Lecture 13 Effects of glycosylation on protein structure and function

Learning objects

- Experimental approaches to studying the effects of glycans on protein structure and function
- How glycans affect protein folding and stability
- The effects of glycans on interactions of proteins

- In many instances, glycosylation directly affects the properties of proteins and membranes, sometimes with important bioogical consequences
- The examples illustrate some of the ways in which glycoprotein functions are modulated by glycosylation, usually by affecting their stability or surface properties.

1 Various approaches can be used to study the effects of glycosylation

Altering glycosylation to determine what effect the alternation has

- elimination of all sugars from all the attachment sites on the protein
- Elimination of sugars at specific attachment sites
- Removal of portions of the sugar structures
- N-linked sugars can be removed completely from glycoproteins by TfOH or anhydrous HF

drawbacks

- Difficulty of ensuring that sugar release is quantitative
- Damage to the protein in the form of occasional cleavage of the polypeptide chain
- Overall denaturation of the folded structure under harsh conditions
- Loss of biological function can't confidently be ascribed to the removal of sugar

Other methods

Eg1: Peptide:N-glycanase (PNGase)
Or other endoglycosidases

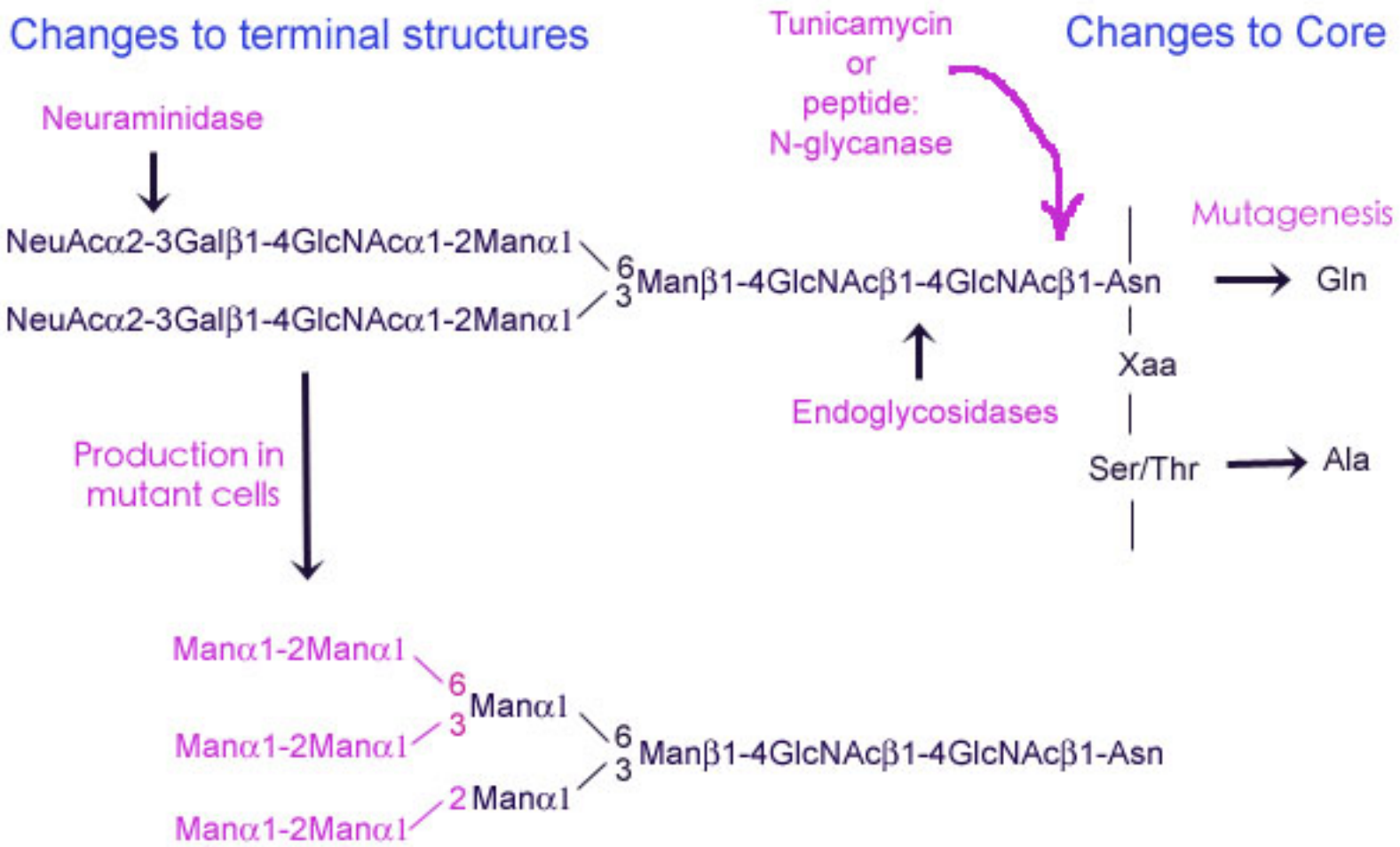
Remove sugars under non-denaturing conditions,

Although many glycoproteins contain glycans that are inaccessible to these enzymes unless the folded structure is disrupted

Eg 2: Inhibitors of glycosylation such as tunicamycin is possible to be employed

Inhibit the initial step in the formation of the dolichol-linked precursor oligosaccharide to prevent glycosylation in the first place

Eg 3: proteins isolated from tissues consist of mixtures of glycosylated and unglycosylated forms that can be separated and studied


Changes to terminal structures
Changes to Core
Tunicamycin
or
peptide:
N-glycanase
Neuraminidase
NeuAcα2-3Galβ1-4GlcNAcα1-2Manα1
NeuAcα2-3Galβ1-4GlcNAcα1-2Manα1
6
3
Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn
Mutagenesis
Gln
Xaa
Ser/Thr
Ala
Endoglycosidases
Production in
mutant cells
Manα1-2Manα1
Manα1-2Manα1
Manα1-2Manα1
6
3
2
Manα1
Manα1
6
3
Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn

- Simultaneously probe the role of all of the glycans attached to a protein at the same time
- Other methods for specific sites:
- Site-directed mutagenesis to alter glycosylation target sequences in proteins
- Possible to study the effects of individual glycans
- N-linked glycosylation at a particular site can be eliminated by changing either the asparagine attachment residue or the Serine or threonine side chain

