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MINI-REVIEW

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Polycarnitine—a new biomaterial

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Abstract The natural product L-carnitine is—due to its biotechnological accessibility and specific properties—on the way to becoming an attractive biobased bulk product. L-Carnitine is a natural betaine with vitamin properties. Carnitine is an essential part of the fatty acid metabolism of human beings and animals. Carnitine was first isolated in 1905 from meat extract and important recent developments include the biosyntheses of L-carnitine from L-lysine or γ-butyrobetaine. Our synthesis routes are designed to maintain the primary structure and specific properties of carnitine, such as hydrophilicity and "stiffening" effects for polymeric structures and applications. L-Carnitine is converted via lactonization or olefinization into polymerizable basic molecules. The properties and the applications of carnitine polymers are described.

Introduction

Carnitine (CAS:1-propanaminium, 3-carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-hydroxide, inner salt) exists in the form of optically active enantiomers (*R*)-carnitine [L(−)-carnitine; Fig. 1, compound 1] and (*S*)-carnitine [D(+)-carnitine; Fig. 1, compound 2] and as the racemic compound (*RS*)-carnitine (DL-carnitine). L-Carnitine (Fig. 1, compound 1) is widespread in the tissues of animals, plants and microorganisms.

L-Carnitine (Fig. 1, compound 1) is an extremely hydrophilic compound. The solubility of L-carnitine in water shows a value of 250 g in 100 ml water at 20°C). Carnitine is soluble in alcohols, but insoluble in acetone. Despite its hydrophilicity, carnitine is a stable substance. L-Carnitine is non-toxic (rat oral LD_{50} >5,000 mg kg^{-1}), non-mutagenic (Ames-test negative) and shows almost no skin irritation (rabbit: mild irritant).

L-Carnitine (Latin: caro, carnis = meat) is found in high concentrations in the muscle of both vertebrates and invertebrate animals (Murray et al. 1980) and is a characteristic component of the skeletal muscles of animal tissues and the liver. L-Carnitine is an essential cofactor of fatty acid metabolism. The effect of L-carnitine is based on its ability to stimulate fatty acid oxidation, thereby increasing oxygen consumption. Within fatty acid metabolism, carnitine serves as a carrier for acyl groups through the mitochondrial membrane. The acyl groups are transferred by acyltransferase from acyl-coenzyme A onto the hydroxyl group of L-carnitine (Fig. 1, compound 1). Transporting L-carnitine and acyl-L-carnitin through the membrane is performed via the transport protein, translocase (Fritz et al. 1963). L-Carnitine is essential for certain insects, e.g. mealworm and therefore it was formerly called Vitamin B_T (T for *Tenebrio molitor*) or mealworm factor (Budavari 1989).

Today, L-carnitine and acetyl-L-carnitine are applied in cosmetics (water reservoir, electrolyte, "stiffening" effects; Konrad 1995) and in pharmacy, e.g. in myocardial disorders (Bahl and Bressler 1987), geriatrics (Bartolomucci and Weltevreden 2000) and certain types of diabetic disease (Bresica et al. 2002). Carnitine (in particular L-carnitine) is prepared in different ways by extraction (Friedmann et al. 1960), by chemical methods, e.g. from carbohydrates (Bellamy et al. 1990) or from glycerin (Marzi et al. 2000), and by microbial conversion (in particular by the resolution of racemic carnitine by e.g. *Acinetobacter*, *Pseudomonas*,

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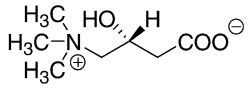

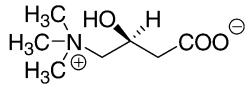

Fig. 1 Enantiomers of carnitine

Escherichia coli, *Corynebacterium*), from its precursors by microorganisms and enzymes and by enantioselective synthesis from achiral precursors (Jung et al. 1993).

Biosynthesis pathways of L-carnitine

L-Carnitine is synthesized from lysine and methionine in the human liver. Biosynthesis research based on L-carnitine started a long time ago: γ-butyrobetaine was administered to dogs, resulting in 3% carnitine in the urine (Linneweh 1928). The origin of the body's own γ-butyrobetaine is ε-*N*-trimethyllysine, which is in turn closely related to lysine (Fraenkel and Friedmann 1957). Based on these studies, a biosynthesis of carnitin was developed, starting with lysin in *Neurospora crassa* (Broquist 1980).

