molecule. Furthermore, asp 32 appears to be in close range for interaction with some parts of the pepstatin molecule. Details regarding the structural features involved in enzyme-pepstatin interactions are currently under investigation by X-ray crystallographic techniques at NIH.

Similar structures for all acid proteases

What conclusions can one draw from the above, regarding the molecular structure of pepsin itself? Physico-chemical properties shared with the fungal acid proteases all point to the conclusion that pepsin also should have a molecular structure very similar to that described above. It is noteworthy that in the electron density map for the *Rhizopus* enzyme, most of the aromatic side chains were located using the amino acid sequence for pepsin [11] and by assuming that the number and location of these residues were conserved for most acid proteases!

Currently available data support the generalization that all acid proteases, irrespective of their source, have essentially similar three-dimensional structures and any differences in their substrate specificity or rate of catalysis might be explained on the basis of the following structural considerations: (a) the hair-pin loop, constituting a 'flap' for the hydrophobic specificity pocket, could move in or out, thus altering the extent of secondary interactions with substrate molecules; (b) the two lobes could move in or out relative to each other, and thus alter the nature and extent of the secondary interactions with substrate molecules; (c) some of the residues lining up the cleft region may be altered from one member of the class to another, without altering the catalytic mechanism.

Tentative proposals have been made by James et al. [23], concerning the mechanism of action, for the acid protease from *Penicillium janthinellum*. Space does not permit a detailed discussion of these proposals. Moreover, it would be premature to review the catalytic mechanism of the acid proteases in general, until amino acid sequence data are available for the other two fungal enzymes. We must await also the structure determination of pepsin for which sequence is already known in order to verify the general validity of mechanistic proposals.

Acknowledgement

I wish to thank Dr. David R. Davies for helpful discussions.

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Structure of simple viruses: specificity and flexibility in protein assemblies

Stephen C. Harrison

Recent X-ray diffraction studies have shown how flexibility can be built into protein subunits or assemblies without losing specificity of subunit interactions.

Protein subunits in the shells of simple viruses are arranged according to a few basic principles [1,2]. Indeed, these principles govern the formation of all protein assemblies. The first is specificity: subunits must 'recognize' each other in quite definite ways, forming an exact interface of noncovalent interactions – just as they must fold in a definite way, forming an exact tertiary structure. Otherwise, the sequence of amino acids in a protein would not determine a precise assembly. The second principle is economy: large structures are composed of many copies of a few kinds of subunit. In the case of small viruses, economy is enforced by the size of their nucleic acid, sufficient to encode only one or a few structural proteins [1]. These principles together imply symmetry: specific, repeated bonding patterns for identical building blocks lead to a symmetrical final structure. The simplest viruses thus exhibit either helical symmetry, for rod-like structures, or icosahedral symmetry, for spherical structures.

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The demands of economy and specificity conflict when it becomes impossible to build a reasonable structure of sufficient size with identically bonded elements. For example, a very long helical structure will probably bend or supercoil in order to achieve some degree of hydro-dynamic compactness, perturbing the exact similarity of all intersubunit contacts. In the case of the spherical viruses, departure from precise identity of subunit packing must occur whenever more than sixty subunits are required, for there is no symmetry describing a compact, essentially isodimensional structure that can accommodate more than sixty strictly-related protein molecules. This highest possible symmetry, of order sixty, is that of the icosahedron, and it indeed describes all the simple spherical viruses. Yet a large number of these are built from *multiples* of sixty chemically-identical proteins. Thus, the elaboration of large, self-assembling structures – even quite *rigid* structures – re-

