POLYSACCHARIDES AS BIOLOGICAL RESPONSE MODIFIERS
STRUCTURE - ACTIVITY RELATIONSHIP

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When chemical synthesis of the substrate A (or the product C!) is very difficult (impossible ?, inefficient ?, extremely expensive?) → we use biosynthesis
Outline

• Polysaccharides - structural diversity
• Functional versatility and biological activity
• Clinical use
• Structure-activity relationship
• Methods for obtaining of polysaccharides
• Chemical and biotechnological modifications
• Astonishing effects of modifying the structure of polysaccharides
Polysaccharides - structural diversity
Much more complex structures than proteins and nucleic acids!

- Composed of \( n > 10 \) monomers (11 up to several thousands)
- Each unit can be combined with another in many ways
- They can have straight or branched chains (to varying degrees), create cyclic forms ...

- **Molecular weight:** \( 10^3 \leq MW \leq 10^7 \) (?)

  Low-molecular-weight polysaccharides \( MW \leq 10^4 \)
  
  (up to 10 kDa)

  High-molecular-weight polysaccharides \( MW > 10^4 \)
  
  (over 10 kDa, i.e. \( 10^5 \sim 10^6 \) (?))
• They may consist of the same monomers (homoglycans) or different monomers (heteroglycans)

• They may contain a protein component, lipid, etc. (eg, lipopolysaccharides, proteoglycans)

Conclusion: an infinite number of combinations
To determine the primary structure of polysaccharides, it is necessary to know:

- the **degree of polymerization**,  
- the **structure** of individual monosaccharides (including absolute configuration),  
- the **sequence** of their linkage to each other,  
- the **points of branching**,  
- the **type of substitution**,  
- the **character and distribution of modifying groups** (e.g. phosphates, sulfates, methyl- and acetylgroups),  
- the **configuration** ($\alpha$- or $\beta$-) of glycosidic bonds.
Lentinan

Branched β-D-glucan
1-3-β-linked backbone, 1-6-β-branched
MW = ~ 500 kDa

Immunostimulator
Secondary structure

- the conformation of individual monosaccharide residues and
- the geometry of their linkage to each other by glycosidic bonds

Helical structure
Twisted structure
Ribbon-like structure
Labile structure
Helical structure

β-1,3-D-glucan

α-1,4-D-glucan
Twisted and ribbon-like structure

β-1,2-glucan  β-1,4-glucan
Labile structure

$\beta$-1,6-D-glucan
The **Tertiary structure** is defined by spatial conformation of the chain.

Factors determining the polysaccharide tertiary structure:

- interactions with nearby monomers along the molecular chain,
- distal three-dimensional interactions,
- entropic effects,
- interactions with the environment.

Tertiary structure is stabilized by **hydrogen bonds** and in the presence of electrically charged groups by **electrostatic forces**.

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Helical structure stabilized by hydrogen bounds
Quaternary structure

Quaternary structure is formed by association of individual molecules.

Examples:
• supramolecular formations of ribbon-like structures due to numerous hydrogen bonds and van der Waals forces
• double and triple helixes
• aggregations of helixes and ribbons

Structure regular in three dimensions, characteristic of crystals
Triple-helical structure
(β-1,3-glucans)
Schizophyllan

Curdlan

(a)

Diameter: 2.6 nm
Pitch: 1.8 nm

Side view

Top view

Schizophyllan triple helix

(b)
Triple helical structure

response to chemical and physical treatment

Functional versatility
Functional versatility –
effect of the structural diversity

Differences in **physical** and **chemical** properties:

• solubility,

• viscosity,

• swelling capacity,

• resistance to acids, bases and enzymes,

• chemical character (acidic, basic, neutral)
Differences in **biological** activity:

- antioxidants,
- lipid-lowering agents,
- cholesterol-lowering agents,
- antitumor agents,
- cytotoxic agents,
- immune-stimulating agents,
- immunosuppressants,
- chemopreventive agents   *etc.*
Antitumor and chemopreventive activities of the polysaccharides

- direct effect on the tumor cells
  (induction of apoptosis, cytotoxicity)

- immunomodulatory activities
  (immunostimulation)

- protection against the oxidative stress
  (antioxidant activity)
Immune activation induced by β-glucans