Three main approaches are known using biological systems for the enantioselective synthesis of L-carnitine from achiral precursors: they are described in detail in an excellent review (Jung et al. 1993) and therefore are only briefly mentioned now . First, enzymes are used which are involved in L-carnitine biosynthesis from lysine and methionine (Fig. 2, reaction I). Second, microorganisms and enzymes are applied which are involved in the assimilation of achiral quarternary ammonium compounds, where L-carnitine is produced as an intermediate, thereby blocking the reaction after L-carnitine formation (reaction IV). Third, the return reactions are integrated in L-carnitine degradation under physiological conditions (reactions II, IIIa).

The enantioselective hydroxylation of γ-butyrobetaine and crotonbetain via an *Agrobacterium*-like strain is also used for L-carnitine synthesis. Detailed studies shows that it proceeds via a conventional β-oxidation pathway involved in fatty acid metabolism (Jung and Kleber 1991; Jung et al. 1993).

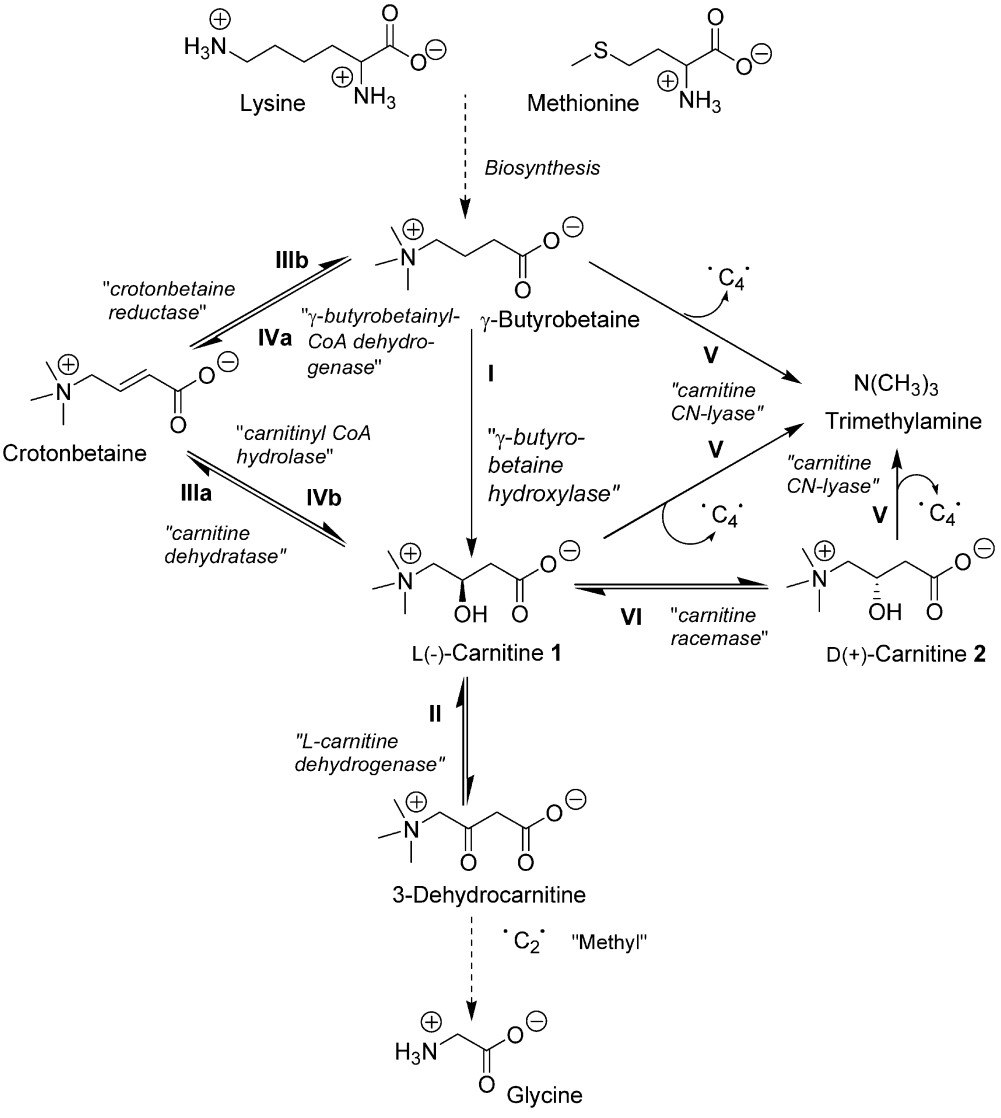

Fig. 2 Microbial metabolism of L-carnitine

Furthermore, strains belonging to the genera *Proteus* (Yokozeki et al. 1988) and *Escherichia* (Castellar et al. 1998) are of interest for the biotransformation of crotonbetaine into carnitine. In the last case, investigations have been performed in batch reactors continuously coated with immobilized cells. However, the achieved carnitine yields of 26% are too low for a large-scale application. γ-Butyrobetainyl-CoA and crotonobetainyl-CoA, which are involved in the carnitin metabolism of *E. coli*, have been isolated and identified to clarify the detailed reaction mechanism (Ellsner et al. 2000; Ellsner and Kleber 2000).

By the enzymatic stereo-inversion of (biologically inactive) D-carnitine (Fig. 1, compound 2) into the target, the bioactive L-enantiomer was produced, using partially purified D- and L-carnitine dehydrogenases. Due to the fact that both dehydrogenase enzymes are NAD(H)-dependent, the end-point of the process is reached within the equilibrium state. Thus, a process yield of only 11% is obtained with permeabilized cells, a yield of 50% with highly stereospecific D- and L-carnitine dehydrogenases from *Agrobacterium* sp. (Hanschmann and Kleber 1997) and 44% with resting cells of *E. coli* O44 K74 (Castellar et al. 1998).

Another group of methods for L-carnitine synthesis from achiral precursors involves the microbial reduction of acetoacetic acid ester derivatives, followed by different chemical reactions (Jung et al. 1993). Thus, γ-chloroacetoacetate is reduced by bakers' yeast (*Saccharomyces cerevisiae)*, thereby converting the keto group stereoselectively into a hydroxyl group (Zhou et al. 1983). In addition, the size of the ester alkyl groups can be varied to achieve high stereochemical purity.

