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Structure–function relationship of trichosanthin

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Abstract

The crystal and molecular structures of two forms (i.e., C2 and $P2_12_12_1$ space group) of trichosanthin are described in this paper. There are many similarities in the crystal structures and the amino acid sequences between the RIPs. We found that five most conservative polar amino acid residues Arg122, Gln156, Glu160, Arg163 and Glu189 gather in the concave on the boundary of the large and small domains, thus forming the posible active center of trichosanthin.

It is known that higher plants contain ribosome inactivating proteins (Abbrev. as RIPs), recognized as one kind of plant toxins, which have strong inhibition on the protein synthesis of the eucaryotic intact cell and cell-free system, with particularly the inactivation of ribosome. Trichosanthin (Abbrev. as TCS) belongs to single-chain RIPs.

We have studied the crystal and molecular structure of two crystal forms of TCS. The crystal of C2 space group is crystallized under basic condition (pH ~ 8.6), while the crystal of $P2_12_12_1$ space group is got under acidic circumstance (pH ~ 5.4). The study and comparison of the structure of TCS under different pH condition, and the exploration of the similarities and differences of polypeptide chain folding are of great significance to the thorough study of the relationship of structure and function of TCS.

I. CRYSTAL STRUCTURE OF TWO CRYSTAL FORMS OF TRICHOSANTHIN (TCS)

Crystal structure of C2 space group

We have published a series of papers on crystal and molecular structure of TCS of C2 space group (refs. 1-6), in which we have discussed the polypeptide chain trace, secondary structure unit, the large and small domains, as well as the molecular structure of TCS in detail.

The cell parameters of TCS are a = 75.64, b = 75.52, c = 88.85Å, = 99.51Å Each crystallographic asymmetric unit has two protein molecules, named Mol.A and Mol.B respectively, there is no crystallographic symmetric relation between them. In each protein molecule there are 247 amino acid residues, 1914 nonhydrogen atoms.

We have accomplished the crystallographic refinement at 2.6Å resolution by using PROLSQ program, etc., and have obtained good results with R = 0.223, bond length r.m.s. = 0.023Å. The new molecular model of TCS is in good agreement with the electron density map calculated with the coefficient 2Fo-Fc.

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Crystal structure of $P2_12_12_1$ space group

The crystal of $P2_12_12_1$ space group is cultured by the hanging drop method under acidic condition (pH5.4), with KCl as the precipitating agent. Its cell parameters are a = 38.31, b = 76.22, c = 79.21Å. Each crystallographic asymmetric unit has one TCS molecule.

The molecular model used in determination of the crystal structure by molecular replacement is Mol.B in the crystal structure of TCS of C2 space group, which we have refined at 2.6Å resolution. After building the molecular model, we have completed the refinement at 2.4Å resolution by using XPLOR and PROLSQ programs etc. successively, and have got good results with R = 0.207, bond length r.m.s. = 0.017Å. The molecular model agrees well with the electron density map.

II. MOLECULAR STRUCTURE OF TRICHOSANTHIN (TCS)

Although the crystallizing conditions of the two crystal forms have great differences (from basicity to acidity), their molecular structures are extremely similar after crystallographic refinement.

Fig.1 is the superposition of α-carbon atom skeleton Mol.B of C2 space group andof $P2_12_12_1$ space group. It can be seen from the figure that the two structure are greatly similar. The average error in this superposition is 1.163Å. So it can be said that the change of pH condition does not lead to a violent change in molecular conformation of TCS.

