

Brief report

Production of functionalized oligo-isoprenoids by enzymatic cleavage of rubber

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Summary

In this study, we show the proof of concept for the production of defined oligo-isoprenoids with terminal functional groups that can be used as starting materials for various purposes including the synthesis of isoprenoid-based plastics. To this end, we used three types of rubber oxygenases for the enzymatic cleavage of rubber [poly(*cis*-1,4-isoprene)]. Two enzymes, rubber oxygenase RoxA_{Xsp} and rubber oxygenase RoxB_{Xsp}, originate from *Xanthomonas* sp. 35Y; the third rubber oxygenase, latex-clearing protein (Lcp_{K30}), is derived from Gram-positive rubber degraders such as *Streptomyces* sp. K30. Emulsions of polyisoprene (latex) were treated with RoxA_{Xsp}, RoxB_{Xsp}, Lcp_{K30} or with combinations of the three proteins. The cleavage products were purified by solvent extraction and FPLC separation. All products had the same general structure with terminal functions (CHO-CH₂- and -CH₂-COCH₃) but differed in the number of intact isoprene units in between. The composition and *m/z* values of oligo-isoprenoid products were determined by HPLC-MS analysis. Our results provide a method for the preparation of reactive oligo-isoprenoids that can likely be used to convert polyisoprene latex or rubber waste materials

into value-added molecules, biofuels, polyurethanes or other polymers.

Introduction

Natural rubber has been produced in huge amounts for more than a century by cultivating the rubber tree (*Hevea brasiliensis*), and the material is used for a variety of applications, as an example for the production of rubbers, tyres, sealings, latex gloves and many other items. The main component of rubber is the hydrocarbon poly(*cis*-1,4-isoprene). For most of today's applications of rubber, an important material property is the molecular weight of the polymer that – when high – gives rise to superior material properties that are necessary for example for the production of tyres. However, no attention has been given so far to the use of rubber for the biotechnological preparation of low molecular fine chemicals (Förster-Fromme and Jendrossek, 2010; Kamm, 2014; Akhlaghi *et al.*, 2015; Schrader and Bohlmann, 2015). In this contribution, we describe the proof of concept for the use of rubber oxygenases to cleave polyisoprene-containing (waste) materials to low molecular products and to produce functionalized oligo-isoprenoids with defined structure. The generated products can be used either directly as biofuels or value-added materials which can be obtained by conversion of oligo-isoprenoids to new products such as polyurethanes and related isoprene-containing polymers.

Only two major types of rubber-cleaving enzymes have been described so far. One is the rubber oxygenase RoxA that was first isolated from *Xanthomonas* sp. 35Y (Tsuchii and Takeda, 1990; Braaz *et al.*, 2004) and has been found only in Gram-negative rubber-degrading bacteria (Birke *et al.*, 2013). The genome sequence of *Xanthomonas* sp. 35Y has been determined (Sharma, V., Siedenburg, G., Birke, J., Mobeen, F., Jendrossek, D., Srivastava, T.P. unpubl. data). RoxA of *Xanthomonas* sp. 35Y (RoxA_{Xsp}) is a *c*-type dihaem dioxygenase (≈70 kDa, Fig. 1A) and cleaves poly(*cis*-1,4-isoprene) into 2-oxo-4,8-dimethyl-trideca-4,8-diene-1-al (ODTD), a C₁₅ compound with a terminal keto and aldehyde group as the main product (Fig. 1B) (Braaz *et al.*,

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2005; Schmitt *et al.*, 2010). The structure of RoxA_{Xsp} has been solved (Seidel *et al.*, 2013), and molecular insights in the active site of RoxA_{Xsp} as well as the cleavage mechanism have been obtained by the construction and biochemical characterization of RoxA_{Xsp} muteins (Birke *et al.*, 2012).