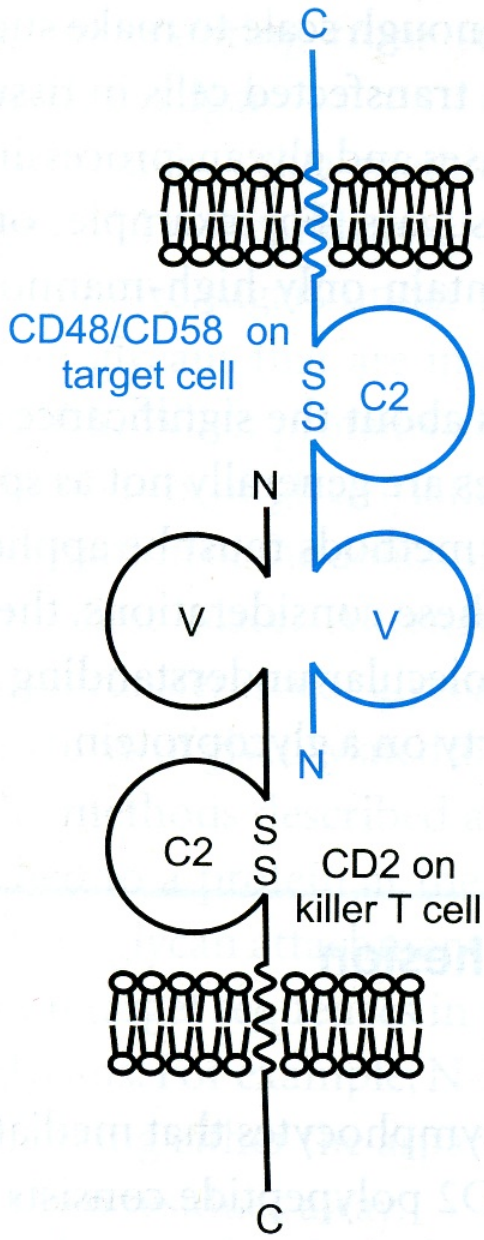
- Role of individual sugars: exoglycosidases
- Particularly useful for exploring the terminal sialic acid
- Sialidases (neuraminidases), work on glycans attached to proteins
- Many glycoforms make separations on a large-enough scale experiments impractical

- Where proteins can be expressed in transfected cells in tissue culture, mutant cell lines that lack specific glycosyltransferases and glycan-processing enzymes can be used to produce proteins with altered sugars
- E.g. Glycoproteins that contain only high-mannose N-linked oligosaccharides instead of complex glycans can be produced

2 Sugars stabilize the structure of the cell-adhesion molecule CD2

CD2 (cluster of differentiation 2)

- A cell-adhesion molecule on the surface of killer T lymphocytes (淋巴细胞) that mediates binding to target cells
- The extracellular portion of the CD2 polypeptide consists of two domains from the immunoglobulin(免疫球蛋白) superfamily, that are linked to the membrane by a C-terminal membrane anchor
- Counter-receptors for CD2 on target cells, CD58 in humans and CD48 in mice, have similar overall domain organization and cell adhesion results from interactions between the N-terminal IG type domains

