ACCESSIBILITY OF DISULFIDE BRIDGES OF RIBONUCLEASE S

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The examination of the steric environment of Cys65-Cys72 was separately carried out and the maximum accessibility of all four disulfide bridges was found on the basis of ribonuclease S drawing. The results obtained showed that Cys65-Cys72 and Cys40-Cys95 are accessible for reagent molecule with maximum diameter of 4·2 Å and 4·4 Å, respectively. Some of the chemical data refering to the reduction of disulfide bridges in RNase A in native state were discussed on the basis of the drawing. A difference in the exposure of Cys40-Cys95 in both enzymes was established.

A method for representation of the steric structure of protein molecules has been recently reported[1]. The procedure consists in exhibition of the three dimensional structure of protein molecules by means of a drawing made by an orthogonal projection on two perpendicular projection planes. The drawing could be produced on the basis of atomic co-ordinates obtained either by X-ray diffraction analysis or by computer methods and permits the determination of a distance between any two atoms of the polypeptide chain, the true location of three atoms as well as the angle of rotation around a single bond. The present paper deals with the application of this method on the molecule of ribonuclease S and especially for the study of the accessibility of disulfide bridges.

METHODS

The drawing of the steric structure of ribonuclease S was made by using the co-ordinates of the atoms of the amino acid residiues obtained by X-ray diffraction analysis with 2·0 Å resolution[2].

For a fixed point of the space at definite co-ordinate system was accepted: In front of the point are located points having larger co-ordinate y that of the point, and at the back those having smaller co-ordinate y respectively. Above the point are these with large coordinate z and under those with smaller co-ordinate z, than z cordinate of the point. On the left of the point are situated points with larger co-ordinate x and on the right those with smaller coordinate x respectively. The positions in front of and at the back of the point might be determined by means of the first projection of the drawing, while above and under are found by the second one. On the left and on the right of the point are identified either by the first or by the second projections.

The study of the steric environment of an atom or group of atoms was carried out by means of a certain geometric figure according to the geometry of the object examined. The most

suitable for the purpose is a cube or parallelepiped, corresponding to the environment studied, the centre of which coincides with the centre of the object. In the geometric figure chosen are located only the atoms the first and the second projections of which are situated in the first and in the second projections of the figure. Only the atoms isolated in this way were studied.

The accessibility of one atom for a certain reagent is determined by means of a geometric figure as sphere, cube or parallelepiped according to the shape of the reagent molecule. The radius of the sphere (if sphere is used) is $R = r + 1{\cdot}8$ Å, where r is van der Waals radius of the reagent molecule and 1·8 is van der Waals radius of protein molecule atoms. The atoms, the first and the second projections of which are situated simultaneously in the first and the second projections of the sphere with radius R, are to the distance not greater than $R\sqrt{2}$ of its centre. In practice the projections of the sphere used are drawn on transparent plates, which are moving on the drawing. If the projections are moved in a way so that no atoms of protein molecule get into their interior it might supposed that the reagent molecule will penetrate unhindered to this direction. An atom of the polypeptide chain could be considered as accessible when the distance between its centre and the centre of the sphere becomes $d = r + 1{\cdot}8$ Å.

RESULTS AND DISCUSSION

The disulfide bridges in ribonuclease S are in positions Cys26-Cys84,Cys40-Cys95, Cys58-Cys110 and Cys65-Cys72 of the polypeptide chain. In Fig. 1 are shown separately the first and the second projections of the steric environment of Cys65-Cys72 with a shape of cube limited by the co-ordinates: $-6{\cdot}0 \leqq x \leqq 8{\cdot}0$; $2{\cdot}0 \leqq y \leqq 16{\cdot}0$; $3{\cdot}0 \leqq z \leqq 17{\cdot}0$. Table I presents the steric arrangement of amino-acid residues of the polypeptide chain refered to Cys65-Cys72. On the whole the polypeptide

TABLE I
Locations of Amino-Acid Residues

Residue	Steric arrangement refered to Cys65-Cys72		
Asn62	on the right,	in front of,	above
Val63	—	in front of,	—
Ala 64	on the right,	in front of,	under
Lys66	—	at the back,	under
Asn67	on the right,	at the back,	under
Gly68	on the right,	—	under
Gln69	on the right,	at the baclk,	—
Thr70	on the right,	in front of,	above
Asn71	—	at the back,	above
Tyr73	—	in front of,	above
Ile107	on the left,	in front of,	under
Val108	on the left,	—	—
Ala109	on the left,	at the back,	above
Cys110	on the left,	—	above

chain from Val63 to Asn71 is situated on the right of the disulfide bridge Cys65-Cys72 while the chain from Ile107 to Cys110 as well as Asp121 and Ala122 are located on the left of it. Evidentely, S^{γ} of Cys65 is accessible only at the back between Asn67, Asn71, Val109 and Asp129.

It was found out that the Cys65-Cys72 is accessible only for the reagent molecule with maximum diameter of 4·2 Å. In Fig. 1 are shown the two projections of its corresponding sphere with radius of 3·9 = 2·1 + 1·8, which touches S^{γ} of Cys65,C^{β} of Ala109,$O^{\delta 2}$ of Asp121 and $O^{\delta 2}$ of Asn67. The bridge Cys40-Cys95 could be reached unhindered by a regent molecule with maximum diameter of 4·4 Å. Its corresponding sphere with radius of 4 = 2·2 + 1·8 Å touches S^{γ} of Cys95,N of Asn94,C′ of Pro93 and C^{α} of Lys37. The other two bridges Cys58-Cys110 and Cys26--Cys84 are in buried state, unaccessible even for molecule with diameter of 2·8 Å (ref.[3]). The data obtained showed that Cys65-Cys72 and Cys40-Cys95 possess almost equal maximum accessibility. A careful examination of the environments of both bridges however shows that the crevices of the disulfide bonds are different. The crevice of Cys65-Cys72 was found shallow, whereas that of Cys40-Cys95 was deep. This location of Cys40-Cys95 does not permit the penetration of long and branched reagent molecules to it.