The second type of rubber oxygenase is a protein designated as latex-clearing protein (Lcp) (Rose *et al.*, 2005; Hiessl *et al.*, 2012; Yikmis and Steinbüchel, 2012). Lcps (≈ 40 kDa, Fig. 1A) are widespread in or even specific for Gram-positive rubber-degrading bacteria, such as *Streptomyces* sp. K30 (Lcp_{K30}) (Rose *et al.*, 2005), *Gordonia polyisoprenivorans*, *Gordonia westfalica* (Arenskötter *et al.*, 2001; Bröker *et al.*, 2008), and were recently isolated from *Gordonia polyisoprenivorans* VH2 (Hiessl *et al.*, 2014) *Streptomyces* sp. K30 (Birke *et al.*, 2015; Röther *et al.*, 2016) and from *Rhodococcus rhodochrous* RPK1 (Watcharakul *et al.*, 2016). The amino acid sequences of RoxAs and Lcps are not related although both enzymes catalyse the oxidative cleavage of the double bonds in poly(*cis*-1,4-isoprene) and both cleave polyisoprene to products with terminal keto and aldehyde groups (Fig. 1B). In contrast to RoxAs that cleave rubber to only one major end-product (ODTD), Lcps produce a mixture of oligo-isoprenoids (C₂₀, C₂₅, C₃₀ and higher oligo-isoprenoids, Fig. 2) (Ibrahim *et al.*, 2006; Birke and Jendrossek, 2014). Lcps are *b*-type cytochromes and share a common domain of unknown function (DUF2236) (Hiessl *et al.*, 2014; Birke *et al.*, 2015). Recently, the importance of several strictly conserved residues within the DUF2236 domain for stability and activity was determined (Röther *et al.*, 2016).

Very recently, a third type of rubber oxygenase, RoxB, was discovered (Birke *et al.*, 2017). The coding sequence is provided under the accession No KY

498024. RoxB_{Xsp} was identified as a RoxA_{Xsp} homologue in *Xanthomonas* sp. 35Y and shared some properties with RoxAs: RoxB_{Xsp} is also a *c*-type dihaem protein with an apparent molecular weight of around 70 kDa, but it has only a low sequence similarity to RoxA_{Xsp} (38%). However, RoxB_{Xsp} differs from RoxAs in cleaving polyisoprene to a mixture of oligo-isoprenoids (C₂₀, C₂₅, C₃₀ and higher oligo-isoprenoids, Fig. 2B). This has previously been described only for Lcps. Therefore, RoxB_{Xsp} combines properties of RoxAs and Lcps (Birke *et al.*, 2017). RoxB is related in amino acid sequence to the *latA* gene product of *Rhizobacter gummiphilus* (83%) (Kasai *et al.*, 2017). The *latA* gene was recently discovered to code for a protein that is responsible for the cleavage of polyisoprene in *R. gummiphilus*. However, no information on the properties of the expressed LatA protein is yet available.

Recombinant overexpression of rubber oxygenases

Despite the fact that all so far described rubber oxygenases must be post-translationaly modified to incorporate the haem cofactor, overexpression of highly active rubber oxygenases is surprisingly easy: RoxA_{Xsp} can be expressed extracellularly in quantities of ≈ 15 mg l⁻¹ from recombinant *Xanthomonas* sp. 35Y strains which harbour a *roxA_{Xsp}* gene on the chromosome under the control of an rhamnose-inducible promoter (Hamsch *et al.*, 2010; Birke *et al.*, 2012). We assume that the amount of produced rubber oxygenase can be increased by a combination of medium optimization, inducer concentration and the time point of addition and harvest. Lcps have been successfully overexpressed intracellularly in recombinant *E. coli* using conventional induction by rhamnose (Birke *et al.*, 2015; Watcharakul *et al.*, 2016) or via autoinduction (Andler and Steinbüchel, 2017). Secretion of the