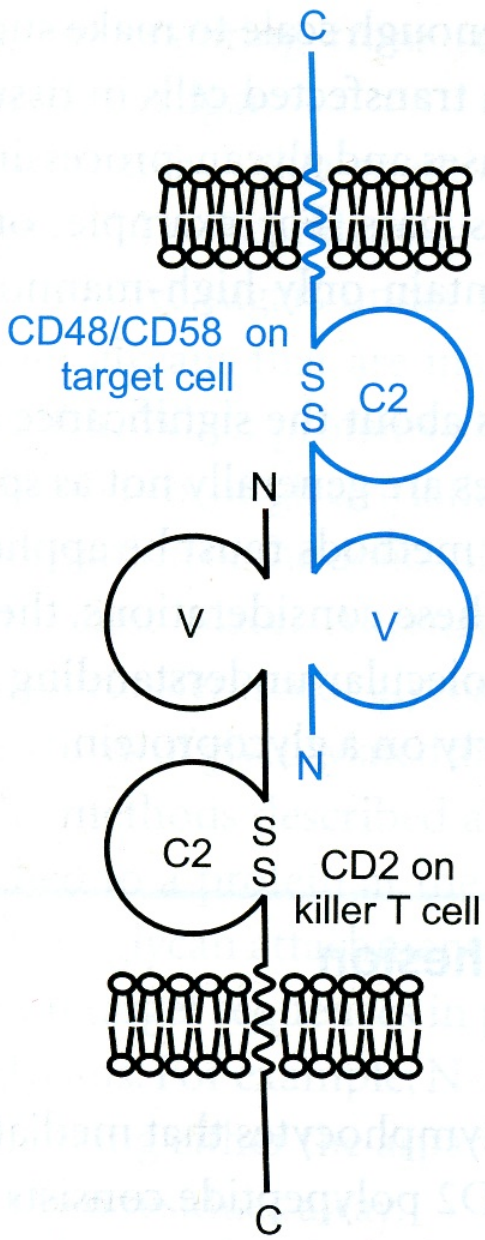


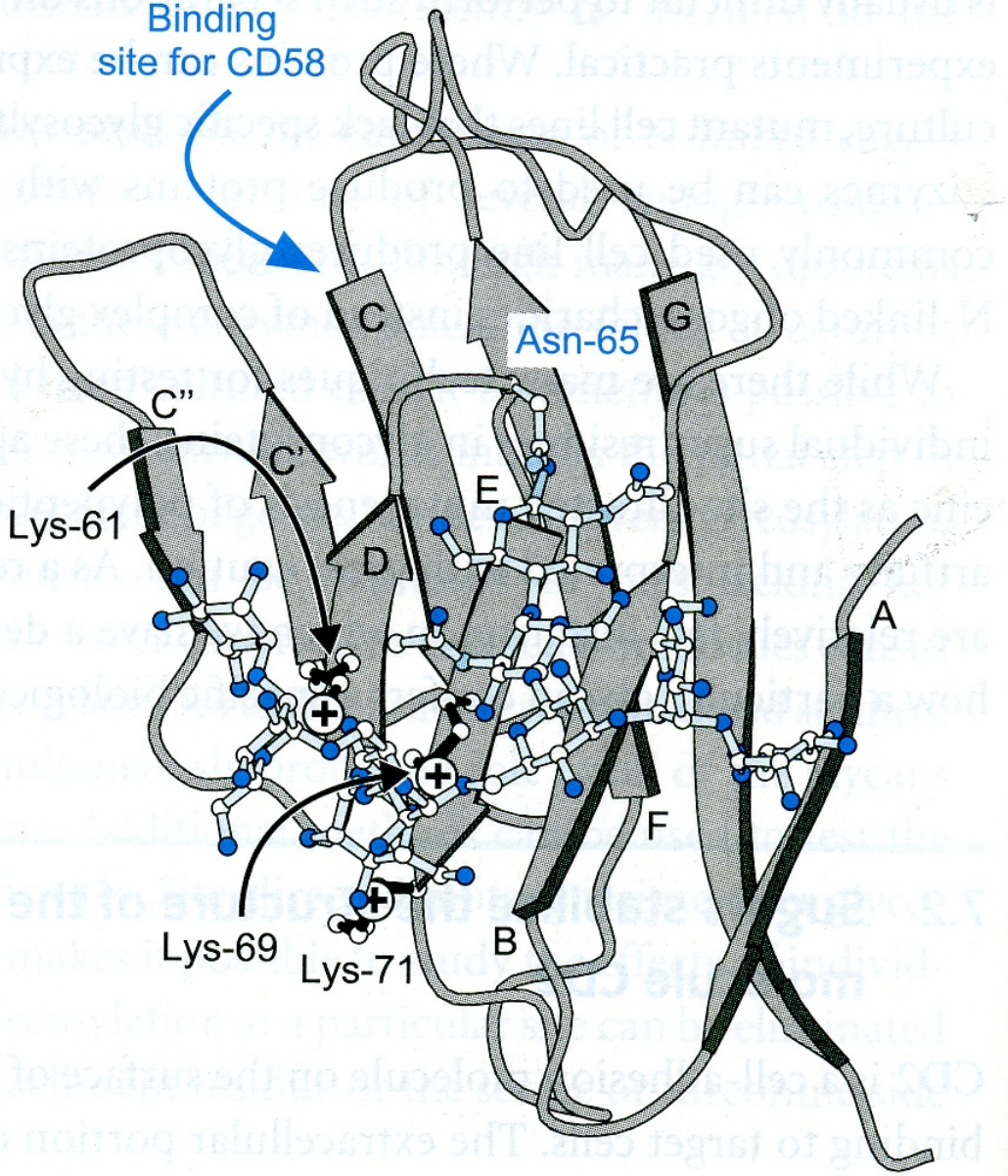
Current Protein and Peptide Science, 2001, *2*, 1-17

- Three N-linked glycosylation sites in CD2:
- One in each of the immunoglobulin-type domains and one in the interdomain linker sequence
- Essential role of glycosylation for CD2:
- Treatment of CD2 with PNGase to remove all N-linked carbohydrate results in complete loss of binding to CD58
- Mutation of the glycosylation site in the N-terminal domain, either by changing the glycosylated Asn-65 to Glu or by changing Thr-67 to Ala, also eliminates binding activity

Indicating an essential role for the N-linked carbohydrate at position 65.

structural analysis shows its occupation by high-mannose oligosaccharides bearing five to nine mannose units.


C
CD48/CD58 on
target cell
S
S
C2
N
V
V
N
C2
S
S
CD2 on
killer T cell
C


Binding
site for CD58
C
Asn-65
G
C"
C'
Lys-61
E
D
A
F
B
Lys-69
Lys-71

Comparison of rat and human CD2

- Rat CD2 is not glycosylated at the position corresponding to Asn-65 in human CD2
- In human protein, lysine at 61, 69, and 71 all project from the same side the molecule
- In rat, this cluster is interrupted by a glutamic acid in position 61
- Role of carbohydrate: to stabilize the cluster of positive charges by making hydrogen bonds with the amino groups of the lysine residues
- Human CD2 lysine at 61 replaced by glutamic acid

- Essential role for carbohydrate does not necessarily imply a direct role in mediating that function
- Glycosylation is needed to stabilize the protein structure but does not participate directly in the interaction with a counter-receptor
- Glycosylation is not the only way to achieve stabilization

3 An oligosaccharide replaces an α-helix in some variant surface glycoproteins of trypanosomes (锥体虫)

Trypanosomes (锥体虫) are large, single-celled eukaryotes (真核细胞) that move between multiple hosts (宿主) and cause sleeping sickness in humans