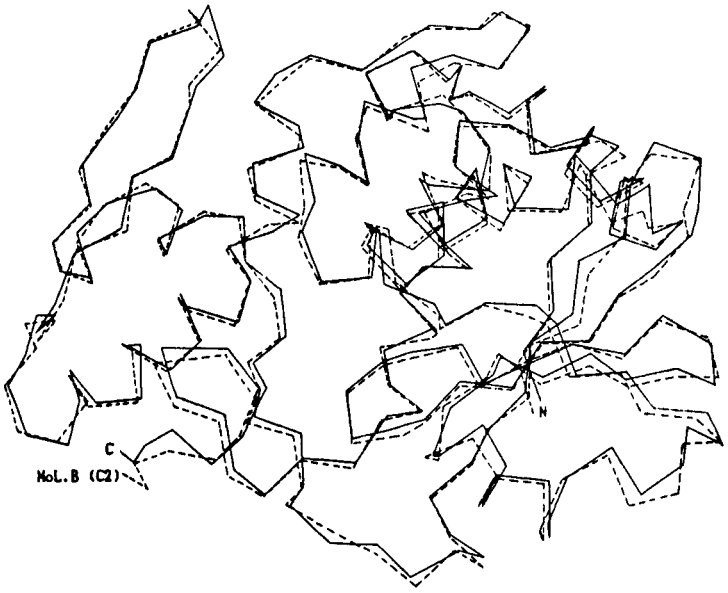

Fig. 1. The least squares superposition of α-carbon atoms in Mol.B of C2 space group and of $P2_12_12_1$ space group of TCS.

There are two molecules of TCS in the asymmetric unit of C2 space group, their crystallographic circumstances are different. Their molecular structures are very similar except some differences in the orientation of some extended polypeptide chain regions and some side chains. We carried out an equivalent superposition of these two molecules which have no symmetric relation between them (there are 1914 non-hydrogen atoms in each molecule) by the Homolog program. The error in superposition is small, the average error is 1.247Å. This indicates that the molecular structures of these two molecules of TCS are also greatly similar despite their differences in crystallographic circumstances.

Therefore, for the sake of convenience, we shall discuss only one of these two molecules (namely Mol.B of C2 space group) in the following discussion of structure.

Fig.2 illustrates the trace of the polypeptide chain of TCS. The protein molecule is composed of eight segments of α-helices and four β sheets which composed of thirteen β strands. These α-helices comparatively concentrate in the center of the molecule whereas β sheets distribute on its surface. This is an outstanding feature of the structure of TCS.

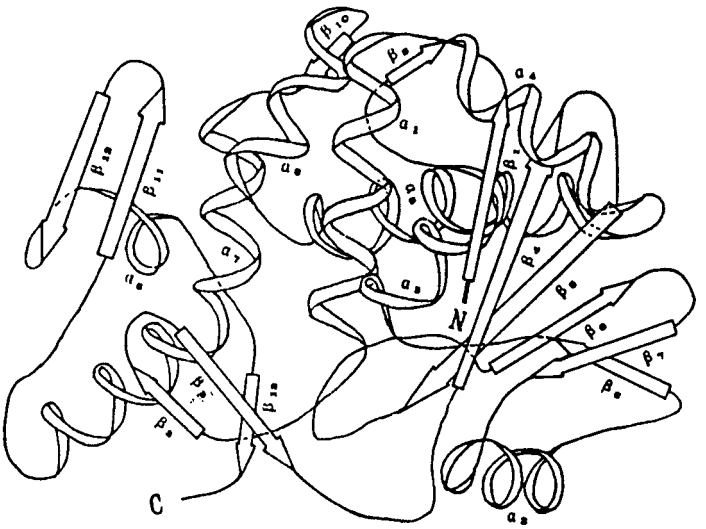

Fig. 2. The trace of polypeptide chain of TCS.

It can be seen from the figure that there are two domains, the large one begins from N-terminal to Pro181, including one big β sheets and two small β sheets as well as 6 segments of α-helices (Note: ordinal numbers are $\beta 1$, $\beta 2$, ..., $\beta 13$; $\alpha 1$, $\alpha 2$, ..., $\alpha 8$ respectively); the small domain starts from Ser182 C-terminal, including two segments of α-helices, in which $\alpha 7$ is a helix with turning, and one β sheet. There are also parallel β sheet composed of $\beta 3$ and $\beta 13$ between the large and the small domains. They are the only secondary structural connection between these two domains.

The secondary structure in the molecular structure of TCS is relatively abundant. There are 104 amino acid residues in eight segments of α-helices, accounting for 42.1% of all amino acid residues; there are 65 amino acid residues on four β sheets composed of thirteen β strands, taking 26.3%. These two types of secondary structural units have 169 amino acid residues altogether, accounting for 68.4% of the whole molecule.