Futhermore, microbial agents (*Alcaligenes*sp.) are used for the stereoselective synthesis of both enantiomers of carnitine from chlorohydrocarbons. Thus, L-carnitine (Fig. 1, compound 1) is generated (as a hydrochloride) through a four-step synthesis using a bacterial solution of 2,3-dichloropropanol (Kasai and Sakaguchi 1992).

Biotechnological production of L-carnitine

An excellent biotechnological procedure for L-carnitine production from γ-butyrobetaine and crotonbetaine was developed by Kulla and coworkers (Kulla and Lehky 1985; Kulla et al. 1986; Kulla 1991). They isolated a microorganism from oil that was able to grow on L-carnitine, γ-butyrobetaine and crotonbetaine as the sole carbon, nitrogen and energy source under aerobic conditions. An important selection criterion was the inability of the strains to utilize D-carnitine. Strain HK4 was phenotypically characterized, but could not be assigned to a genus. Taxonomically, the strain is situated between *Agrobacterium* and *Rhizobium* (Kulla 1991). The production strain for the fermentative production of L-carnitine was obtained by irreversible inactivation of the carnitine dehydrogenase in HK4 by a frame-shift mutation. The yield of L-carnitine was nearly 100%. The product was enantiomerically pure.

Based on these findings, a procedure was developed which allows L-carnitine production from γ-butyrobetaine in a continuous manner (Kulla 1991; Zimmermann et al. 1997). A high biotransformation efficiency is realized by a cell-recycling system which provides a high biomass concentration. Cells in this bioreactor show high metabolic activity at low growth rates. Starting from γ-butyrobetaine, the obtained yield of carnitine is approximately 92%, with a high volumetric productivity (130 g l^{-1} day^{-1}) in the steady state. But two recrystalizations from isobutanol are necessary after the continuous biotransformation process, in order to obtain L-canitine of the purity required by the market (>99%). Compared with this procedure, fed-batch operation provides higher product yields (approximately 99.5%), although the volume productivity (30 g l^{-1} day^{-1}) is significantly lower. To improve the low product to substrate ratio of the continuous process, a multi-stage continuous culture was evaluated, more precisely two bioreactors with two cell-recycling systems. Finally, Lonza AG opted for a fed-batch process, in which lower productivity was compensated by cheaper and simplified downstream processes and a lower investment cost (Zimmermann et al. 1997).