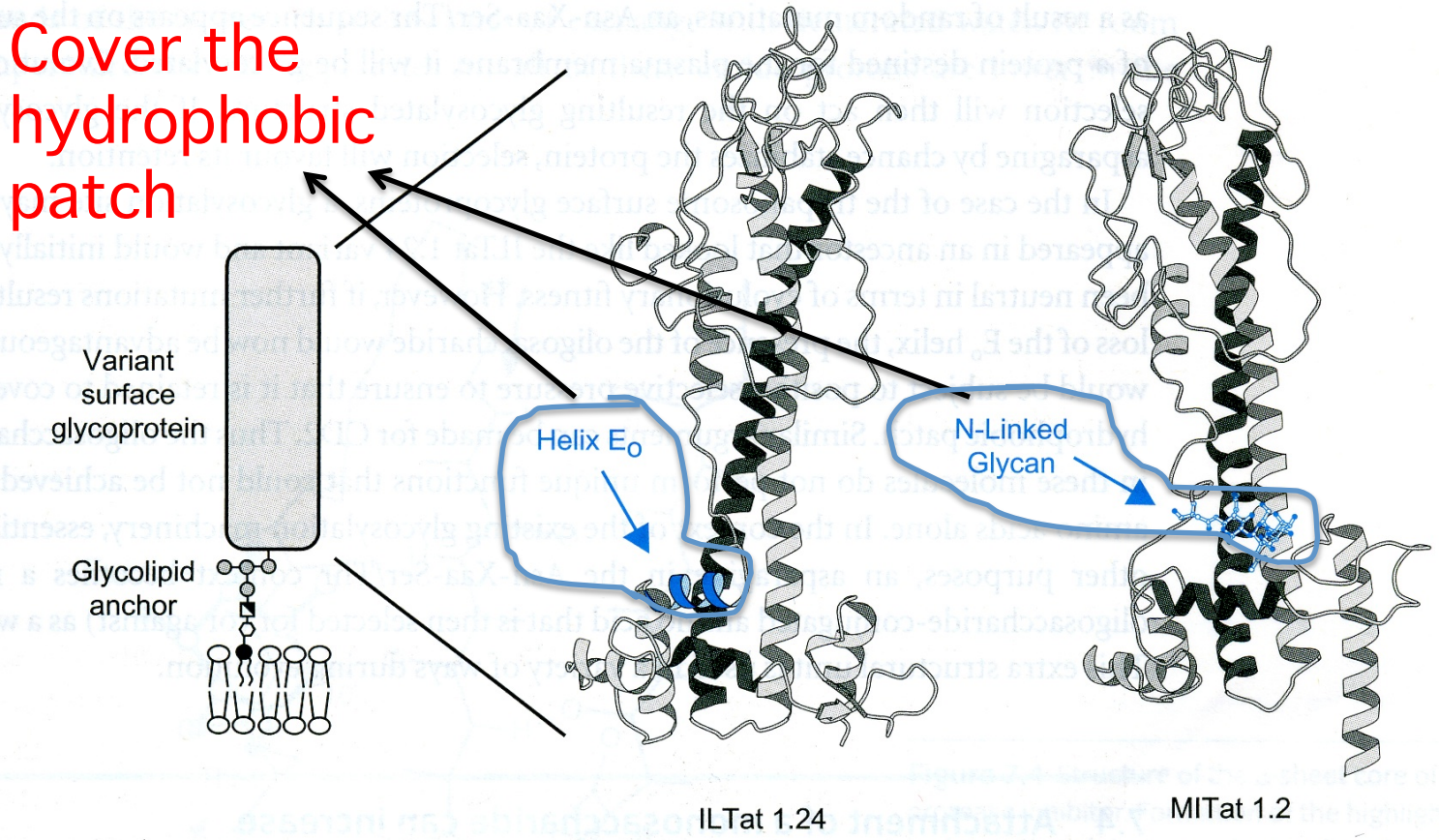
They move about in the circulation in one phase of their life cycle.

They evade host immune responses by sequentially expressing a series of different coat proteins.

Under strong evolutionary pressure, when the host antibodies and T cells begin to recognize the trypanosomes, the parasite switches to expression of a different variant.

They always stays one step ahead of the immune response of the host, which results in the characteristic waves of fever associated with trypanosomal infections

Variant surface glycoproteins that coat the surface of the trypanosome parasite



The necessary Asn-Xaa-Ser/Thr sequence is not present

Asparagine-linked high-mannose oligosaccharide

Perplexing: role of glycosylation / changes to amino acid sequence

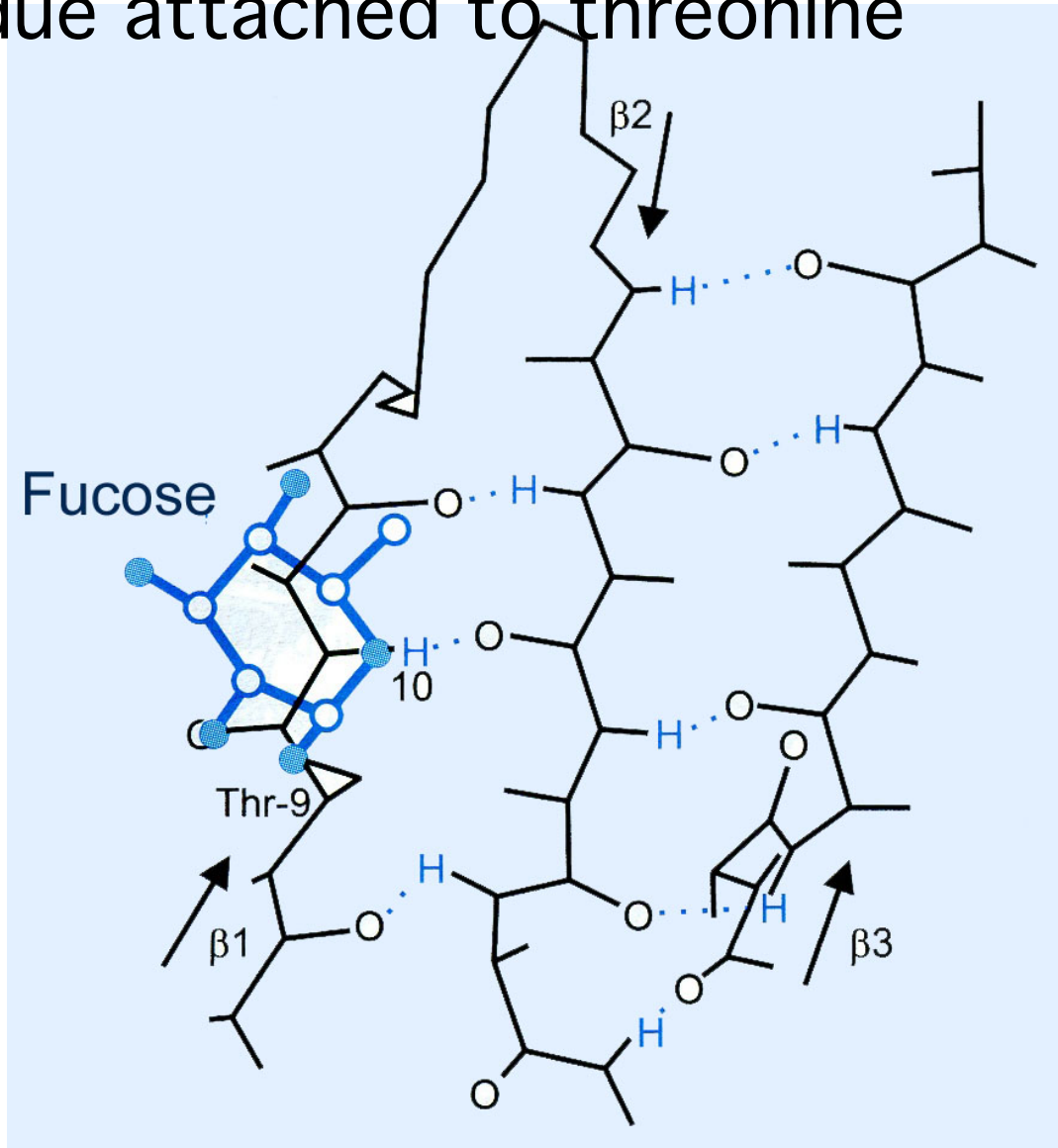
- Complex manchinery for synthesis of sugars is not evolved
- Once established, the machinery will work on all proteins that pass through the ER
- An Asn-Xaa-Ser/Thr appeared on the surface of protein will be glycosylated
- If it by chance stabilizes the protein, selection will favour its retention