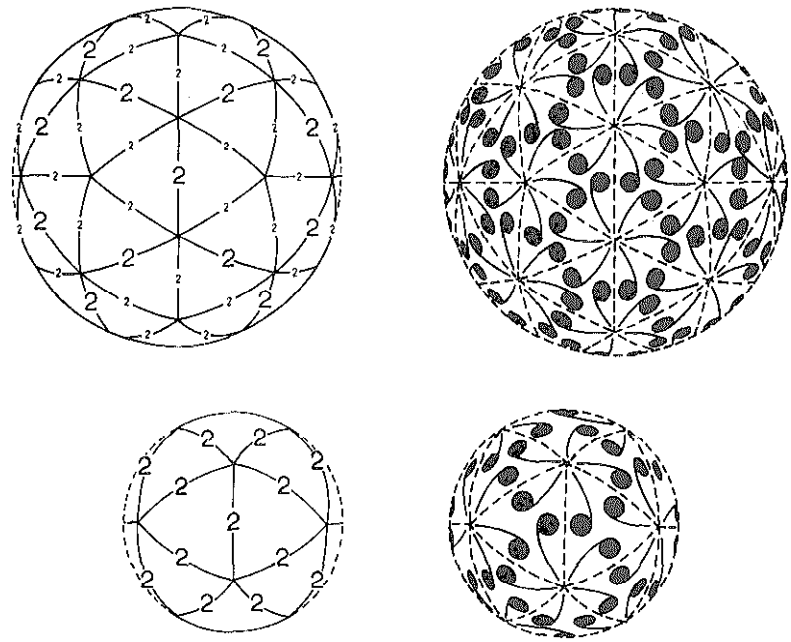

Fig. 1. Icosahedral surface lattices, showing the packing of 60 strictly equivalent units (bottom) and 180 quasi-equivalent units (top). The figures on the left show two-fold symmetry axes – large numbers indicate strict dyads and small numbers indicate local ('quasi') dyads. Strict axes describe the entire structure; local axes relate only adjacent subunits, in a nearly precise (but still approximate) way.

quires a certain flexibility in the subunits; these proteins must be capable either of alternative modes of bonding to their neighbors (i.e., different sets of amino-acid side-chain contacts) or of some degree of internal bending. A major goal of structural studies of spherical viruses is to see how their coat proteins have evolved to incorporate the requisite flexibility without losing the specificity necessary for accurate folding and self-assembly.

Quasi-equivalence

The structure of tomato bushy stunt virus (TBSV), recently determined at 5.5 Å resolution [3], shows one very clear solution to the specificity/flexibility problem. In order to describe this result, it is necessary to discuss the geometry of icosahedral structures in slightly greater detail.

As stated above, icosahedral symmetry implies exactly sixty equivalent locations – for each piece of the structure, there must be fifty-nine others in identical, symmetry-related environments. The structure shown in Fig. 1 (bottom) contains sixty comma-shaped subunits, icosahedrally related. Each comma makes three sorts of nearest-neighbor contacts: head-to-head across a two-fold axis of symmetry, head-to-tail about a three-fold axis, and tail-to-tail about a five-fold axis. The structure in Fig. 1 (top) contains 180 units, and at first sight the contacts appear identical to those in Fig. 1 (bottom). But notice that some of the tail-to-tail contacts are in rings of five, while others are in rings of six. In a real, three-dimensional protein assembly, this would imply some sort of bending of the subunits or some small alteration of the non-covalent bonds between them. The basic similarity of the contacts is preserved, and hence the fundamental biochemical specificity maintained, but distortions (e.g., 'squeezing' six tails instead of five around a particular axis) must be introduced to accommodate more than sixty subunits.

Since the structure has icosahedral symmetry, the subunits must appear strictly equivalent in sets of sixty – for example, members of a given set of sixty must be flexed in exactly the same way. Members of different sets are only 'quasi-equivalent' to each other, however, they are flexed or bonded slightly differently.

Caspar and Klug [2], who introduced the idea of quasi-equivalence, showed that certain (but not all) multiples of sixty subunits could be accommodated in such designs, the simplest example, containing 3×60 subunits, being the one just described. The fact that many, very different, spherical viruses conform to one or another of these designs confirms the basic validity of their idea: specificity of subunit bonding is necessary for self assembly, and a limited flexibility, either of constitutent subunits or of their contacts, is sufficient to take care of situations where some variability of packing is required.

Tomato bushy stunt virus

The TBSV particles contain 180 protein subunits of M.W. 40000, and one molecule of single-stranded RNA (4800 bases) [4]. It thus conforms to the simplest quasi-equivalent design, illustrated in Fig. 1 (top). Structural studies of TBSV have a long history, since Bernal and Frankuchen performed the earliest X-ray diffraction studies in 1938, only a year or two after the first crystallization [5,6]. The most recent stage, calculation and interpretation of an electron density map at 5.5 Å [3], has given an answer to the question of how its protein subunit achieves flexibility for quasi-equivalent packing. In brief, the subunit folds into two essentially rigid domains, connected by a short, flexible hinge. Two different hinge angles, differing by about 20°, are found in the virus particle; 60 subunits have one configuration and 120, the other. This internal bend permits 180 subunits to pack in such a way that most of the inter-subunit contacts remain invariant. The 'strain' for quasi-equivalent bonding is concentrated in the protein hinge region, involving inter-domain contacts within a single subunit.

