pepsin [5], and the N-terminal group is looking into the solvent. While these proteases have no zymogen, for many acid proteases of higher organisms, having zymogens, Glu-4 seems to be conserved*.

The disposition of the N-terminus in the hydrophobic interior of the domain requires conformational change during the activation process. These changes have to include the movements of the A_1 and A_2 hairpins [4] (fig.1) which have to be arranged in the zymogen or in the products of its partial activation somewhat differently thereby providing more space near the D_2 [4] second strand (fig.1). Undoubtly the formation of the internal ion pair protects the N-terminal strand from hydrolysis and stops the stepwise process of activation. It also decreases the isoelectric point of the enzyme. However the complete role of this ion pair is difficult to evaluate at this stage. As pepsinogen and various products of its partial activation are active [8], the formation of this ion pair is not neccessary for the appearance of the activity. One can think that its formation promotes an additional small adjustment of the conformation of the B_1 [4] active loop, which makes the enzyme more active. This adjustment can take place due to the disruption of the hydrogen bond between Glu-4 and the NH of the peptide group 30–31. It can also include changes in the contacts between the B_1 active loop and the D_2 hairpin second strand which has hydrogen bonds with the NH_3^+-terminus. X-Ray studies of pepsinogen, developing those in [9], will help in understanding the role of Glu-4 and the NH_3^+-terminus in the activation process.

The other ion pair meriting attention is Asp-11–Arg-308. Comparison of pepsin data to those of penicillopepsin shows this pair to be conserved. Further experimental studies of this ion pair and its role in pepsin function are necessary.

The arrangement of the Arg-316 side chain near the loop 122–132, protecting the active site from the solvent can explain the loss of enzyme activity during modification of Arg-316 [10].

* One exception is chymosin which has two additional residues at the N-terminus and valine at position 4, in pepsin notation [7]. At the same time the 1-position is occupied by a glutamic acid residue, which can neutralize the charge of the N-terminus

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