- A glycosylation site may have appeared in an ancestor that looked like the ILTat 1.24 variant and would initially have been neutral in terms of evolutionary fitness
- However, if further mutations resulted in loss of the E_o helix, the presence of the oligosaccharide would now be advantageous and would be subject to positive selective pressure that is retained to cover the hydrophobic patch

4 Attachment of a monosaccharide can increase protein stability

- Glycosylation can modulate the stability and the dynamics of proteins in subtle ways
- Understanding the basis for such effects requires a rather detailed knowledge of the structure of the glycoprotein and its unglycosylated counterpart
- Small proteins, e.g. protease inhibitors (蛋白酶抑制剂) are often favoured for model studies

PMP-C protease inhibitor isolated from locusts is a 36-AA polypeptide that contains a single O-linked fucose residue attached to threonine at position 9



NMR

- Chemically synthetic versions
- A three-stranded β-sheet that is essentially unaffected by the presence of the fucose residue
- Comparison of the chemical shifts of individual atoms, reveals that only conformational differences between the glycosylated and unglycosylated versions are within 6 Å of the fucose, including the adjacent AA Thr-8 and Phe-10

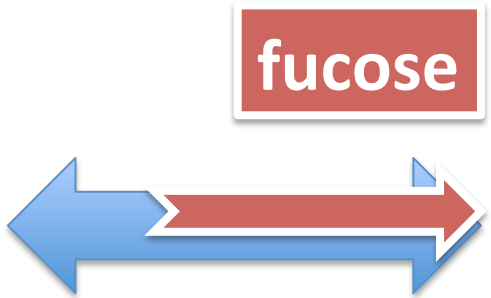
Functional consequences

- Stability study of the inhibitor as a function of temperature
- Denaturation temp. of unfucosylated form is 20°C lower than the fucosylated form
- The interactions of the fucose contribute to the stability of the folded form of the protein
- The long-range effects of local interactions with sugar are demonstrated by deuterium exchange of amide protons in the polypeptide backbone

- Which reflects the degree to which these atoms are exposed to solvent
- Residues involved in H-bonding between strands of the β-sheet show low rates of exchange with deuterated water
- At rt, the exchange rates for all of these amide protons are lower in the fucosylated than the unfucosylated protein

- Global and cooperative nature of protein folding

- A fully unfolded form in which the β-sheet has come apart



- A fully folded form in which the β-sheet is present

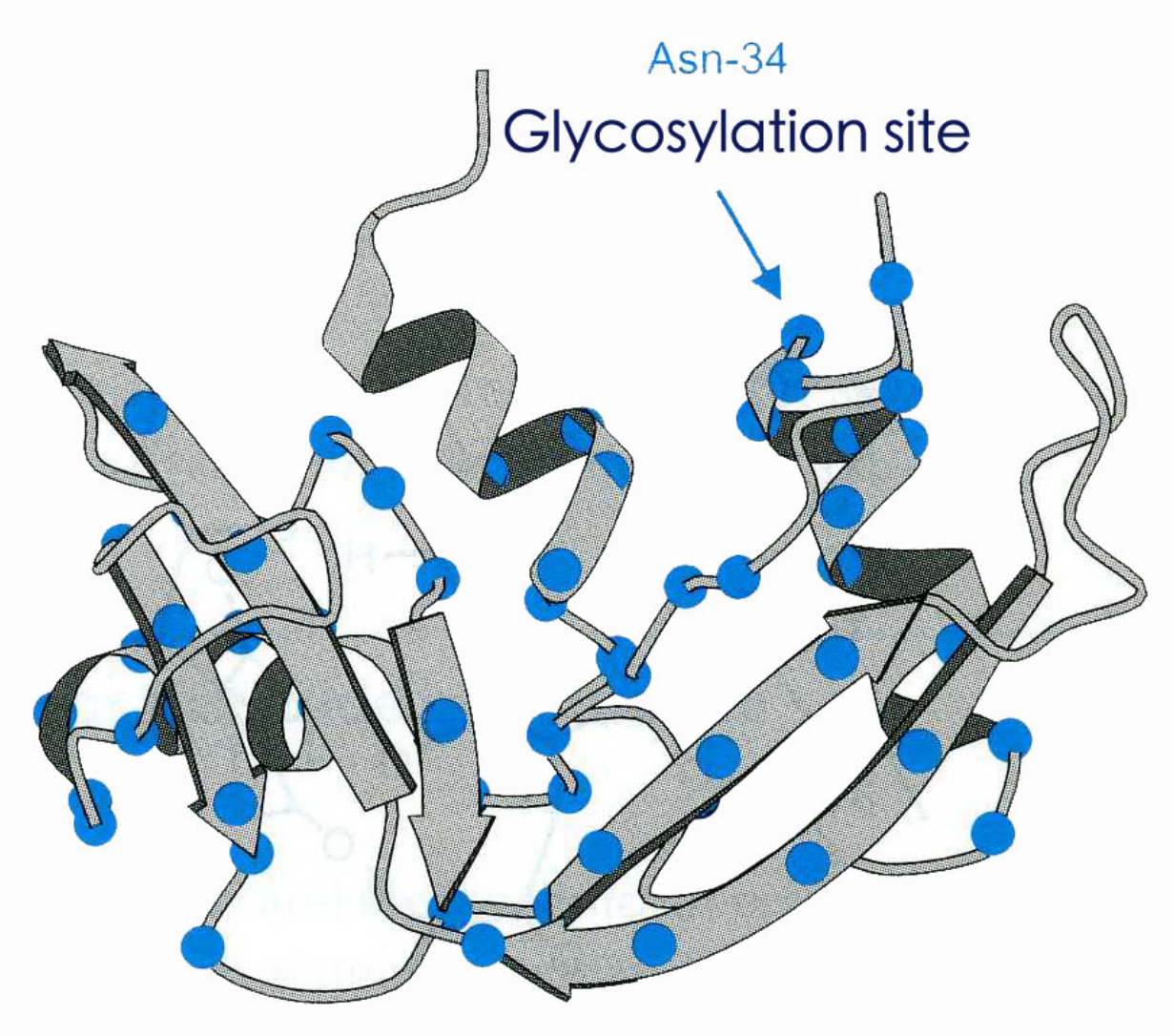
- Even though it is located only at one edge of the β-sheet
- Fucose stabilizes H-bonds on the far side of the β-sheet by making local interactions that determine the equilibrium state of the entire module