The β strand ($\beta 1$) is formed starting from the N terminal of TCS. It consists of a big β sheet together with $\beta 4$, $\beta 5$, $\beta 6$, $\beta 7$, $\beta 8$ strands, where $\beta 1$ and $\beta 4$, $\beta 7$ and $\beta 8$ are parallel while $\beta 4$, $\beta 5$, $\beta 6$ and $\beta 7$ in the middle are anti-parallel with each other respectively. It is interesting to note that a cleft is formed at the entrance of $\beta 5$ and the exit of $\beta 6$, and there is no hydrogen bond between them. Therefore a typical left hand twist of sheet is resulted. $\beta 2$ and $\beta 3$ are anti-parallel while $\beta 3$ and $\beta 13$ are parallel, they form a small β sheet which extends across the large and the small domains. $\beta 11$ and $\beta 12$ form an antiparallel β sheet in the small domain, it has two pairs of hydrogen bonds, and it is not a hairpin structure but a small loop at the turn. $\beta 9$ and $\beta 10$ is a small β sheet connected only by a pair of hydrogen bonds between Leu127 and Phe179. Most of these β sheets distribute on the molecular surface.

The eight segments of α-helices lie in different circumstances in the molecule. The first six segments lie in the large domain, while the last two segments are in the small one. On the boundary of the two domains, there is mainly an interconnection between $\alpha 5$, $\alpha 6$, $\alpha 7$ helices and their segments of extended polypeptide chains. $\alpha 4$, $\alpha 5$ and $\alpha 6$ are three successive segments of α-helix. $\alpha 5$-helix lies in the center of the molecule, almost deeply embedded in the interior of the molecule. This helix starts from Gln156 and forms an irregular helix. The O atom of Gln156 does not form a hydrogen bond with the N atom of Glu160. The helix begins to bend outward, thus making Gln156 locate at the

bottom of the concave formed by the large and the small domains' boundary and expose on the surface of the molecule. Meanwhile, Glu160 and Arg163 residues also locate on the surface of this concave. α7-helix starts from Ser182 with its turning at Ser191. The upper half and the lower half part of the helix form a turning of about 60°. The Ser191 at the turning point and the upper and lower segments of the helix are connected by hydrogen bonds. The N atom of Ser191 and O atom of Ser187 form a hydrogen bond. The O atom of Ser191 and N atom of Ala194, however, form the lower half segments of α-helix starting from 3_{10} helix. Therefore, although this helix has a big angle turn, the upper and lower segments of the helix can still be regarded as one α-helix with a turning, rather than two α-helix segments. This kind of α-helix with angle turn is conspicuous in the molecular structure of TCS.

There are two concentrated hydrophobic areas in TCS, each in the large and the small domains. In the large domain, a big hydrophobic area is enclosed by the boundary of α1, α4, α5 and the big β sheet. The hydrophobic area in the small domain is formed in the boundary of α7-helix, β11 and β12 antiparallel β sheet and some segments of extended polypeptide chain. The interaction of the hydrophobic residues facilitates the folding of the polypeptide chain of the protein and the maintaining of the stability of protein structure as well as the forming of the large and the small domains of the molecule.

III. STRUCTURE OF THE ACTIVE CENTER OF THE MOLECULE

Both TCS and ricin A-chain belong to RIPs with similar amino acid sequences and many common conservative residues (see Table 1). Their crystal structure also show many similarities. This can be seen from the comparison between the crystal structures of TCS and ricin A-chain (ref. 7).

There are many similarities in amino acid sequences between the single-chain RIPs and the A-chain of double-chain RIPs. It is very important to compare the amino acid sequences between the homologous proteins so as to find out which amino acid residues are important in the relationship of the structure and function in TCS.

The amino acid sequences of RIPs from ten different kinds of plants already published in literature are listed in sequences order and comprehensively compared in Table 1. Among these plant toxins, it is essential to know which are the most conservative residues in the evolution of plants, which is certainly very important in the study on the relationship of structure and function of all these proteins. Then, based on the molecular structure of TCS, we can go further into investigation on the relationship of its structure and function by analyzing and comparing the locations and surrounding environments of these most conservative residues in the molecular structure.