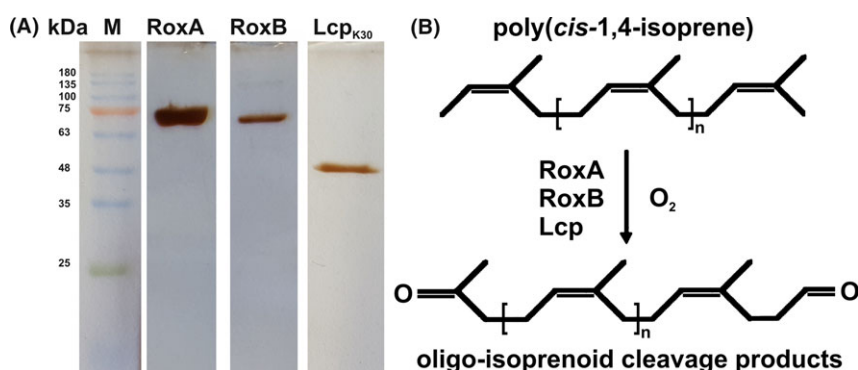


Fig. 1. SDS-PAGE of purified rubber oxygenases. RoxA_{Xsp} and RoxB_{Xsp} were purified from filter-concentrated supernatants of L-rhamnose/LB-medium-grown Δ *roxA* *Xanthomonas* sp. 35Y cells with genome-integrated *roxA_{Xsp}* or *roxB_{Xsp}* gene respectively. Lcp_{K30} was purified from soluble French-press extracts of L-rhamnose/LB-medium-grown *E. coli* (p4782.1::*strep-lcp_{K30}*) via Strep-Tactin HC gravity flow affinity chromatography.

A. Purified proteins were separated by SDS-PAGE and stained with silver. A molecular mass standard (M) with kDa values indicated is shown. B. Oxidative cleavage of rubber. Poly(*cis*-1,4-isoprene) ($100 < n \approx 10\,000$) is oxidatively cleaved by rubber oxygenases to oligo-isoprenoids with terminal keto- and aldehyde groups. The methanol-soluble products differ in the number of intact isoprene units (n) with $1 \leq n \approx 12$.

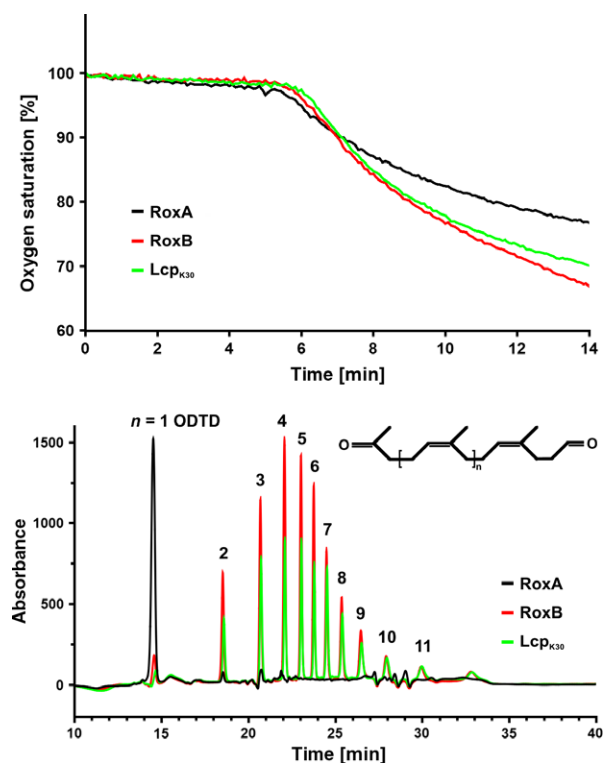


Fig. 2. Activities and product analysis of rubber oxygenases. Activities of purified rubber oxygenases (Lcp_{K30}, RoxA_{Xsp} and RoxB_{Xsp}) were determined by following the consumption of dissolved oxygen at 37°C in a Oxy4 V2 apparatus, Presens, Regensburg, Germany, as described recently (Röther *et al.*, 2017) (top). 4 µg each of Lcp_{K30}, RoxA_{Xsp} or RoxB_{Xsp} was added to 1 ml of an emulsion of polyisoprene latex in potassium phosphate buffer (100 mM, pH 7) at ≈5.5 min. The initial slopes correspond to specific activities of 6.2, 2.6, 6.4 U mg⁻¹ for Lcp_{K30}, RoxA_{Xsp} or RoxB_{Xsp} respectively. One unit corresponds to the consumption of one molecule of dioxygen per minute. The products of polyisoprene cleavage were determined by HPLC-based analysis of the ethylacetate-extracted cleavage products (bottom). For Lcp_{K30} and RoxB_{Xsp}, a typical pattern of oligo-isoprenoids varying in the number of subunits ($n = 2-11$) was observed. For RoxA, 12-oxo-4,8-dimethyltrideca-4,8-diene-1-ol (ODTD, $n = 1$) was detected as the major cleavage product.