5 The stability of ribonuclease (核糖核酸酶) is increased by N-glycosylation

Digestive enzyme ribonuclease (RNase)

- Secreted from the pancreas (胰腺) into the intestine (肠) in both an unglycosylated form called RNase A and the RNase B form bears a single high-Man oligosaccharide attached to asparagine-34
- Glycoforms containing 4-9 mannose residues are found in preparations from bovine pancreas
- The structure of the protein portions are identical

Unglycosylated bovine pancreatic ribonuclease A (RNase A) with the attachment site of a high-mannose oligosaccharide in RNase B



- Glycosylation decreases exchange rates for the backbone amide protons, not just locally near the glycosylation site but in distant parts of the protein as well
- The presence of the carbohydrate makes the glycosylated structure more thermodynamically stable than the unglycosylated form
- Owing to the cooperative nature of protein fold, this difference has a global effect on the frequency with which the protein changes from the folded state to an unfolded state

- Not possible to specify the local contacts between the sugar and protein
- The critical interactions must involve the portion of the glycan closest to asn-34, because exoglycosidase digestion to remove all but the two core GlcNAc and one Man has no effect on the stability of RNase B
- The first GlcNAc can interact with the polypeptide backbone (results from other proteins)

Evolution view

- RNase is secreted from the pancreas in both glycosylated and unglycosylated forms
- They were selected together during evolution
- A relatively neutral modification in this case
- The greater stability may have led to some slight selective pressure favouring the retention of Asn-34, but not sufficient
- All forms of the enzyme work reasonably well for digestive purposes

6 Protein-protein interactions can be modulated by oligosaccharides

Sugars on the surfaces of proteins

- Would not be expected to have a significant effect on the ability of small-molecule substrates to reach active sites
- Can reduce accessibility of the proteins to larger probes such as antibodies, proteases and other proteins
- Shielding of hydrophobic regions of a protein surface from the aqueous solvent can also increase solubility

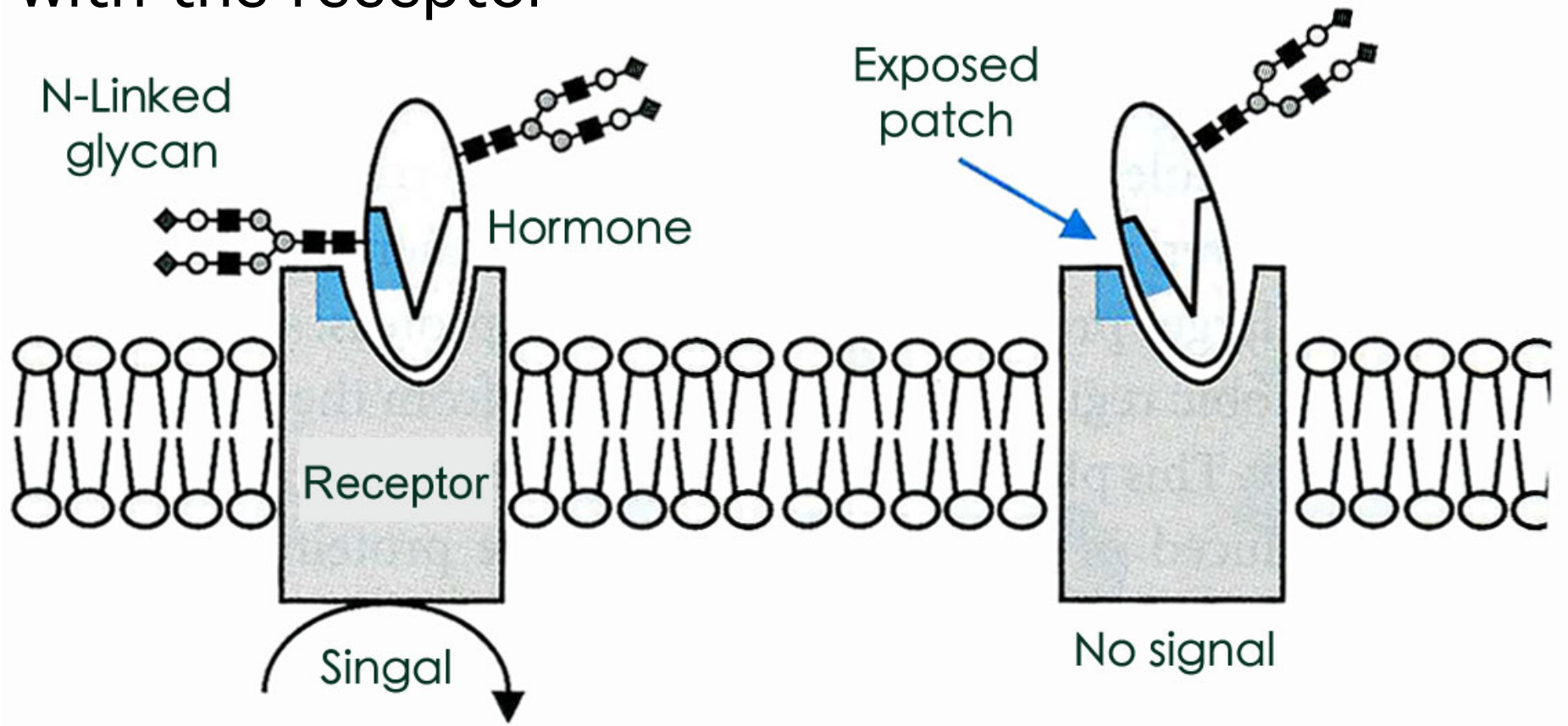
Glycoprotein hormones produced in the pituitary gland (脑垂体) and the placenta (胎盘)