Among these plant toxins, only ricin and abrin belong to the A-chain of double-chain RIPs, the other are single-chain RIPs. Each of them comes from different kinds of plant: (1) Trichosanthin (Abbrev. tcs) (ref. 8), (2) α-Momorchain (Abbrev. mmc) (ref. 9). (3)-Luffin(Abbrev. lufa) (ref. 10). (4) β-Luffin(Abbrev. lufb) (ref. 11). (5) Ricin A chain (Abbrev. ric) (ref. 12). (6) *Ricinus communis* agglutinin (Abbrev. rca) (ref. 13). (7) Abrin A chain (Abbrev. abr)[14]. (8) Pokeweed Antiviral Protein (Abbrev. pap) (ref. 15). (9) Mirabili Antiviral Protein (Abbrev. map) (ref. 16). (10) Saporin-6 (Abbrev. so6) (ref. 17).

It can be seen from Tab.1 that in the amino acid sequences of these ten kinds of RIPs, there exist some most conservative residues, which remain unchanged in their biological evolution (marked by "*" in Tab.1) either in A chain of double-chain RIPs or in single-chain RIPs. There are seventeen altogether: Tyr14, Phe17, Arg22, Tyr70, Tyr111, Arg122, Gly128, Leu132, Ala148, Gln156, Glu160, Arg163, Lle167, Glu189, Trp192, Val232 and Leu241.

Among these seventeen most conservative residues, there are three kinds of residues: the polar residues with electric charges, Arg22, Arg122, Glu160, Arg163 and Glu189; the polar residues without electric charges, Tyr14, Tyr70, Tyr111 and Gln156; and the nonpolar residues, Phe17, Gly128, Leu132, Ala148, Ile167, Trp192, Val232 and Leu241. The roles they play in the relationship of structure and function are different.