A more detailed description of the structure is necessary to make these conclusions clearer. Fig. 2a shows the general shape of the protein subunit of TBSV; the two domains appear to connect through a single, short length of polypeptide chain and to make rather limited contact at their interface. This suggests that the energy needed to change the relative orientation of domains 1 and 2 might not be very great.

The packing of subunits in the TBSV particle is shown in Fig. 2b. To recognize the requirement for a hinge, notice that domains 2 are bonded in sets of three and that these groups form two kinds of contacts with adjacent groups of three: at positions such as q2, where there is a dihedral angle between groups of about 40°, and at positions such as s2, where the dihedral angle is about 0°. If domain 1 always had a fixed orientation with respect to domain 2, the contacts between domains 1 would have to be very different at q2 and at s2 (Fig. 2c): for example, an orientation designed to give an extensive interface for non-covalent bonding at q2 would cause the domains to miss each other entirely at s2. By hinging the subunit between domains, it is possible to conserve the *inter*subunit domain 1 – domain 1 bonding, at the expense of some *intra*subunit do-

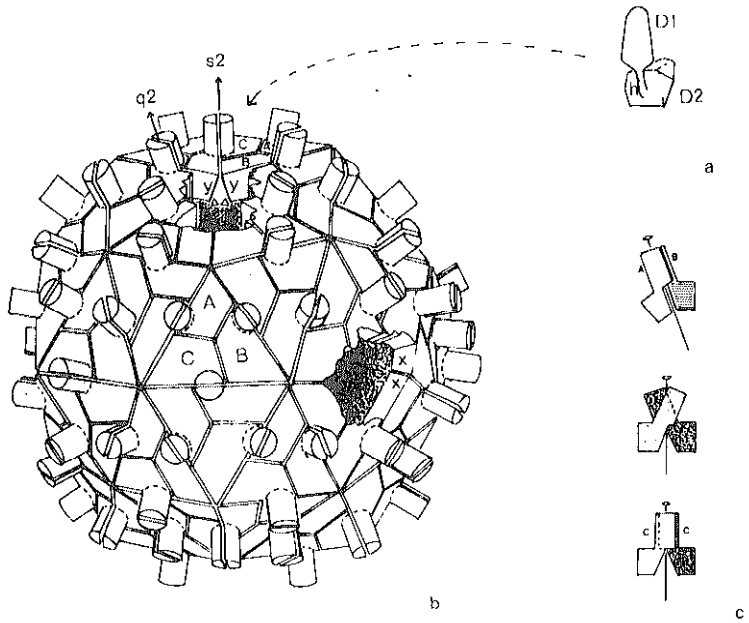

Fig. 2. (a) Schematic drawing of the TBSV protein subunit, showing its two-domain structure. The subunit would pack into a viral shell as indicated by the arrow. D1, D2: domains 1 and 2; h, inter-domain hinge. (b) Packing of subunits in the TBSV particle. Domain 1 is shown by a projecting half-cylinder and domain 2 by a block-like base. Domains 2 form tightly-bonded three-fold groups, 'clamped' together by the pairwise-clustered domains 1. The subunits in one such group are lettered A,B,C. The letters s2 and q2 indicate strict- and quasi-two-fold positions; at x, close contact between domain 2 surfaces leads to strong curvature; at y, a cleft is opened between adjacent domains 2, with a bit of the polypeptide chain (apparently disordered at x) bonded to the sites thus exposed. (c) Drawings showing how a subunit with flexibly connected domains can adapt to positions of different surface curvature while conserving inter-subunit bonding. The top diagram shows subunit contacts at positions such as q2 ('sharp' dihedral angle); flattening the surface (as at s2) without hinging the subunit would create a poor contact as in the middle diagram; change in position of the inter-domain hinge leaves contacts between domains 1 undisturbed by the flattening (bottom diagram).