- exposure of extra surfaces by deglycosylation or underglycosylation of proteins can lead to unexpected behaviour
- This family of related hormones includes lutrophin (促黄体素), follitrophin (促滤泡素) and chorionic gonadotrophin (绒毛膜促性腺激素)
- Primary role of N-linked glycans: direct their clearance from circulation, which is mediated by a receptor in liver

- Binding of the intact, glycosylated hormones to target cell receptors leads to the activation of adenylate cyclase (腺苷酸环化酶)
- Removal of the N-linked glycans abolishes their ability to activate these receptors
- Inactivation is visible, when the entire glycan is absent or key glycosylation sites are removed by mutagenesis
- However, removal of just the terminal sugars does not affect hormone activity

- The cores of the N-linked oligosaccharides play a direct role in hormone binding to target cell receptors
- However, the affinity of the deglycosylated hormone actually increases even though the bound hormones do not cause receptor activation

The absence of the glycans probably exposes new surfaces that interact in abnormal ways with the receptor

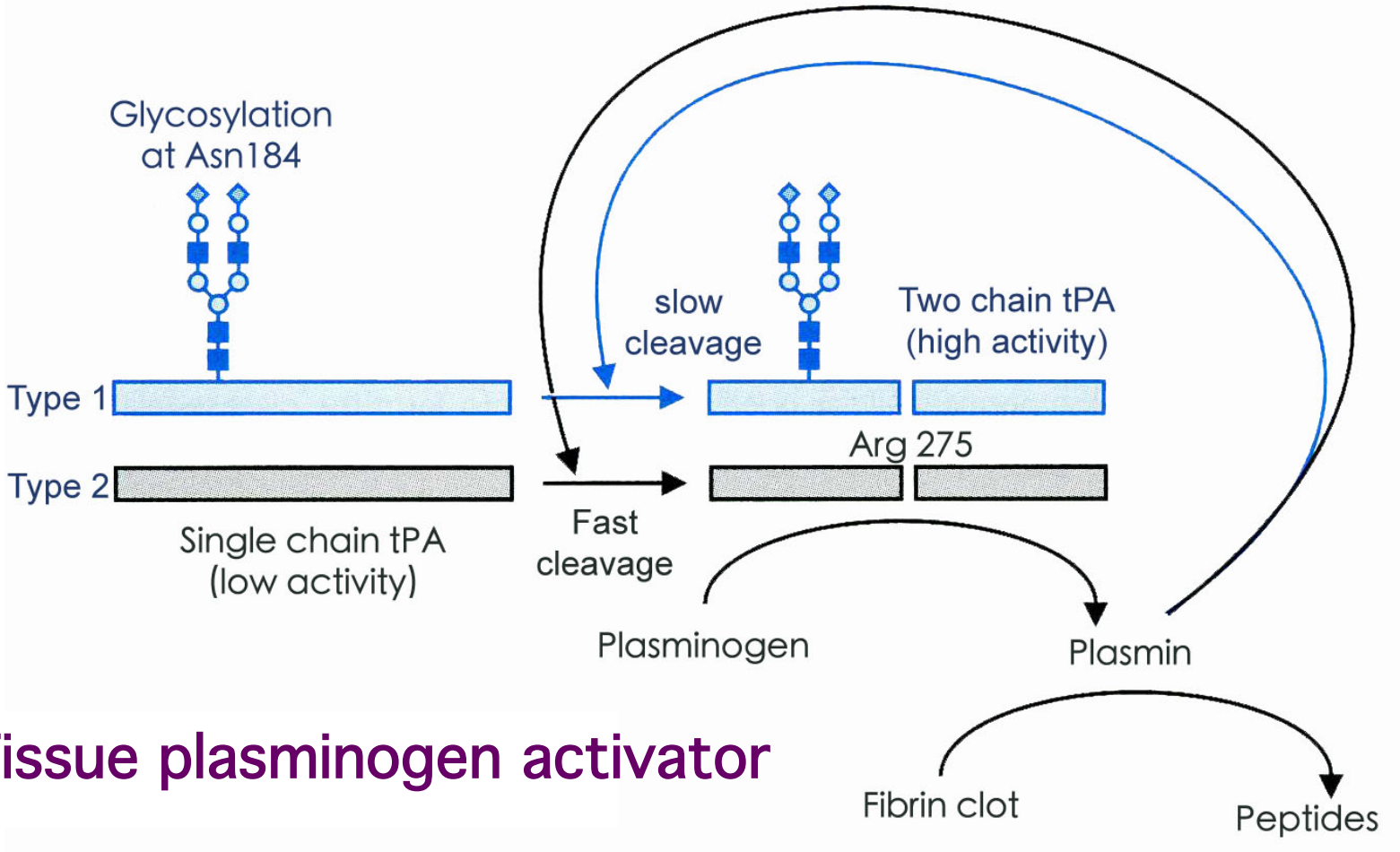


Increased affinity but shift the hormone out of the position needed for receptor activation

7 Oligosaccharides covering surfaces of proteins can protect against proteolysis (蛋白质水解)

- If a protein-glycan linkage is flexible rather than fixed, the glycan may, at least partially, cover a large portion of the protein surface
- Although the effect to any given part of the protein may be transient
- Explains that glycosylation can sometimes confer increased resistance to proteolysis without preventing it altogether
- Such modulation of proteolysis is illustrated by tissue plasminogen (血浆酶原) activation