Today, Lonza AG produces in industrial scale L-carnitine in a factory in Kourim (Czech Republic) in a plant with five 15 m^3 fermenters and five 50 m^3 fermenters. In addition, a separate downstream processing plant (for electrodialysis, purification by active carbon, drying, recrystallization) has been installed (Lonza 1999). A comparative ecobalance of the biotechnological processes compared with the Lonza pilot plant for the chemical production of L-carnitine shows clearly less waste and effluents (Brass et al. 1997).

Chemical conversion of carnitine into monomers and polymers

A goal was established to investigate whether structure-changing properties, in particular the high hydrophilicity and the "stiffening" effects of carnitine could be transferred to polymeric products. Thus, synthesis strategies for the formation of polymerizable structures were developed and their feasibility was tested (Fig. 3).

Carnitine (Fig. 3, compound 1), a β-hydroxy-carbonic acid (inner salt) is able to form the four-ring-lactone (Fig. 3, compound 3), which can subsequently perform a ring-opening polymerization. A second synthesis route is the generation of crotonbetaine (apocarnitine; (Fig. 3, compound 4) via the dehydration of carnitine and its radical polymerization. The effort to polymerize crotonbetaine (Fig. 3, compound 4) and its ester (Fig. 3, compounds 5–7) receives further motivation from the fact that crotonbetaine is an intermediate of the biosynthesis of carnitine from lysine (see Fig. 2). The third synthesis route aims to maintain the primary structure of carnitine completely as a side-chain in a polymer. Thus, the possibility of esterification of the carboxyl group in the carnitine molecule can be checked by means of relevant hydroxy-substituted olefin monomers and subsequently the ability to polymerize

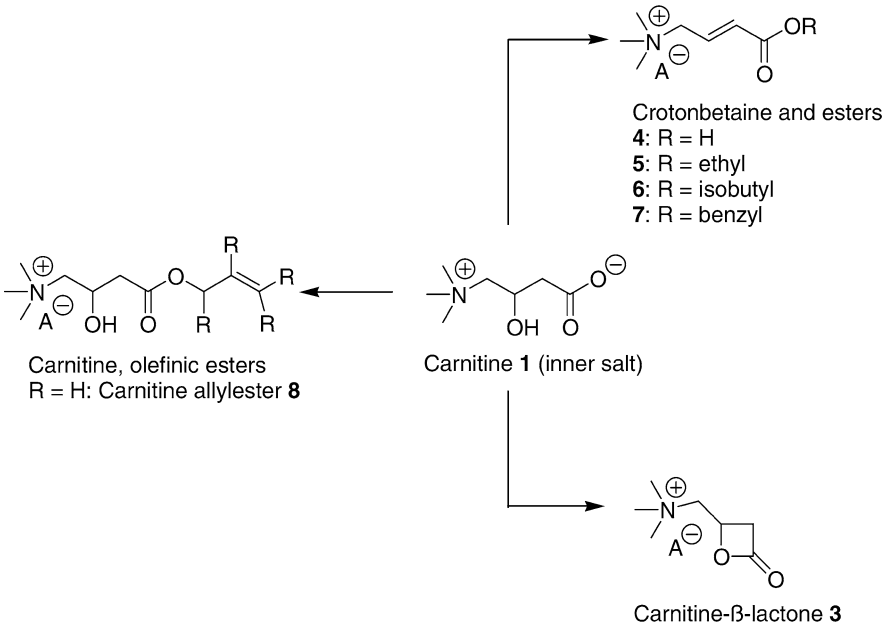

Fig. 3 Structures for polymerization using the natural substance carnitine

the resulting olefin-substituted carnitine structures (Fig. 3, compound 8) can be tested.