„The exact immunological actions and signaling pathway induced by β-glucan are still unclear and have to be further defined”
The uptake and subsequent actions of β-glucan on immune cells
Clinical use
Drugs used in the cancer treatment isolated from *Basidiomycota* type medicinal mushrooms

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Krestin</th>
<th>Lentinan</th>
<th>Sonifilan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbreviation</td>
<td>PSK</td>
<td>-</td>
<td>SPG</td>
</tr>
<tr>
<td>Common Name</td>
<td>Krestin</td>
<td>Lentinan</td>
<td>Schizophyllan</td>
</tr>
<tr>
<td>Company</td>
<td>Sankyo, Kureha</td>
<td>Ajinomoto, Yamanouchi, Morishita</td>
<td>Taito, Kaken</td>
</tr>
<tr>
<td>Marketed date</td>
<td>May 1977</td>
<td>December 1985</td>
<td>April 1986</td>
</tr>
<tr>
<td>Fungus (origin)</td>
<td><em>Trametes versicolor</em> (mycelium)</td>
<td><em>Lentinus edodes</em> (fruit body)</td>
<td><em>Schizopyllum commune</em> (medium product)</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>β-glucan-protein -1,6-branching -1,3: 1,4-main chain</td>
<td>β-glucan -1,6-branching -1,3-main chain</td>
<td>β-glucan -1,6-branching -1,3-main chain</td>
</tr>
<tr>
<td>Structure</td>
<td>100,000</td>
<td>500,000 + 14-22° (NaOH) 1-mg vial</td>
<td>450,000 + 18-24° (water) 20-mg ampoule (2 ml)</td>
</tr>
<tr>
<td>MW</td>
<td>-</td>
<td>9,500 i.p., i.v.</td>
<td>¥ 9,500 i.p., i.v.</td>
</tr>
<tr>
<td>Specific rotation</td>
<td>1-g sack</td>
<td>1-mg vial</td>
<td>20-mg ampoule (2 ml)</td>
</tr>
<tr>
<td>Pharmaceutical</td>
<td>¥ 1,000</td>
<td>¥ 9,500</td>
<td>¥ 9,500</td>
</tr>
<tr>
<td>Price</td>
<td>p.o.</td>
<td>Cancer of digestive organ, lung and breast</td>
<td>Cancer of stomach</td>
</tr>
<tr>
<td>Dose route</td>
<td></td>
<td>Cervical cancer</td>
<td></td>
</tr>
<tr>
<td>Cancer treated</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[http://www.anticancer-drug.net/BRM/lentinan.htm](http://www.anticancer-drug.net/BRM/lentinan.htm)

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Clinical data (LNT)

Prolongation of life by various prognosis factors (Ajinomoto Co. 1984)

<table>
<thead>
<tr>
<th>Extension of tumour</th>
<th>Background</th>
<th>Treatment</th>
<th>No. of cases</th>
<th>50% survival (day)</th>
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</thead>
<tbody>
<tr>
<td>Abdominal localisation</td>
<td>Tegafur group</td>
<td>13</td>
<td>166 days</td>
<td></td>
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<tr>
<td>LENTINAN + tegafur group</td>
<td>19</td>
<td>237 days</td>
<td></td>
<td></td>
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<tr>
<td>Hepatic or peritoneal metastasis</td>
<td>Tegafur group</td>
<td>47</td>
<td>68 days</td>
<td></td>
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<tr>
<td>LENTINAN + tegafur group</td>
<td>48</td>
<td>169 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>Tegafur group</td>
<td>8</td>
<td>170 days</td>
<td></td>
</tr>
<tr>
<td>LENTINAN + tegafur group</td>
<td>9</td>
<td>133 days</td>
<td></td>
<td></td>
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<tr>
<td>Histology</td>
<td>Well-differentiated adenocarcinoma</td>
<td>31</td>
<td>105 days</td>
<td></td>
</tr>
<tr>
<td>Tegafur group</td>
<td>34</td>
<td>223 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LENTINAN + tegafur group</td>
<td>34</td>
<td>91 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorly-differentiated adenocarcinoma</td>
<td>Tegafur group</td>
<td>34</td>
<td>169 days</td>
<td></td>
</tr>
<tr>
<td>LENTINAN + tegafur group</td>
<td>40</td>
<td>133 days</td>
<td></td>
<td></td>
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<tr>
<td>Bormann types 1 and 2</td>
<td>Tegafur group</td>
<td>7</td>
<td>119 days</td>
<td></td>
</tr>
<tr>
<td>LENTINAN + tegafur group</td>
<td>7</td>
<td>391 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bormann types 3 and 4</td>
<td>Tegafur group</td>
<td>32</td>
<td>100 days</td>
<td></td>
</tr>
<tr>
<td>LENTINAN + tegafur group</td>
<td>41</td>
<td>163 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Clinical use