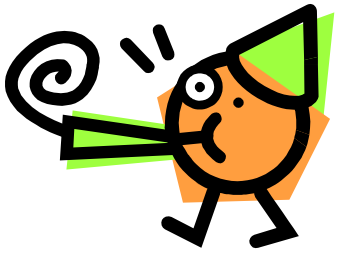
The protease plasmin (血浆酶) mediates removal of blood clots by degrading fibrin ((血)纤维蛋白) when tissue integrity has been restored and the clot has served its purpose in preventing blood loss



tPA: Tissue plasminogen activator

Differential glycosylation of prion proteins may be linked to the conformational changes leading to CJD and related diseases

Glycobiology of disease



Prion (朊病毒) disease

- Fatal neurodegenerative disorders
- Cretzfeldt-Jakob disease (CJD) in humans
- Scrapie in sheep
- Bovine spongiform encephalopathy (BSE) in cattle
- Transmissible spongiform encephalopathies (传染性海绵状脑病)
- Characterized by the accumulation of an abnormal form of the prion protein in the central nervous system

identification

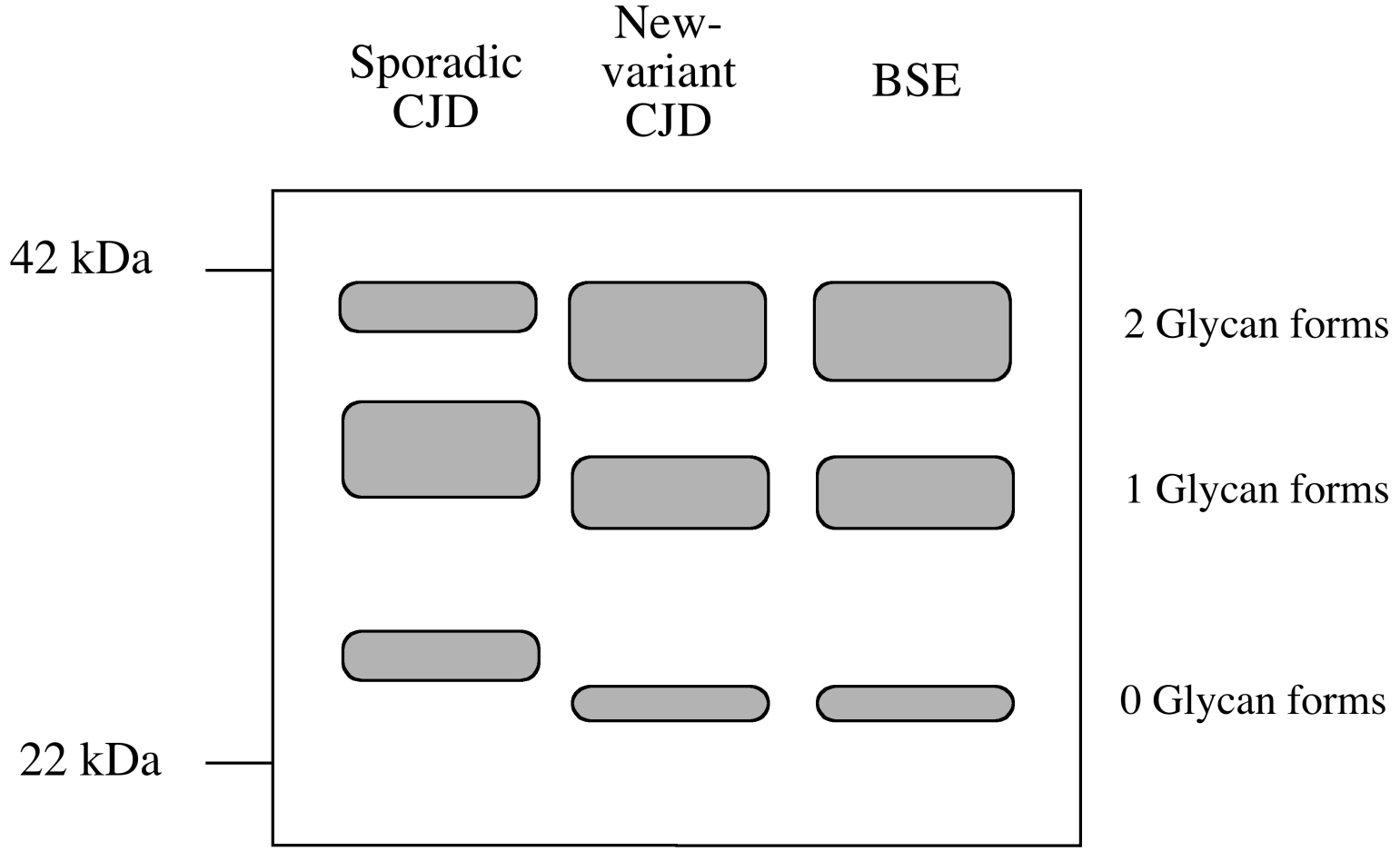
- No viruses or bacteria have been identified in association with these diseases
- treatments that destroy nucleic acid do not prevent transmission of the disease
- Infectious microorganisms are not involved
- Abnormal form of the prion protein itself is the infectious agent that causes disease

- A change in conformation of the normal cellular prion protein, PrP^{c}, to form the disease-related prion protein, PrP^{Sc}, is the key event in the pathogenesis (发病机理) of transmissible spongiform encephalopathies
- PrP^{Sc} having a high content of β structure, differs from PrP^{c}, a mainly α-helical conformation
- a high content of β structure is less soluble and more resistant to proteolysis

- Whether glycoysylation plays any part in the conformational change associated with conversion of PrP^{c} to PrP^{Sc} ?
- The prion protein has two N-linked glycosylation sites and is anchored to the plasma membrane through a GPI anchor
- Variable occupancy , glycofomrs of 0, 1, or 2 glycans
- Glycosylation has little effect on either the conformation or stability of PrP^{c}

A useful marker for categorizing prions isolated from different species or from different types of human prion disease

Treatment with proteinase (蛋白酶) K followed by gel electrophoresis (凝胶电泳) and western blotting



The new-variant of CJD, which affects young people, is acquired by eating beef from BSE-infected cows

- PrP^{Sc} from the brains of new-variant CJD patients have an identical pattern of proteinase K fragments to that seen from prions isolated from BSE-infected cows
- Both the sizes of the fragments and the relative amounts of each glycoform are identical and this pattern is different to that seen for PrP^{Sc} from brains of patients with sporadic CJD