Polyester based on carnitine

First, L(−)-carnitine-isobutylester chloride (Fig. 4, compound 9) is synthesized as a starting material for the target synthesis of lactone (Fig. 4, compound 3). L-Carnitine (Fig. 4, compound 1) is solved in a 5-fold volume of 2 N HCl and refluxed for 30 min. L-Carnitine hydrochloride (Fig. 4, compound 1_{HCl}) is then received after isolation in an almost quantitative yield. Subsequently, L-carnitine hydrochloride is esterified in a 5-fold amount of isobutanol, yielding 98% of L(−)-carnitine-isobutylester chloride (Fig. 4, compound 9).

Isobutyl ester (Fig. 4, compound 9) is preferentially sulfonated by reaction with an excess of methanesulfonic anhydride at a temperature of 80°C in the melt. The L(−)-β-mesylate-isobutylester salt (Fig. 4, compound 10) is isolated in a yield of 93% as a white, slightly opalescent solid. By means of 2 N HCl, this product is saponified at 50°C within 20 h. After isolation, the L-(−)-β-carnitine mesylate salt (Fig. 4, compound 11) is obtained in a 87–90% yield. Then, there are different ways for ring closure. After evaluation of the corresponding literature (Bernabei et al. 1994a,b, 1996), we selected the approach using an aqueous system and a basic ion-exchange resin. Lactone (Fig. 4, compound 3) is received under inversion at the asymmetric C-atom in a yield of 81% as a white crystalline product (Kamm 2004).

This first-time synthesis of D(+)-carnitine-β-lactone (Fig. 5, compound 3) can be tested regarding a ring-opening

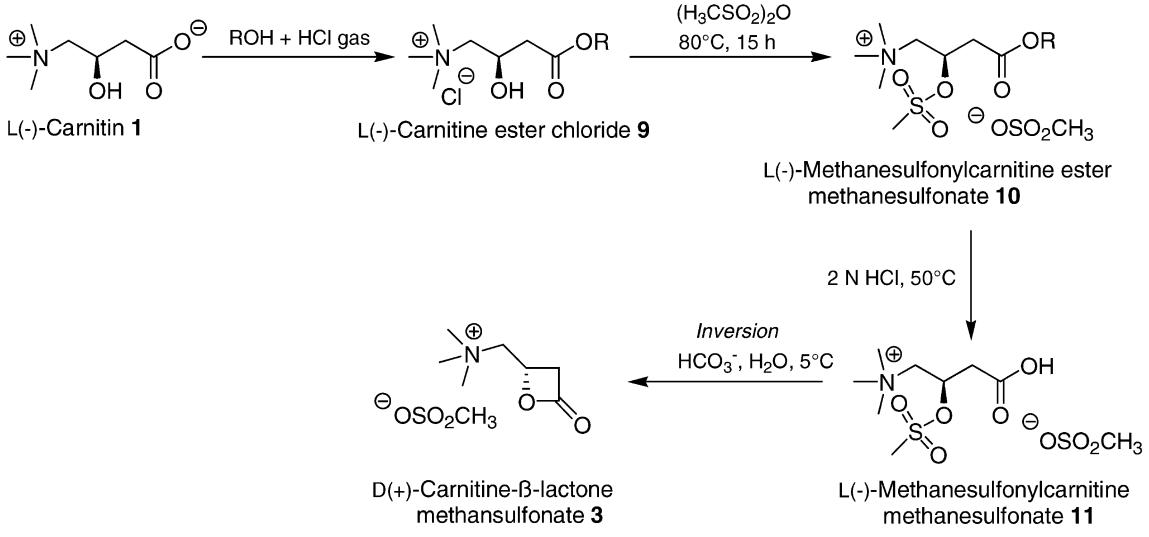

Fig. 4 Synthesis of D(+)-carnitine-β-lactone (*3*) from L(−)-carnitine (*1*)