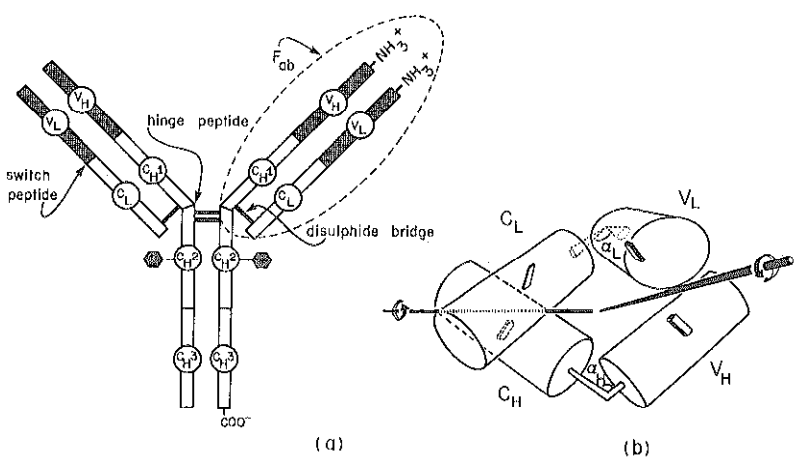

Fig. 3. (a) Schematic diagram of the immunoglobulin (IgG) molecule. V_L-variable half of light chain; C_L-constant half of light chain; C_{H1}, C_{H2}, C_{H3} - the three constant domains of the heavy chain; Fab-antigen binding fragment, released by papain cleavage at the hinge peptide. (Drawing adapted from ref. 9.) (b) Schematic diagram of the packing of domains in Fab and in the Mcg Bence-Jones dimer. Each cylinder represents an approximately 100-residue β-barrel, with two such domains per chain. The upper chain (a light chain in both Fab and in the Bence-Jones protein) has a rather acute angle, α_L, between its variable and constant regions. The lower chain (V_H, C_{H1} in Fab, but another V_L, C_L in the Bence-Jones dimer) has a more obtuse angle, α_H, between its two domains. Thus, in the McG Bence-Jones dimer, the second light chain adopts a heavy-chain-like bend. (Drawing adapted from ref. 10.)

main 1 – domain 2 strain. We have said already that the domain 1-domain 2 interface is rather tenuous, as if designed for bending. By contrast, the interface between domains 1 is an extended region of close contact.

In addition to bending the hinge within a subunit, one other perturbation or non-equivalence is necessary to generate the observed packing. If surfaces of domains 2 are tightly apposed at positions such as x (Fig. 2b), then at y, corresponding surfaces must move apart in order to create the much flatter dihedral angle at s2. This leaves a 'cleft' between domains 2 at y. The map shows ordered density in this cleft that appears to force open the subunit contacts, thereby increasing the overall radius of curvature.

(Note: A 2.9 Å resolution electron density map of TBSV just obtained suggests that this feature represents a portion of the polypeptide chain that is ordered in one of the three quasi-equivalent positions – C in Fig. 2b – and disordered in the other two.)

The formation of a structure of correct size thus requires opening up of one set of domain 2 contacts as well as a change in the hinge angle, as shown in Fig. 2c. Were the contact at y not so opened, the overall curvature would be sharper: the protein would form a smaller, spherical structure with just 60 subunits, all exactly equivalent in packing and hinge angle (cf. Fig. 1a). Such 60-subunit particles have indeed been observed in studies of reassembly of the closely-related turnip crinkle virus [7].

The packing diagram in Fig. 2b shows that the structural roles of domains 1 and 2 are quite distinct. We can imagine the protective shell for viral nucleic acid to be constructed of triangular modules, the trimer clusters of domains 2. Because of the cleft at positions such as y, this shell must be 'clamped' together by the pairwise-bonded domains 1. In addition, each domain 2 may have binding sites for RNA on its inward-facing surface.

Domains and hinges

The TBSV subunit is not the only example of a protein with domains of fixed conformation connected by a flexible hinge. The immunoglobulins and their fragments are the classic examples: IgG light chains have two similarly folded domains (V_L, C_L) and heavy chains have four (V_H, C_{H1}, C_{H2}, C_{H3}) (Fig. 3a). The structure of these domains has been called a β-barrel, since the polypeptide chain forms a flat barrel of 7 to 9 stretches of β-pleated sheet [8]. Contacts between chains are very strong, and the domains are tightly paired: V_L with V_H, C_L with C_{H1}, and C_{H3} with a second C_{H3} across the molecular dyad [9]. The so-called 'switch' and 'hinge'

regions between domains along a chain are apparently flexible, as shown by a series of comparative crystallographic studies described in a recent TIBS by Huber [9].