mature Lcps via the TAT secretion pathway in *E. coli* (Yikmis *et al.*, 2008) or *Bacillus subtilis* (van Dijk and Hecker, 2013) should be possible. However, the secretion pathways used for RoxA and RoxB proteins have not yet been identified. If pure proteins are necessary, tagged versions of Lcps can be purified in high yields using a one step affinity chromatography (≈ 15 mg Lcp_{K30} l⁻¹ culture for Strep-tagged Lcp). The tag also offers the opportunity for enzyme immobilization. Furthermore, over-production of haem containing rubber oxygenases might be limited by the intracellular availability of the cofactor. An increase in the efficiency of haem biosynthesis, e.g., by the expression of gamma-aminolevulinic acid synthase and gamma-aminolevulinic acid dehydratase could be used to overcome this limitation (Doss and Philipp-Dormston, 1975).

Purification of rubber oxygenases

We purified each one representative of the three types of rubber oxygenase (RoxA_{Xsp}, RoxB_{Xsp} and Lcp_{K30}, Fig. 1) and used the purified proteins alone or in combination for the production of oligo-isoprenoids from polyisoprene latex. Produced oligo-isoprenoids were purified by HPLC and FPLC, and the identity of the isolated products was confirmed by ESI-MS analysis.

Untagged RoxA_{Xsp} and RoxB_{Xsp} were purified from the culture fluid of recombinant Δ roxA *Xanthomonas* sp. 35Y strains which harboured either the roxA_{Xsp} or the roxB_{Xsp} gene integrated into the chromosome under the control of an L-rhamnose-inducible promoter using a two-step purification procedure as described recently (Birke *et al.*, 2012, 2017). Lcp_{K30} was expressed intracellularly in form of an N-terminal Strep-tagged protein and was purified from recombinant *E. coli* as described previously (Röther *et al.*, 2016). Fig. 1A shows that all three proteins were of high purity and activity determinations confirmed high specific activities of 2.6 U mg⁻¹ (RoxA_{Xsp}), 6.2 U mg⁻¹ (Lcp_{K30}) and 6.4 U mg⁻¹ (RoxB_{Xsp}) at 37°C for the three purified rubber oxygenases (Fig. 2 top). HPLC analysis of the solvent-extracted products confirmed the cleavage of polyisoprene to ODTD (C₁₅ oligo-isoprenoid) as major product by RoxA_{Xsp} and the formation of a mixture of C₂₀ and higher oligo-isoprenoids in case of RoxB_{Xsp} and Lcp_{K30} (Fig. 2 bottom). ODTD was present only in minor amounts in the products obtained from RoxB_{Xsp} and Lcp_{K30}.

The finding of only one cleavage product (C₁₅ oligo-isoprenoid ODTD) for the RoxA_{Xsp}-catalysed reaction and the identification of multiple cleavage products (C₂₀ and higher oligo-isoprenoids) in case of the RoxB_{Xsp}- or Lcp_{K30}-cleaved polyisoprene suggested that RoxA_{Xsp} on the one side and RoxB_{Xsp} and Lcp_{K30} on the other side employ different cleavage mechanisms. We assume that RoxA_{Xsp} has a 'molecular ruler' and uses an *exo*-type mechanism to cleave the polyisoprene chain (Seidel *et al.*, 2013). This explains the formation of only one main cleavage product of a defined length (ODTD). In contrast, in case of RoxB_{Xsp} and Lcp_{K30}, the formation of multiple products of different length suggests that these rubber oxygenases do not have such a molecular ruler and cleave the polyisoprene chain randomly in an *endo*-type mechanism resulting in the observed mixture of oligo-isoprenoids of different lengths.