Let us go through some of the chemical data referring to the reduction of disulfide bridges of ribonuclease A in native state. Neumann and coworkers[4] succeeded in cleaving with phosphothionic acid only the two of cystine bridges (65–72 and 58–110). The modified enzyme was more than RNase A active towards 2′,2′-cyclic phosphate, but was digestible by trypsin. Sperling and coworkers[5] have been able to reduce some of the disulfide bonds of RNase A by dithioerythritol. It was found that ribonuclease reacts in two steps: a fast step followed by a slower one. Cys75-Cys72 was cleaved during the fast reduction step. The ribonuclease carboxymethyl derivative obtained after reduction of Cys65-Cys72 is enzymatically as active as the native protein and resistant to digestion by trypsin. Ribonuclease A was desulfurized by Ra–Ni in native state[6]. Six of the 12 sulfur atoms were removed, but 3 of them within 5 min, whereas the other 3 in about 8 hours of interaction. These steps were accompanied by a decrease of the enzyme activity. There are no chemical data available to indicate the reduction of Cys40-Cys95 of ribonuclease A in the native state. Dickerson and Geis[7] have shown the large structural similarity between RNase A and RNase S on the basis of the stereopairs drawings of the α carbon chains of these enzymes. However the complete comparison of the structures of both enzymes has not been made yet[8].

The chemical data obtained for RNase A could be commented on the drawing of RNase S provided both enzymes are similar in the region of Cys65-Cys72 and Cys58-Cys110. One of the SH groups of dithioerythritol molecule might penetrate freely up to S^{γ} of Cys65 and the other part of the molecule should not sterically hinder this contact since the bridge is very exposed (Fig. 1). The diameters of the

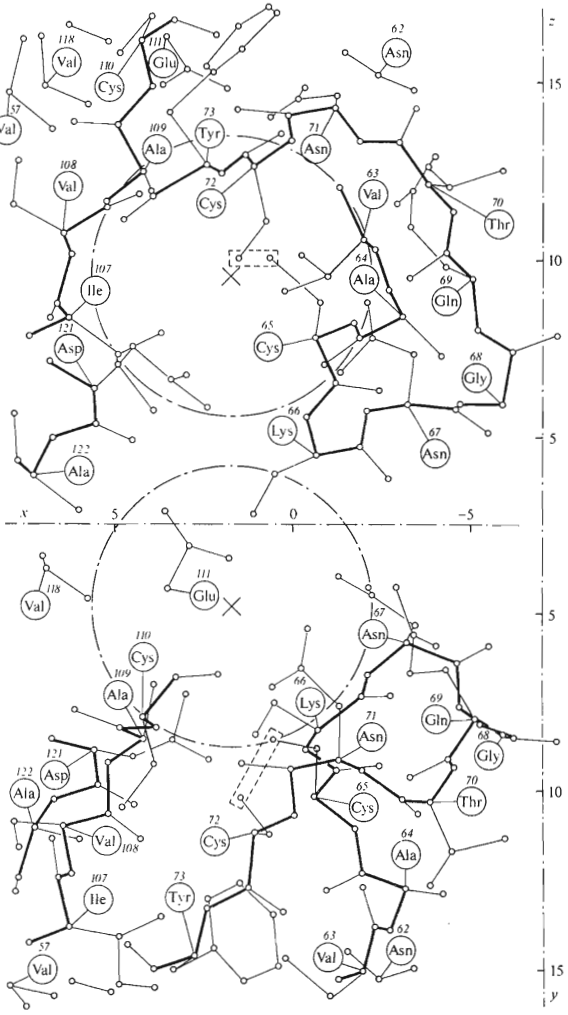

FIG. 1

The Steric Environment of Cys65-Cys72 and Examination of Its Accessibility

—·— Projections of auxiliary sphere; —— main polypeptide chain; —— side chains; [----] disulfide bridges.

end of dithioerythritol molecule and the SH group are equal and do not exceed 3·7 Å. The cleavage of this bond is probably the so-called fast step of reduction[5]. The slow is supposed to involve the conformational changes as a result of the conditions of the reaction. The diameter of phosphothionic acid is 7·0 Å and evidently the reagent is not able to penetrate up to Cys65-Cys72. Even less possible appears the reduction of Cys58-Cys110 which is in unaccessible state. The particles of Ra—Ni vary from 40 to 100 Å. Since the reaction of desulfurization is performed in heterogeneous phase, it might be expected that this process is preceded by some changes of enzyme molecule.

From the chemical data available for ribonuclease A* and the data obtained on the basis of ribonuclease S drawing, one could suppose a difference in the exposure of Cys40-Cys95 in both enzymes. This difference might be looked up as a result of the proteolytic cleavage between 20 and 21 residues of the polypeptide chain of RNase S. Tamburro and coworkers[9]: supposed that one or both disulfide bonds Cys40-Cys95 and Cys26-Cys84 are playing the role of stabilizing points for the native conformation of the enzyme. On the other hand it has been suggested[5] that Cys65-Cys72 appears unnecessary for enzymic activity as well as for the maintenance of the ribonuclease A native conformation. Evidently, the rupture of Cys40-Cys95 in RNase S will lead to decrease or loss of enzyme activity.

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* Unfortunately, we have not been able to receive any information about the list of atomic co-ordinates of RNase A.