The Meg Bence-Jones protein, a dimer of identical light chains studied by Schiffer et al. [10], is a particularly remarkable illustration of this flexibility: instead of associating into a symmetrical dimer, the chains adopt different conformations with respect to bending at the switch peptide (Fig. 3b). The dimer in fact looks like the immunoglobulin fragment Fab (a light chain plus the V_{H1}–C_{H1} portion of a heavy chain). One light chain in the Bence-Jones dimer has the normal Fab light-chain configuration, the other 'masquerades' as the V_H and C_{H1} domains of a heavy chain. In other words, it is important to conserve the V:V and C:C pairing interactions, even at the expense of distorting contacts between V and C domains in the 'heavy-chain-like' subunit. The β-barrel fold appears to be especially suited for such pairing interactions, since each domain presents an extended, sheet-like surface to the other. The strong interactions between apposed domains resist the stress generated by changes in hinge configuration. Domain 1 of the TBSV subunit has a β-barrel fold, probably quite similar to that of the immunoglobulin domains, and its structural role is likewise to pair tightly with a homologous domain of another subunit (Fig. 2).

Tobacco mosaic virus

The hinged subunit of TBSV illustrates one way of achieving a multi-state system: most inter-subunit contacts are conserved, and shifts occur at domain boundaries within a single protein. The assembly of tobacco mosaic virus (TMV) provides an excellent example of another mode of switching: changes are achieved by shifting contacts between relatively rigid subunits. There are two important structures to consider: the 2-layer, 34-subunit disk aggregate of TMV protein, an intermediate in the formation of virus particles, and the helical assembly found in the virus particles themselves (Fig. 4; ref. 11–13).

These two structures represent distinct states of assembly of the TMV subunit (MW ~ 17000). They have both been studied by X-ray diffraction – the disk by single-crystal methods [11] and the virus by diffraction from oriented gels [13]. In the disk, subunits in the two layers have the same axial orientation (i.e., the same surface is 'up' in both cases), but units in the B-layer are tilted by about 10° with respect to units in the A layer (Fig. 4b). This tilting requires some 'play' in the azimuthal contacts: that is, the side-to-side bonding of subunits in the B-layer is a bit distorted from that in the A-layer. The structure of the disk allows simple rearrangement to the helical configuration characteristic of the virus. In this process, which actually occurs during assembly, the two layers shift over each other by about 10 Å, and all subunits acquire an identical axial tilt (about 10°, but in the opposite direction to the original B-layer tilt). Thus, all subunits end up equivalently bonded, but in a manner distinct from either the A- or the B-layer of the disk (Fig. 4b, c; ref. 12).

It can be seen from Fig. 4c that in the disk-to-helix transition two sorts of bonding changes occur: a large displacement across axial contacts (note the changed position of LR in the A layer with respect to LS and RS in the B layer), and a much smaller flexing of azimuthal contacts (due to the change in subunit tilt and to the fact that the units form a gentle helical ramp rather than a closed disk). At present, it appears as if these changes occur without large distortions of the subunits themselves, but a detailed comparison at atomic resolution of virus and disk, and of A- and B-layers within the disk, will be necessary for precise definition of the different patterns of contact.