Synergistic effect of RoxB and of Lcp on polyisoprene cleavage by RoxA

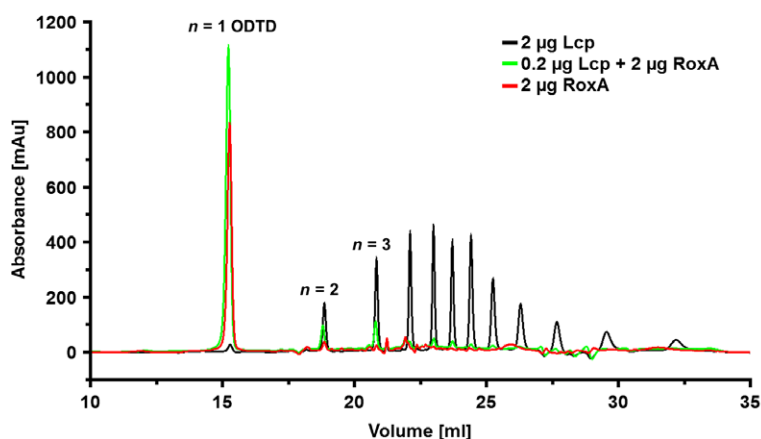
The generation of oligo-isoprenoids by *endo*-cleavage of polyisoprene molecules (with RoxB_{Xsp} or Lcp_{K30})

increases the number of free polyisoprene chains. A higher concentration of polyisoprenoid ends should enhance the efficiency of polyisoprene cleavage by rubber oxygenases with an endo-type cleavage such as RoxA_{Xsp}. We therefore determined whether the amount of ODTD produced by RoxA could be increased by the presence of trace amounts of RoxB_{Xsp} or Lcp_{K30}. The presence of 0.2 µg ml⁻¹ purified RoxB_{Xsp} or Lcp_{K30} in the assay mixture did not lead to the formation of substantial amounts of ODTD (factor being < 0.02 relative to 1.0 by 2 µg of RoxA_{Xsp}, Fig. 3). However, when combined, 2 µg ml⁻¹ RoxA_{Xsp} and 0.2 µg ml⁻¹ purified RoxB_{Xsp} or Lcp_{K30} increased the amount of produced ODTD by a factor of 1.4 or 1.5, respectively, in comparison with the values obtained with 2 µg RoxA_{Xsp} or Lcp_{K30} alone (Fig. 3). Furthermore, the synergistic effect was investigated with respect to a kinetic effect enhancing the speed of the cleavage reaction, representing a major factor to be considered upon industrial employment of the reaction. To this end, the oxygen consumption rates by Lcp_{K30} (0.4 µg) and RoxA_{Xsp} (4 µg) alone were determined, combined (added) *in silico* and were then compared to an experiment in which both enzymes were simultaneously present. As evident from Fig. 4, the simultaneous presence of low amounts of Lcp_{K30} increased the specific oxygen consumption by a factor of 1.4 (2.6 U mg⁻¹) relative to the *in silico* combined oxygen consumption rates (1.8 U mg⁻¹). These results also showed that the presence of terminal aldehyde and keto groups did not inhibit the cleavage of these oligo-isoprenoids to ODTD by RoxA_{Xsp}. Furthermore, the efficiency of rubber degradation was enhanced when each

an *endo*- and *exo*-type rubber oxygenase were simultaneously present. These data provide a plausible explanation for the presence of the *roxA* and *roxB* gene in *Xanthomonas* sp. 35Y due to a synergistic effect; in the presence of both gene products, ODTD is the only observed cleavage product for the facilitated uptake into the cells and use as a source of carbon and energy.