- The clinical data are available for lentinan (LNT), schizophyllan (SPG), PSK,
- In cancer treatment used as adjunctive in the postoperative chemo- and radiotherapy
- Breast, cervix, prostate, stomach, colon, lung cancer
- Dose (LNT): intravenous 2mg/week, orally 3 mg/day
- Much more effective in the early stages of tumor development
• The listed above preparations are registered as the medicinal products in Japan, China, other Asian countries

• In US some of them undergo I/II phase of clinical trials

• In EU - lack of clinical trials; available wide preclinical animal studies

• There are companies (e.g. Norwegian GlycaNova) that specialize in the development and production of polysaccharide preparations

• Marketed in Europe preparation are dietary supplements or clinical cosmetics. The polysaccharide doses are several times lower than in Japanese drugs

• The most effective polysaccharide preparations have to be administered i.p. or i.v.
Structure-activity relationship
• Extensively investigated in the past three decades
• Results of these studies are often ambiguous and concern mainly the β-glucans
• For the other types of immuno-active polysaccharides data are not available
**Structure – activity relationship**

well-known (?) in β-D-glucans, poorly in other types of polysaccharides (e.g. α-glucans)

**Factors affecting the immunomodulatory activity:**

- Water solubility
- Monosaccharide composition
- Molecular weight (optimum 200-500 kDa) (?)
- Degree of branching (DB) (optimum 0.2 – 0.33) (?)
- Type of glycosidic boundings (optimum β-1,3-) (?)
- Conformation of the higher order polysaccharide structure (e.g. triple helical structure)
Methods for obtaining
of polysaccharides

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Chemical synthesis of high-molecular-weight polysaccharides is practically impossible. These compounds are either isolated from natural sources or obtained by biotechnological methods and in certain cases chemically modified.
Location of PS in bacteria cell wall
Location of PS in fungal cel (cel wall)
Location of PS in plant cell wall

https://thebuddychemists.wordpress.com/tag/polysaccharide/
Solubility of polysaccharides

• Water-soluble glycans

• Alkali-soluble glycans – (S-glycans)
  often β-1,3-D-glucans or β-1,4-D-glucans with β-1,6-D-glucan side-chains, β-1,4-D-glucans

• Alkali-insoluble glycans (R-glycans)
  often β-1,3-D-glucans with β-1,6-D-glucan side-chains
Isolation of *lentinan* according G. Chihara

**Fresh fruit bodies of *Lentinula edodes***

- *Extraction with hot water*
- *Ethanol 1 vols.*

**Fraction L (ppt.)**

- *Solubilization in water*
- *Precipitation with 0.2 M CTA-OH*

**Precipitate**

- *Extraction with 20% acetic acid*

**Soluble part** (Fractions LC-1A, 1B, 1C, 1D, 1E, 11, 12, 13)

**Insoluble part (LC-3)**

- *Extraction with 50% acetic acid*

**Insoluble part**

- *Solubilization with 6% NaOH*
- *Ethanol 3 vols.*

**Precipitate**

- *Deproteinization*

**White powder – *Lentinan***
Why use biotechnological methods?

- short cultivation time
- highly repeatable conditions
- regulation of metabolism by optimizing the culture conditions
- preservation of biochemical and genetic identity

*Lentinula edodes* – fruit-bodies

http://fungus.org.uk/nwfg/fungbiot.htm

mycelial cultures

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Not always biotechnological methods are better than cropping or harvesting!!