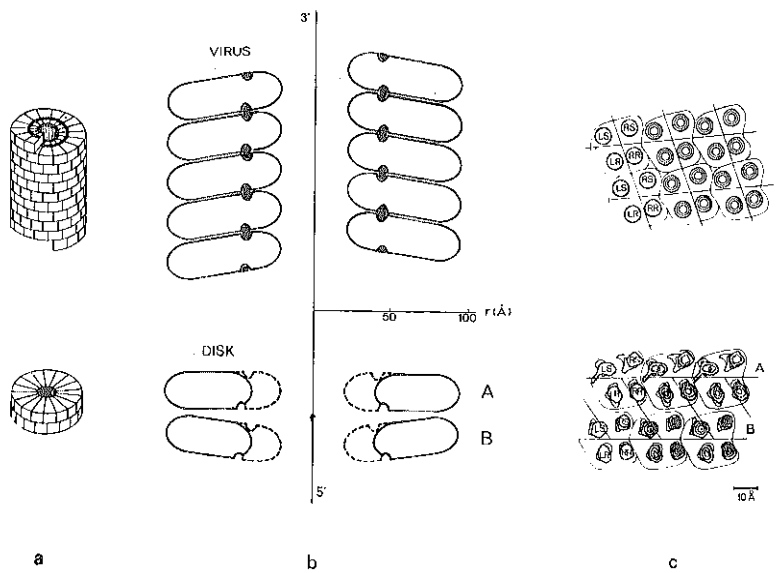

Fig. 4. Packing of subunits in the TMV protein disk (below) and in the virus (above). (a) The disk contains 34 subunits in two rings of 17; the virus 16 1/3 subunits per turn of the helix. The disk has a polar structure: all subunits have the same axial orientation. Therefore, the exposed surface of subunits in the upper (A) layer is buried in the lower (B) layer, and vice versa. RNA is present in the virus particle, sandwiched between the helically arranged subunits as shown. (b) Subunits in A (top) and B (bottom) layers of the disk have different tilts with respect to the axis, and subunits in the virus have a still different tilt. The innermost part of the subunit appears to be disordered in the disk (dashed outline), perhaps to facilitate incorporation of RNA (black dot in upper diagram) in the disk-to-helix transition that occurs during assembly. (c) Sections through subunits in the disk and in the virus, viewed end-on. The disk sections are taken from the electron density map [11]; the virus section is schematic. The major part of each subunit consists of four α-helical rods, running in a roughly radial direction, and denoted RS, LS, RR, LR; the sections have been taken through these rods. The figure shows that in the transition from a 17-fold disk to a 16 1/3 fold helix, subunits slide over each other by about 10 Å. Because of the change in subunit tilt, lateral contacts will be bent somewhat, but not completely shifted like the axial bonds. (Drawings courtesy of A. Bloomer and A. Klug.)

Specific flexibility

Formation of subcellular structures often appears to involve several distinct states of a macromolecular aggregate [14]. TMV and TBSV show how multi-state assemblies or multi-state subunits can be designed without loss of specificity. Both structures will soon be known to quite high resolution (2.8 Å or better), and the stereochemistry of switching from one state to another – perhaps even aspects of its energetics – can then be defined in more detail.

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Abrin and ricin - two toxic lectins

Sjur Olsnes and Alexander Pihl

Abrin and ricin are two peculiar proteins, each having two peptide chains with different functions. One chain, the 'effectomer', is an enzyme capable of inactivating eucaryotic ribosomes. The other chain, the 'haptomer', is a lectin which binds the toxin to cells and facilitates the entry of the effectomer into the cytoplasm. The toxins are useful in studies of cellular uptake of proteins and may be valuable in the treatment of cancer.

The lectins are a poorly defined group of proteins which are able to bind to and agglutinate cells. The best characterized lectins are present in plant material and particularly in plant seeds, but lectins have also been found in animal tissue such as rat liver and rabbit muscle [1–3]. The mammalian lectins are present in low amounts in the plasma membrane and possibly play a role in cellular recognition and interaction. Plant lectins like concanavalin A and phytohemagglutinin from *Phaseolus vulgaris* are often found in high amounts (up to a few per cent of the total dry weight) in the endosperm of the seeds, but their physiological role in the plant is not known. Most lectins so far isolated are not very toxic to animals, except when very high doses (milligrams per kg body weight) are given.

Abrin and ricin, the first two lectins described, are present in the seeds from *Abrus precatorius* and *Ricinus communis*. In contrast to most other lectins, they are highly toxic. In fact, they are amongst the most toxic substances known. Their concentration in the seeds, in comparison to their toxicity, is high. Thus, about 1 mg of pure toxin can be isolated from 1 g of seed.

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The properties of abrin and ricin were extensively studied in the late 19th century by many workers, including Paul Ehrlich, who discovered some of the fundamental principles in immunology by working with abrin, ricin and antisera against them (for review, see ref. 4).

Lethal doses of abrin and ricin are about 1 μg toxin/kg body weight in the mouse, rat and dog, whereas the rabbit is about 10 times more sensitive. There is always a lag period of several hours before the animals become sick. This lag period increases with decreasing doses of toxin. Experiments with cells in culture have shown that the earliest demonstrable effect of the toxins is inhibition of protein synthesis [5], an effect seen even at concentrations considerably below 1 ng/ml. In accordance with findings made with other inhibitors of protein synthesis, the intoxicated cells may survive for many hours, as measured by dye exclusion tests after protein synthesis has completely ceased. As will be outlined below, the toxins have many features in common with diphtheria toxin, which also inhibits protein synthesis.