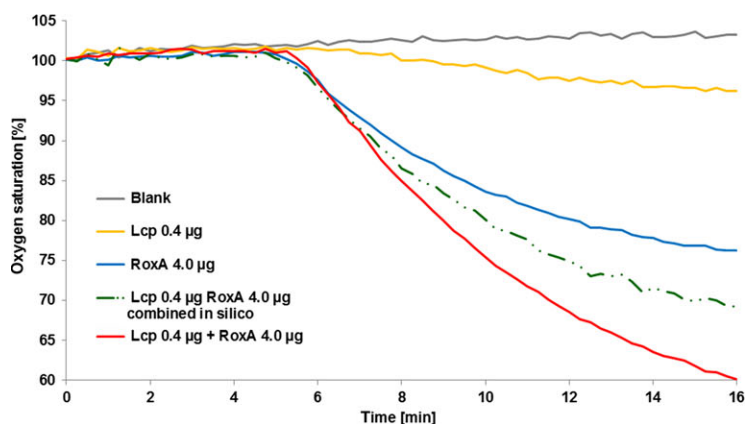
Separation and purification of oligo-isoprenoids

As shown in Fig 2B, the cleavage of polyisoprene by RoxB_{Xsp} or by Lcp_{K30} yielded a mixture of oligo-isoprenoids (C₂₀ and higher oligo-isoprenoids). For the application of these compounds as fine chemicals or as building blocks for (polymer) plastic synthesis in organic chemistry, the preparation of large amounts of pure oligo-isoprenoids is preferable. To demonstrate the isolation of isoprenoids at a higher scale, we increased the volume of polyisoprene latex and replaced the HPLC-based separation of oligo-isoprenoids by an FPLC separation system because FPLC systems can be up-scaled more easily than HPLC-based separations. As a proof of principle, we treated 1 litre of 5% (wt/vol) polyisoprene latex in 100 mM potassium phosphate buffer, pH 7 with 4 mg of purified Lcp_{K30} and incubated the assay mixture for 24 h at room temperature while stirring at 200 rpm. The produced oligo-isoprenoids were solvent-extracted with 100 ml ethylacetate. The solvent was evaporated, and the products (≈100 mg) were dissolved in 5 ml methanol. 200 µl of the dissolved products was then applied to a PEP RPC HR5/5-FPLC column that had been equilibrated with 50% methanol: water and eluted



Enzyme combination	Relative amount of ODTD
Lcp 0.2 µg	<0.01
RoxB 0.2 µg	<0.02
Lcp 2 µg	0.03
RoxB 2 µg	0.15
RoxA 2 µg	1.00
RoxA 4 µg	1.70
Lcp 2 µg + RoxA 2 µg	2.11
RoxB 2 µg + RoxA 2 µg	2.15
Lcp 0.2 µg + RoxA 2 µg	1.40
RoxB 0.2 µg + RoxA 2 µg	1.52

Fig. 3. Synergistic effect during rubber cleavage. Polyisoprene latex was cleaved by different amounts and combinations of rubber oxygenases as indicated (left). The amounts of produced ODTD (Table on the right) were determined from the ODTD areas in HPLC chromatograms (exemplary shown in the image on the left). The ODTD-specific area obtained for 2 µg of RoxA_{Xsp} was set as 1.0. The addition of only 0.2 µg Lcp increased ODTD formation by 2 µg of RoxA_{Xsp} by a factor of 1.4 and only trace amounts of higher oligo-isoprenoids ($n = 2$ and 3 ; n indicates the number of intact isoprene units, see structure shown in Fig. 1B) were determined. A similar effect with 1.5-fold higher amount of produced ODTD was observed for a combination of 2 µg RoxA_{Xsp} and 0.2 µg RoxB_{Xsp}.



Enzyme combination	Specific activity [U/mg]
Lcp 0.4 µg	1.7
RoxA 4 µg	1.5
Lcp and RoxA combined <i>in silico</i>	1.8
Lcp 0.4 µg + RoxA 4 µg	2.6

Fig. 4. Synergistic effect of the presence of Lcp_{K30} on the specific activity of RoxA_{Xsp}. The oxygen consumption rates of 0.4 µg Lcp_{K30}, of 4 µg RoxA_{Xsp} and of a mixture of 0.4 µg Lcp_{K30} and of 4 µg RoxA_{Xsp} were recorded. The values for 0.4 µg Lcp_{K30} and 4 µg RoxA_{Xsp} were combined *in silico* and the slope of the resulting curve was calculated to determine a theoretical specific activity. Note that the specific activities of the reaction in the presence of both enzymes were 1.4-fold higher (2.6 U mg⁻¹) compared to the *in silico* combined values of the two reactions with the single enzymes (1.8 U mg⁻¹).