Tab.1. Amino acid sequences of ten kinds of RIPs

	1 10 20 30 40 50 60 70 80
tcs	DVSFRLSGATSSSYGVFISNLRKALPNER.KLYDIPLLRSSLPGSQ..RYALIHLTNYADETISVAIDVTNVYIMGYRAGDTS.....YFF...NE
mmc	DVSFRLSGADPRSYGMFIKDLRNALPFRE.KVYNIPLLLPSVSGAG..RYLLMHLFNYDGKTITVAVDVTNVYIMGYLADTTS.....YFF...NE
lufa	DVRFSLSGSSSTSYSKFIGDLRKALPSNG.TVYNLTILLSSASGA...RYTLMTLSNYDGKAITVAVDVSQLYIMGYLVNSTS.....YFF...NE
lufb	ANVSFSLSGADSKSYSKFITALRKALPSKE.KVSNIPLLLPSASGAS..RYILMQLSNYDAKAITMAIDVTNVYIMGYLVNSTS.....YFA...NE
ric	IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVGLPINQRFILVELQNHAELSVTLALSVTNAYVVGYRAG.NS....AYFFHPDNQ
rca	IFPKQYPIINFTTAGATVESYTNFIRAVRSHLTTGADVRHEIPVLPNRVGAPISQRFILVELQNHAELSVTLALSVTNAYVVGCRAG.NS....AYFFHPDNQ
abr	EDRPI.KFSTEGATSQSYKQFIEALRERLRGGL..IHDIPVLPDPTTLQERNRYITVELSNSDTESIEVGIDVTNAYVVAYRAGTQS.....YFLR.DAP
pap	INTITFDAGNATINKYATFMESLRNEAKDPSLKGYGIPMLPNTNS.TI..KYLLVKLQGAL.KTITLMLRRNNLYVMGYSDPYDN.KCR.YHIFADIK
map	APTLETIASLDLNNPTTYLSFITNIRTKV....ADKTEQCTI.QKISKTFTQRYSYIDLIVSSTQKITLAIDMADLYVLGYSDIANN.KGRAFFFKDVTE
so6	VTSITLDLVNPTAGQYSSFVDKIRNNVKDPNLK.YGGTDIAV.IGPPSKEKFLRIN.FQSSRGTVSLGLKRDNLYVVAYLAMDNTNVNRAYYFRSEIT
	* * * *
	90 100 110 120 130 140 150 160 170
tcs	ASA.TE.AAKYVF.KDAMRKVTLPYSGNYERLQTAAGKI.....RENIPLGLPALDSAITTLFYYNANSA......ASALMVLIQSTSEAARYKFIEQQIGKR
mmc	PAA..ELASQYVF.RDARRKITLPYSGNYERLQIAAGKP.....REKIPIGLPALDSAISTLLHYDSTAA......AGALLVLIQTTAEAARFKYIEQQIQER
lufa	SDA..KLASQYVF.K.GSTIVTLPYSGNYEKLQTAAGKI.....REKIPLGFPALDSALTTIFHYDSTAA......AAAFLVILQTTAEASRFKYIEGQIIER
lufb	SDA..KLASQYVF.K.GSTLVTIPYSGNYERLQNAAGKI.....RΓKIPLGFRALDSALTSIFHYDSTAA......AAAFLVILQTTAEASRFKYIEGQIIER
ric	EDA..E.AITHLF.TDVQNRYTFAFGGNYDRLEQLAGNL.....RENIELGNGPLEEAISALYYY.STGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTR
rca	EDA..E.AITHLF.TDQVNSFTFAFGGNYDRLEQL.GGL.....RENIELGTGPLEEAISALYYY.STCGTQIPTLARSFMVCIQMISEAARFQYIEGFMRTR
abr	SSA.SD....YLF.TGTDQHSLPFY.GTYGDLERWAHQS.....RQQIPLGLQALTHGIS..FFR.SGGNDNEEK.ARTLIVIIQMVAEAARFRYISNRVRVS
pap	GTEYSDVENTLCPSSNPRVAKPINYNGLYPTLEKKAGVT....SRNEVQLGIQILSSDIGKISGQ.SFTE..KIE.AKFLLVAIQMVSEAARFKYIENQVKTN
map	AVA..N..N.FFPGATGTNRIKLTFTGSYGDLEKN.GGL.....RKDNPLGIFRLENSIVNIYGK.AG..DVKKQ.AKFFLLAIQMVSEAARFKYISDKIPSE
so6	.SA..ELTA.LFPEATTANQKALEYTEDYQSIEKNAQITQSDKSRKELGLGIDLLLTFMEAVNKK.ARV.V.KNE.ARFLLIAIQMTAEVARFRYIQN.LVTK
	* * * * * * * * *
	180 190 200 210 220 230 240 247
tcs	V...DKTFL.PSLAIISLE.NS.WSALSKQIQIASTNNGQFESPVVLINAQNQRVTITNVDAGVVTSNIA..LLLNRN.NMA
mmc	A...YRDEV.PSLATISLE.NS.WSGLSKQIQLAQGNNGIFRTPIVLVDNKGNRVQITNVTSKVVTSNIQ..LLLNTR.NIAEGDVSTTHGFSSY
lufa	I...SKNQV.PSLATISLE.NSLWSALSKQIQLAQTNNGTFKTPVVITDDKGQRVEITNVTSKVVTKNIQ..LLLNYKQNVA
lufb	I...PKNEV.PSPAALSLE.NSLWSLLSKQIQLAQTNNGAFRTPVVIIDNKGQRVEITNLASKVQIKDVNSKLLLN.KQNIA
ric	IRYNRRSAPDPS.V.ITLE.NS.WGRLSTAIQ..ESNQGAFASPIQL..QR.DRNGSKFSVYDV..S.....ILLPIIALMVYRCAPPPSSQF
rca	IRYNRRSAPDPS.V.ITLE.NS.WGRLSTAIQ..ESNQGAFASPIQL..QR.DRNGSKFSVYDV..S.....ILLPIIALMVYACAPPPSSQF
abr	IQTGTAFQPDAA.M.ISLENN..WDNL.RGVQ..ESVQDTFPNQVTL..TNI.RNE..PVI..V.DSLSHPTVVLALMLFVCN...PPN
pap	F..NRDFSPNDK.V.LDLEEN..WGKISTAIH..NSKNGALPKPLEL...KNADGT.KWIVLRV.DEIKPDVGLLNYVNGTCQAT
map	.KY.EEVTVDEY.MT.ALENN..WAKLSTAVY..NSKPSTTTATKCQLATSPVTISP.WIFKTV.EEIKLVMGLLKSS
so6	NFPN.KFDSDNK.V.IQFEV.S.WRKISTAIY.GDAKNGVFNKDYDFGFGK.VR........QV..KD.LQMGLLMYLGKPK
	* * * *