Structure–function relationships

Abrin and ricin are both glycoproteins and consist of two polypeptide chains, the A-chain and the B-chain. The two chains are connected by a single SS-bond (Fig. 1). When we treated the two toxins with 2-mercaptoethanol, the SS-bond was easily reduced and the two chains could be separated by ion-exchange chromatography [4]. The separated A- and B-chains proved to contain less than 1% of the toxicity of the intact toxins and also a mixture of the chains was not very toxic to animals or to cells in culture. If 2-mercaptoethanol was removed by dialysis, the interchain SS-bond was reformed, as revealed by polyacrylamide gel electrophoresis and, significantly, there was an almost complete restoration of the toxicity. Furthermore, in experiments where mixtures of abrin A-chain and ricin B-chain, or vice versa, were dialyzed to remove 2-mercaptoethanol, highly toxic hybrid molecules were formed in good yield. It is therefore clear that the requirement for toxicity to animals and cultured cells is an A-chain bound by a disulfide bond to a B-chain. It is of little importance which of the A-chains is bound to which of the B-chains. Some physical properties of the intact lectins and the separated chains are given in Table 1, together with their toxicities.

The effector moiety is an enzyme

Our early experiments showed that abrin and ricin are potent inhibitors of protein synthesis in cell-free systems, due to an irreversible inactivation of the ribosomes [6,7]. Quantitative considerations suggested that one toxin molecule inactivated many ribosomes, indicating a catalytic activity of the toxins. This was even clearer after 2-mercaptoethanol treatment of the toxins. In contrast to the situation in living animals and in cells in culture where such treatment resulted in an almost complete loss of toxicity, the ability of the reduced toxins to inhibit cell-free protein synthesis was strongly increased. Experiments with the isolated chains established that only the free A-chain is able to inhibit protein synthesis in cell-free systems [8,9]. Clearly this was the effector-moeity of the toxin and it was termed 'effectomer'.

Further experiments by Sperti et al. [10] and in our laboratory [11] have shown that the target for the toxins is the 60S ribosomal subunit, which is modified in a still unknown way. This modification somehow interferes with the GTPase site on the ribosomes. Thus, the ability of toxin-treated ribosomes to support elongation factors, EF-1 and EF-2, dependent hydrolysis of GTP was clearly reduced [4]. Apparently, the binding site for EF-2 on the ribosomes is identical with or partly overlaps with the target for the toxin A-chain. Thus, prebound EF-2 strongly protects the ribosomes from being inactivated

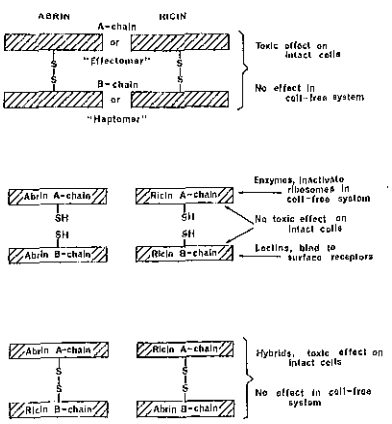

Fig. 1. Relationship between structure and function of the toxins.

by the toxins and, furthermore, once the ribosomes are inactivated after toxin treatment, their ability to bind EF-2 is strongly reduced [12–13].

The inactivation of pure ribosomes by the isolated A-chains was studied in a quantitative way by Olsnes et al. [14]. The data showed that one A-chain molecule is able to inactivate about 1,500 ribosomes per minute. The Q_{10} was found to be 1.8 and the K_m about $1–2 \times 10^{-7}$ M. The inactivation could be stopped at any time by adding specific anti-A-chain antibodies. This clearly shows that the A-chains are enzymes acting directly on the 60S subunits without any intermediates. The most easily demonstrable effect of abrin and ricin A-chains in cell-free systems is inhibition of peptide chain elongation [6,7]. Our recent data indicate that inactivation of one or a few ribosomes per polysome is sufficient to stop the elongation [15].

Recently, we have also shown that protein synthesis in systems made dependent on initiation of new peptide chains is strongly inhibited by the toxin A-chains. The association of the 40S initiation complex with the 60S subunit to form the 80S initiation complex is the only initiation step inhibited by the toxins. Interestingly, this process, which is dependent upon hydrolysis of GTP, appears to be even more sensitive to the toxin A-chains than the process of chain elongation (Skorve, Abraham, Olsnes and Pihl, submitted for publication).