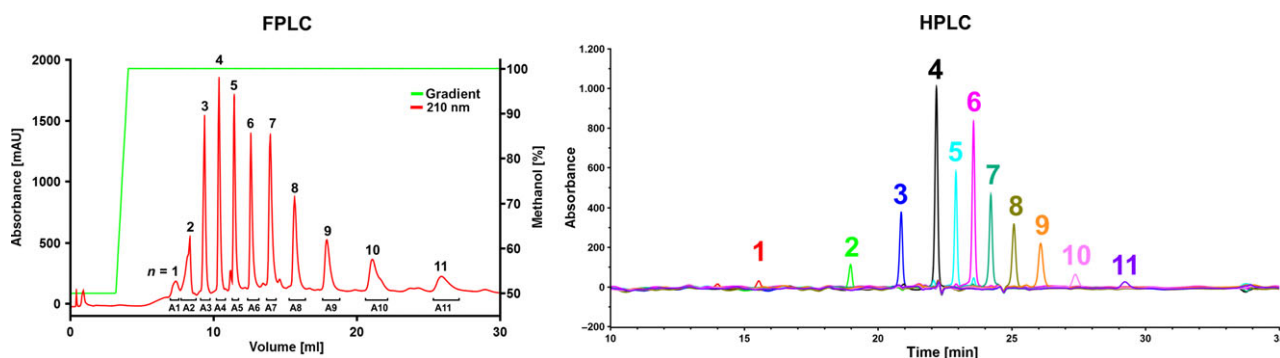


Fig. 5. Separation of oligo-isoprenoid mixtures by FPLC, HPLC and HPLC-MS. 200 µl oligo-isoprenoid solution in methanol (prepared by digestion of polyisoprene with Lcp_{K30} as described in the main text) was applied to a reversed-phase FPLC column (Pep RPC HR 5/5, 1 ml bed volume) and separated by a step gradient from 50% water: methanol to 100% methanol (green line) (left image). Absorption at 210 nm (red line) was used to fractionate peaks representing different oligo-isoprenoids (A1–A11, corresponding to $n = 1–11$). Aliquots of each separately collected fraction (A1 to A11) were applied to analysis via HPLC. An overlay image consisting of all eleven HPLC chromatograms is shown on the right. The superposition of the chromatograms shows the high resolution power of the used FPLC column. The masses (m/z values) of each isolated compound were confirmed by HPLC-ESI-MS and are provided in Table 1.

by the application of an increasing step gradient to 100% methanol at a constant flow rate of 1.5 ml min⁻¹. Peaks were automatically fractionated (≈ 2 ml per peak) by monitoring the absorbance at 210 nm. As shown in Fig. 5 left, the same eleven individual peaks were identified that had been detected on the analytical HPLC column (Fig. 2). The compound of each of the separated peaks was collected individually, concentrated by evaporation and dissolved in 100 µl of methanol. When each of the isolated compounds was separately run on the analytical HPLC column, the successful isolation of each oligo-isoprenoid was demonstrated by the appearance of one homogeneous peak (Fig. 5, right). The m/z values of the isolated oligo-isoprenoids were determined by HPLC-MS and were in agreement with the structural

formulas and the theoretical values for the individual oligo-isoprenoids (Table 1).

Conclusions and outlook

Polyisoprene in form of natural rubber latex is a cheap bulk compound and is available in the ton-scale. Cleavage of polyisoprene by rubber oxygenases and separation of produced oligo-isoprenoids is fairly possible. In this study, eleven oligo-isoprenoids of the 'ODTD-family' with one to eleven central isoprene units (n) between the terminal aldehyde and keto functional groups could be separately prepared. The highest yields were obtained for ODTD (RoxA_{Xsp} alone) and for the C₃₀ to C₅₀ compounds (Lcp_{K30} or RoxB_{Xsp} alone). Purification of oligo-

Table 1. Oligo-isoprenoids produced by enzymatic cleavage of polyisoprene using purified rubber oxygenases.