Abbr: tcs--Trichosanthin, mmc--Momorcharin, lufa--α-Luffin, lufb--β-Luffin, ric--Ricin A chain, rca--Ricinus communis agglutinin, abr--Abrin A chain, pap--Pokeweed antiviral protein, map--Mirabili antiviral protein, so6--Saporin-6.

Based on the summarization of the regularity of the amino acid sequences of ten kinds of RIPs, together with the crystal and molecular structure of TCS, seventeen most conservative amino acid residues are surveyed and analyzed. We found that five most conservative polar amino acid residues Arg122, Gln156, Glu160, Arg163 and Glu189 gather in the concave on the boundary of the large and the small domains, and located on the molecular surface. They are supported by the actions of the resulting ion pair, hydrogen bonds and Van der waals' force, thus forming the possible active center of TCS, which plays a key role in catalysis, as shown in Fig.3. Meanwhile, Tyr14, Arg22, Tyr70, Tyr111 and Trp192 lie around this active center. Some hydrophobic residues Phe17, Gly128, Ala148, Trp192 and Leu241 take part in the formation of hydrophobic areas in the large and the small domains, support the folding of polypeptide chain of protein and form the above-mentioned active center structure.

On the surface of the concave on the boundary of the large and small domains, there is mainly the neighboring contact region of C-terminal of α7-helix. The C-terminal of 5-helix bends a little outward, leading the terminal of this α-helix located almost in the center of the molecule to expose on the molecular surface. Gln156 and Glu160 of this helix as well as Arg163 located on the extended segment of polypeptide chain rather than in α5-helix, all lie on the surface of the concave. The Glu189 of α7-helix in the small domain also lies on the surface of this concave. These five polar residues come into contact with each other, as shown in Fig.3. In this region, Arg122 and Glu189, Arg163 and Glu160 form an ion pair; OE atom of Glu189 and NE2 atom of Gln156 form a hydrogen bond. Therefore, the active center region where these polar residues interact with each other is formed.

