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Partner in Research

Biosynthesis of polysialic acid for the preparation of artificial scaffold materials

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Structure, occurrance and function

A number of polysaccharides consisting of 9-C-sugar acids are summarized under the name of polysialic acids (polySia, PSA). All oft their particular structural parts are derivatives of neuraminic acid (sialic acids) linked by glycosidic bonds to either homo- or heteropolymers. The most common natural sialic acids are shown in figure 1. In nature, polySia can be found in vertebrates as a modification of the outer cell membrane especially in nerve cells. Here, it controls the cell adhesion and can be regarded as a kind of growth support matrix in the developing brain. Depending on the species the composition of the generated polySia differs more or less. In humans only the homopolymer of 2,8-linked 5-N-acetyl-neuraminic acid can be found (figure 2). This special kind of polySia is also known as colominic acid (CA). No other higher developed creature posesses the same kind of polySia. Nevertheless, some neuroinvasive bacteria like *Escherichia coli* K1 or *Neisseria meningitidis* serogroup B are encapsulated by polySia and the capsule provides an important virulence factor. The capsular polysaccharide is identical to colominic acid found in the human body. Due to this molecular mimicry the bacterium is protected against the host immune system.

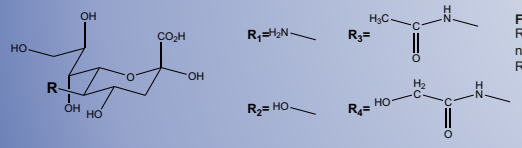

Figure 1: Basic structure of sialic acids, R_1=neuraminic acid, R_2=5-deamino-3,5-dideoxy-neuraminic acid, R_3=5-N-acetyl-neuraminic acid, R_4=5-N-glycolyl-neuraminic acid

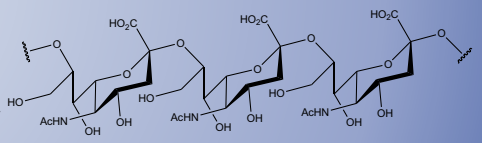

Figure 2: Human polySia of 2,8-linked 5-N-acetyl-neuraminic acid (colominic acid)

Biosynthesis

A natural way for the recovery of polysialic acid is the isolation from bacteria. The cultivation of these microorganisms is very inexpensive and the product is consistent. Starting with glucose (Glc) the biosynthesis of sialic acid in bacteria is illustrated in figure 3.

Glc + Gln --> GlcN
GlcN + CoA-Ac + UTP --> UDP-GlcNAc
UDP-GlcNAc --> ManNAc + UDP
ManNAc + PEP --> NeuNAc + P_i

Figure 3: Scheme of the sialic acid synthesis in bacteria.

Important metabolites and intermediates are glutamine (Gln), N-acetyl-mannosamine (ManNAc) and phosphoenolpyruvate (PEP). N-acetyl-neuraminic acid (NeuNAc) is then polymerized in the cytosole by a multi enzyme complex and transported to the cell surface. There it is fixed in the outer cell membrane by a phospholipid-anchor.

Growth medium

For the *E.coli* K1 strain B2032/82 the influence of different media on the polySia productivity has been investigated. All media were of the same buffer and mineral-concentration but varying substrate and N-source. Among others, substrates and N-sources were chosen which are directly or indirectly involved in the biosynthetic way of bacterial polySia production. The results are shown in figure 4. The highest yield was achieved using xylose/L-proline. The most inexpensive way is the application of glucose/$(NH_4)_2SO_4$. A good compromise with average yield and costs is a medium containing xylose/Na-glutamate. For a further optimization a combined application of different substrates and N-sources is possible. Also the development of a feed strategy in fed-batch cultures may improve the production process.

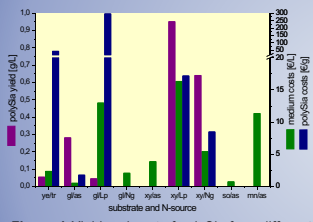

Figure 4: Yield and cost of polySia from different media. No bar means polySia concentration was below the limit of detection. (ye - yeast extract; gl glucose; xy - xylose; so - sorbitol; mn - mannose; as - $(NH_4)_2SO_4$; Lp - L-prolin; Ng - Na-glutamate)

E.coli K1 strains

In another experiment the productivity of different natural *E.coli* K1 strains has been compared. All strains produce the human-like polySia of 2,8-linked 5-N-acetyl-neuraminic acid. The cultivations were performed in shaking flasks with complex medium containing yeast extract and tryptone. The particular cultivations of the same strains partly showed a great variation. All production rates were in the area of 0,05 to 0,17 g/L polySia.

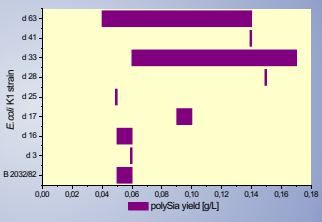

Figure 5: polySia yield for different natural *E.coli* K1 strains in complex medium (d 3, 17, 33, 63 - strains isolated from stool; d 25, 28, 41 - strains isolated from urine; d 16 - strain isolated from cervix)

Detection

The content of polySia has been determined colorimetrically by the thiobarbituric acid (TBA) assay. The method is a further development of the assay for the quantitative determination of sialic acids [Warren/Aminoff, 1959]. In the process, the hydrolyzed polySia is at first oxidized with periodate to -formylpyruvic acid. This intermediate product reacts quantitative with two molecules of thiobarbituric acid to form a pink dye with an absorption maximum of 549 nm. A following extraction with cyclohexanone stabilizes the dye for about 12 hours. Figure 6 shows a dilution series of colominic acid prepared as described above. Depending on the employed amount of TBA there is a linear correlation of the absorption and the polySia-concentration (figure 7). The detection limit of this method is about 0,5 mg polySia. The described assay is very specific as it only responds to 2-keto-3-deoxy sugar acids.

Figure 6: Dilution series of colominic acid coloured with the TBA-assay

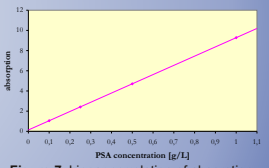

Figure 7: Linear correlation of absorption and polySia-concentration

Application and outlook

Demands on scaffold materials are very high. Polysialic acid fulfills a number of requirements for the application as an artificial growth matrix for cells. It is biocompatible, immunologically inert and there is no specific degradation system in the human body. However, for the specific degradation of polySia, phage born enzymes, the endosialidases, can be used. To ensure a sufficient supply for comprehensive studies of this material the optimized process for the production of polysialic acid in *E.coli* K1 has to be upscaled to large bioreactor scale. Furthermore a downstream strategy based on the purification methods for polysaccharides is currently in development.

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Evaluation eines neuen Werkstoffs als Gerüstsubstanz
polysia
für die Herstellung artifizieller Gewebe