- Ex: not all higher fungal species have the ability to effectively grow in the form of mycelial cultures in the bioreactor

- White truffle
  - *Tuber magnatum* Pico
  - Summer truffle
  - *Tuber aestivum* Vittad.

http://www.borghiautentiditalia.it/bai
- Sometimes there are significant differences in the chemical composition of the fungal fruit-bodies and the mycelial biomass.

**T. Aestivum**, Polyphenolics, fruit-body/mycelium

**L. Edodes**, Chitin, fruit-body/mycelium

**L. Edodes**, Polysaccharides (lentinan), fruit-body/mycelium
Modifications of the structure
Modifiable factors affecting the immunomodulatory activity of polysaccharides:

- Water solubility *
- Monosaccharide composition
- Molecular weight (optimum 200-500 kDa) *
- Degree of branching (DB) (optimum 0.2 – 0.33) *
- Type of glycosidic boundings (optimum β-1,3-)  
- Conformation of the higher order polysaccharide structure (e. g. triple helical structure) *
Chemical modifications of the structure

- Reduction (controlled) of molecular weight by acidic, basic or enzymatic hydrolysis (or ultrasonic degradation)
- Changing of the polarity (sulfonation, carboxymethylation, hydroxymethylation etc.)

Positive effects - improved solubility and bioavailability

Negative effects - reduced activity
Biotechnological modifications of polysaccharides: Incorporation of selenium to the polysaccharide structure (project)

\[ \text{SeO}_3^{2-} \]
Astonishing effects of modifying the structure of polysaccharides
1st goal: to obtain Lentinan by biotechnological method (by extraction from the submerged-cultivated mycelial biomass)

2nd goal: to obtain by biotechnological method a new pharmacologically-active preparation - the Se-enriched Lentinan

Hypothesis: a similar pharmacological effect of polysaccharides and selenium suggests a possible synergism of these two agents
The project include:

- cultivation of mycelial cultures of *L. edodes* in not enriched and Se-enriched media,
- isolation of the polysaccharide fraction acc. to Chihara method („lentinan”) from not enriched and Se-enriched cultures,
- structural analysis of the polysaccharides,
- examination of biological activity
What is the reason of the opposite biological effect??

Probably - the differences in the structure of lentinan and the fraction LC-33

The structure of the polysaccharides has to be identified.
Lentinan
Branched $\beta$-D-glucan
1-3-$\beta$-linked backbone, 1-6-$\beta$-branched
MW = $\sim$ 500 kDa
Immunostimulator
**Mycelial polysaccharide** – fraction that correspond to lentinin

MW = >>>>650 kDa (2000 kDa ?)

**Selective immunosuppressant!!**

1-6-β-linked backbone, 1-3-β–branched

\[
\rightarrow 6)\beta-D-Glcp-(1\rightarrow 6)\beta-D-Glcp-(1\rightarrow 6)\beta-D-Glcp-(1
\]

\[
3 \quad 3
\]

\[
\uparrow \quad \uparrow
\]

\[
\beta-D-Glcp \quad \beta-D-Glcp
\]
<table>
<thead>
<tr>
<th></th>
<th>Lentinan</th>
<th>Mycelial polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M.W.</strong></td>
<td>~ 500 kDa</td>
<td>&gt;&gt;&gt;&gt;650 kDa (2000 kDa ?)</td>
</tr>
<tr>
<td><strong>Monosaccharide composition</strong></td>
<td>Glucan (β-D-glucan)</td>
<td>Glucan (β-D-glucan)</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td>1-3-β-glycosidic bondings (main chain)</td>
<td>1-6-β-glycosidic bondings (main chain)</td>
</tr>
<tr>
<td></td>
<td>1-6-β-branched</td>
<td>1-3-β-branched</td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td>Immunostimulant</td>
<td>Immunosuppressant</td>
</tr>
</tbody>
</table>
XAFS spectra confirm that selenium in the Se-polysaccharide structure is present at –II oxidation stage. Se is organically bounded.

The simulation EXAFS analysis confirm, that selenium is probably introduced into the polysaccharide structure in place of oxygen in β-1,3-glycosidic bond or substitutes oxygen in carbohydrate acetal ring.
Probably the results will enable to design a novel immunosuppressant with a completely different structure than the currently known
Founding source: grant from the Polish National Science Centre DEC-2013/09/B/NZ7/03978

Consortium:

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Thank you for attention