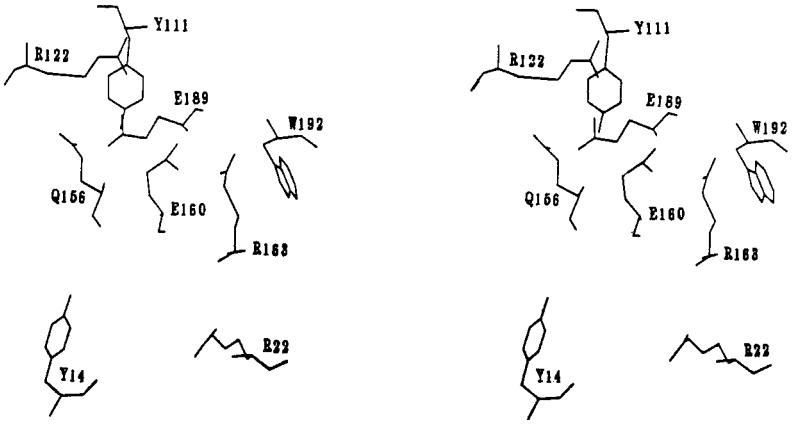

Fig.3. The stereoscopic view of the active center structure in TCS

Two most conservation residues in α1-helix Tyr14 and Arg22 do not lie within the active center region. The side chain of Tyr14 is deeply embedded in the protein molecule. Its OH group and O atom of Ser157 form a hydrogen bond, NH_2 atom of Arg22 and O atom of Ala162 also form a hydrogen bond. These help stabilize the outward bend of the α5-helix crooked from Gln156. In the meantime, Arg22 forms an ion pair with the Glu168 near this active center, and this is advantageous to the stabilization of this active center. The most conservative residue Trp192 in α7-helix lies at the bottom of the concave rather than on the molecular surface. Its side chain is parallel to that of Arg163, and its C atoms come into contact in the Van der Waal's radii region, thus acting an important role in the relative stabilization of Arg163 in the action center region. Another side of the side chain of Trp192 is parallel to and comes into contact with the side chain of another most conservative residue Leu241, making a hydrophobic core of the small domain together. The OH groups of Tyr70 and Tyr111 form hydrogen bonds with N atoms of Gln156 and Arg163 respectively. So it can be regarded that these two residues Tyr70 and Tyr111 play a role in the support of the relative stabilization of active center structure as well.

The back of the concave in which this active center region lies joins together with the hydrophobic area in the large and the small domains. The small domain connect with the hydrophobic area from Trp192 and Leu241, while the large one, from Tyr14 and Tyr70.

IV. DISCUSSIONS

In the crystal structure of TCS of C2 space group, the asymmetric unit consists of two molecules, there is no symmetric relation between them. The molecular structure of these two molecules are very similar although their crystallographic circumstances are different. It can be seen that, despite the differences of crystallographic circumstances, the active centers of biological function of these two molecules both locate on its own molecular surface but not on the boundary of the two molecules consequently, these two molecules have the same molecular structure and have the same biological functions. In acidic circumstance (pH $\sim$5), the conformation of diphtherin (ref. 18)

changes violently and forms 24Å diameter pores, possibly by aggregation into dimeric structure. Therefore, the pore formation is a feature of diphtherin-membrane interaction. However, the increasing of pH decreases the binding of diphtherin with cell membrane and hence indirectly decreases its penetration. The crystallizing conditions of TCS of two different crystal forms change from basicity to acidity and does not lead to any considerable changes in molecular conformation. It is thus clear that, TCS has a rather wide adapting range in playing its biological function in the cell. This also shows that the action mechanism of TCS getting into cytoplasm is not the same as that of diphtherin.

The action mechanism of ricin has been studied by Endo (ref. 19) Ricin A chain catalyzes the cleavage of the N-glycosidic bond of a specific adenine nucleoside residue at position 4324 in 28S rRNA, and produce a hemiacetal group. There is a balance between the hemiacetal conformation and the aldehyde conformation of ribose, and the ß elimination reaction takes place under the action of aniline, releasing a section of about 500 nucleotides' length of 3'terminal. This section is discovered by gel electrophoresis. On this basis, the action point of ricin A chain is determined, and it is proved that ricin A chain is a kind of RIPs of RNA N-glycosidase type. Zhang Jinsong (ref. 20), etc., have studied the action mechanism of TCS and have proved that the action mechanism of TCS and that of the ricin A chain are identical, they both act on the N-glycosidic bond of adenosine at position 4324 of 28S rRNA. Ricin is a kind of double-chain RIPs. Its B chain combines with the galactose on the surface of intact cell, leading A chain to get into the inside of cyton and reach the cytoplasm, attack the ribosome and thus have the above-mentioned RNA N- glycosidase hydrolysis mechanism. TCS belongs to single chain RIPs. Since it lacks B chain, it is difficult for it to transport over the intact cell and combine with ribosome and so have a low activity on the intact cells. In cell free systems, however, TCS has a strong effect on ribosome, thus the synthesis of protein is inhibited, providing that it has the same action mechanism.

Arginine residues are very important to active positions of many enzymes. Its side chain with positive electric charges makes it convenient to distinguish the substrate with negative electric chains. The positive charge of arginine may act to the backbone of phosphate of RNA in the form of ion pair (ref. 21).

Shiga-like toxin I (ref. 22) has the same depurination mechanism with Ricin. Glutamic acid residues play a critical role in the active positions of shiga-like toxin.

There are two ion pairs formed by Arg and Glu in the active center position of TCS. When they combine with the substrate, two Arg residues may combine with the substrate with negative electric charges, and connect with phosphate skeleton of RNA in the form of ion pair. While the two Glu residues play an important role on the hydrolysing of N-glycoside bond.

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