Lectin properties of the B-chains

The A-chain is like a 'war-head' of very high potential impact. Thus, only a few A-chain molecules in the cytoplasm might be sufficient to inactivate the major part of the ribosomes in a cell within one day and thus kill the cell [14]. However, the isolated A-chain as such is not toxic to cells and animals. It needs a carrier which brings the A-chain 'war-head' to the cells and possibly facilitates its entry through the cell membrane.

Ehrlich had already assumed that abrin and ricin must be bound to cells before they can intoxicate them. In recent years, experiments with labelled toxins have directly shown that abrin and ricin indeed bind to cell-surface receptors (for review, see ref. 4). Experiments in our laboratory showed that one HeLa cell contains 3×10^7 binding sites for either toxin, with a K_a of 10^7 M^{-1} to 10^8 M^{-1} depending on the temperature [16,17]. When we tested the isolated A- and B-chains for binding to cells, it was evident that only the isolated B-chain is able to bind and we termed it the 'haptomer'. The B-chain also binds to free galactose or lactose [18], although with a much lower affinity (K_a of the order 10^4 M^{-1}) than to intact cells. In spite of this, lactose is an efficient inhibitor of the binding to cells, indicating that the cell-surface binding sites have features in common with lactose. Apparently, the binding sites contain terminal non-reducing galactose residues.

Several lines of evidence indicate that only toxin molecules bound to the cell surface can intoxicate cells. Thus, addition of lactose to the cell culture medium strongly reduces the amount of toxin bound to the cells and concomitantly the toxic effect is reduced to the same extent [17]. A similar effect is obtained after pretreatment of the cells with isolated (and non-toxic) ricin B-chain to block the major part of the binding sites. Furthermore, treatment of cells with neuraminidase, which removes terminal sialic acid residues often results in the exposure of penultimate galactose residues which may act as binding sites for abrin and ricin. In most cases tested (but not all, see below) the sensitivity of the cells to the toxins increases to the same extent as the increase in the number of binding sites.

Although the B-chains have only a single binding site for lactose per molecule, in concentrations of 10 μg/ml they do agglutinate the cells, probably due to the presence of a small amount of B-chain dimers [18]. Thus the B-chain fulfills the requirements for being a lectin.

Properties of toxin-resistant cell lines

In recent years a number of cell variants have been isolated which tolerate higher ricin concentrations than the parent lines. The variants are obtained by incubating cells (with or without mutagens) in the presence of increasing toxin concentrations and then surviving clones are selected. From the known mechanism of action of ricin there may be various reasons for the resistance. One possible reason would be a change in the ribosomes, rendering them resistant to the toxin A-chain. So far, no such variant has been described. Other possible reasons are changes in the surface receptors resulting in a reduced number of binding sites for the toxins. Finally, the resistance may be due to a decreased rate of internalization of surface-bound toxin molecules.

Gottlieb et al. [19] isolated a variant of Chinese hamster ovary cells which tolerated 80 times higher ricin concentrations than the parent cell line and contained only 1.3% as many binding sites. Obviously, in this case the resistance can be accounted for by the reduction in the number of binding sites. Also some of the variants isolated by Meager et al. [20] have a strongly reduced number of binding sites.

There appear to be at least two common reasons for the reduction in the number of ricin-binding sites. Thus, ricin-resistant variants isolated in 3 different laboratories proved to be deficient in *N*-acetylglycosaminyl transferase [20–22]. Since *N*-acetylglucosamine is often penultimate to terminal galactose residues, such cells have a reduced ability to complete binding sites for abrin and ricin. The same variants have an increased number of binding sites for concanavalin A, apparently due to the presence of incomplete oligosaccharide chains ending in mannose. Other variants contain more membrane-bound sialic acid than the parent strain (ref. 23 and S. Olsnes, submitted for publication). In these variants terminal galactose residues are apparently covered by sialic acid which prevents toxin binding.

In other cases the resistant cell lines bind approximately as much toxin as the sensitive parent lines [20,24]. Since we have shown that at least in some such variants

TABLE I
Properties of abrin, ricin and their constituent peptide chains.

	Abrin			Ricin		
	Intact	A-chain	B-chain	Intact	A-chain	B-chain
Molecular weight	65000	30000	35000	65000	32000	34000
pI	6.1	4.6	7.2	7.1	7.2	4.8
Toxicity to mice ($LD_{50/\mu g}$)	77	<0.1	<0.1	15	<0.1	<0.1