No of intact isoprene units [n]	Elemental formula	m/z	m/z [M+H] ⁺	m/z [M+Na] ⁺	m/z [M+Na+CH ₃ OH] ⁺	FPLC peak area [%]
1	C ₁₅ H ₂₄ O ₂ (ODTD) 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al	236.178	237.185	259.167	291.193	2.9
2	C ₂₀ H ₃₂ O ₂	304.240	305.248	327.230	359.256	6.8
3	C ₂₅ H ₄₀ O ₂	372.303	373.310	395.292	427.318	9.6
4	C ₃₀ H ₄₈ O ₂	440.365	441.373	463.355	495.381	10.5
5	C ₃₅ H ₅₆ O ₂	508.428	509.435	531.417	563.444	10.3
6	C ₄₀ H ₆₄ O ₂	576.491	577.498	599.480	631.506	10.4
7	C ₄₅ H ₇₂ O ₂	644.553	645.561	667.543	699.569	12.4
8	C ₅₀ H ₈₀ O ₂	712.616	713.623	735.605	767.631	10.1
9	C ₅₅ H ₈₈ O ₂	780.678	781.686	803.668	835.694	8.7
10	C ₆₀ H ₉₆ O ₂	848.741	849.748	871.730	903.757	9.5
11	C ₆₅ H ₁₀₄ O ₂	916.804	917.811	939.793	971.819	9.0

Polyisoprene latex was treated with purified rubber oxygenase (Lcp_{K30}), and cleavage products were extracted with ethylacetate and dissolved in methanol. Products were analysed by HPLC-ESI-MS analysis before and after purification of individual peaks by FPLC (Fig. 4). For each compound the theoretical m/z values and the values corresponding to the protonated ([M+H]⁺), the sodium ion adduct ([M+Na]⁺) and for the sodium ion+ methanol adduct forms ([M+Na+CH₃OH]⁺) are indicated. The relative amounts (in %) of each prepared oligo-isoprenoid are also provided.

isoprenoids by FPLC can be easily up-scaled for the mass production of oligo-isoprenoids. The use of tyres and other materials containing vulcanized rubbers as substrates for enzymatic degradation by different rubber oxygenases is also possible; however, the presence of sulfur bridges and other components complicates the efficiency of enzymatic cleavage of vulcanized rubber waste and therefore limit – at present – the use of rubber oxygenases to the cleavage of unprocessed natural rubber latex. Mechanical, chemical and/or physical pre-treatments of rubber wastes (e.g. grinding, solvent extraction, desulphurization) might help to make processed rubber wastes also accessible for enzymatic cleavage. Isoprenoids derived from rubber can be used for the production of fragrances, hormones and pharmaceuticals, creating interest in cheap synthesis pathways see (Förster-Fromme and Jendrossek, 2010; Schewe *et al.*, 2015). Furthermore, they can be also used in chemical or enzymatic cyclization reactions (Siedenburg *et al.*, 2012, 2013) for the production of cyclic compounds or can be used as biofuels (Mewalal *et al.*, 2017). This study provides purified, reactive oligo-isoprenoids that can likely be used to convert rubber waste, e.g., from tires into precursors for the synthesis of value-added compounds. The reactivity of the aldehydes might be directly used to form covalent bonds with other molecules (e.g. with amines). Alternatively, the keto groups of the oligo-isoprenoids can be chemically or enzymatically reduced to the corresponding mono- or di-alcohols. The reduction in the C₁₅ compound ODTD to the corresponding alcohol by enzymatic reduction has been previously demonstrated (Braaz *et al.*, 2005). Enzymatic generation of isoprenoid-diols can help to provide precursors for the production of polymers from sustainably produced

monomers, e.g., for the production of polyurethanes and might be an alternative to chemical methods for the conversion of polyisoprenes to polyurethanes (Anancharoenwong, 2011).

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Conflict of Interest

None declared.

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