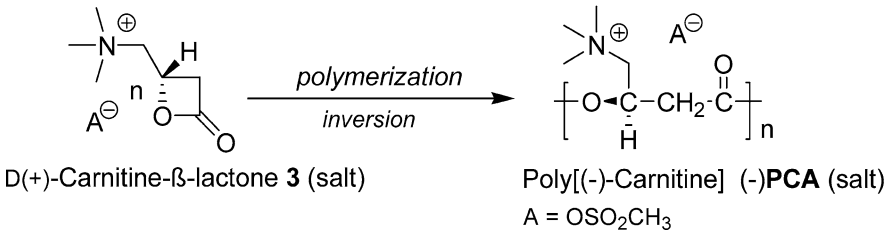

Fig. 5 Ring-opening polymerization of carnitine-β-lactone (*3*) to poly(carnitine), *PCA*

polymerization. In order to achieve high molecular weights, the polymerization of carnitine-β-lactone should be performed in absolutely dry solvents or in the melt. Thus, carnitine-β-lactone (Fig. 5, compound 3) can be converted under absolute exclusion of H_2O, using a nitrogen stream with dimethylformamide as solvent and tetraalkylammoniumcarboxylate as initiator at a temperature of 50°C. Poly(carnitine)—a polyester based on carnitine is separated after purification as a transparent crystalline product in a yield of 75% (Kamm et al. 2000a, 2001a; Kamm 2004; Fig. 5).

Oligomers and polymers based on crotonbetaine

Crotonbetaine hydrochloride (Fig. 6, compound 4) is synthesized from carnitine via the elimination of water with glacial acetic acid/acetoanhydride. Subsequently, crotonbetaine hydrochloride (Fig. 6, compound 4) is esterified with a series of alcohols in a HCl stream, resulting in products such as crotonbetaine ethylester chloride (Fig. 6, compound 5; 88% yield), crotonbetaine isobutylester chloride (Fig. 6, compound 6; 94% yield) and crotonbetaine benzylester chloride (Fig. 6, compound 7; 71% yield; Kamm 2004).

Crotonbetaine hydrochloride (Fig. 6, compound 4) and crotonbetaine ester chloride (Fig. 6, compounds 5–7) were polymerized under radical conditions in aqueous medium, yielding 50–60% oligo- and poly(crotonbetaine) (Kamm et al. 2000b, 2001b; Kamm 2004; Fig. 6).

Thus, crotonbetaine hydrochloride and crotonbetaine ester chloride (Fig. 6, compounds 5–7) were polymerized by peroxodisulfate in water or in methanol/water under a nitrogen stream at a temperature of 90°C within 12–24 h. In the case of crotonbetaine ester (Fig. 6, compounds 5–7, sodium hydrogen carbonate was added to stop saponification of the ester via generated hydrogen sulfate. In addition, a reduction in the reaction time of 2–3 h was achieved under microwave conditions. Also, in order to decrease the reaction temperature to 30–50°C, an ammonium iron-III-sulfate/potassium sulfite redox system was added.

Polymer based on carnitine allylester

The third synthesis strategy leads to polymerizable derivatives with complete maintenance of the carnitinic skeletal structure (Fig. 7, compound 8). L(−)-Carnitine hydrochloride (Fig. 7, compound 1_{HCl} was esterified by allyl alcohol into L(−)-carnitine-allylester chloride (Fig. 7, compound 8), which was then comprehensively characterized (Kamm et al. 2004). Usually, the polymerization of mono-allyl compounds does not lead to higher molecular products, due to chain-termination reactions (Bandrup and Immergut 1999), so it was unexpected that the synthesis of homopolymers like L(−)-carnitine-allylester chloride (Fig. 7, compound 8) was possible by radical initiation.

The polymerization can successfully be performed using peroxodisulfate/sodium hydrogen cabonate in water at temperatures of 70–90°C within 1–3 h under a nitrogen stream; and, after ultrafiltration, the polymer poly(carnitine allylester) is isolated in yields of 85% (Fig. 7).

Properties of carnitine oligomers and polymers

Poly(carnitine)

Poly(carnitine) is a crystalline transparent product. Molecular weights of 6,000–7,000 were determined by gel permeation chromatography and polydispersities of 1.1–1.2 towards a polystyrene standard in dimethylformamide (detection by refractive index, UV light). The structure of the separated polymers was proved by nuclear magnetic resonance spectroscopy, infrared spectroscopy and elemental analysis (Kamm et al. 2000a, 2001a). Differential scanning calorimetry showed a melting temperature of 147–148°C and a crystallization temperature of 85°C. Poly(carnitine)

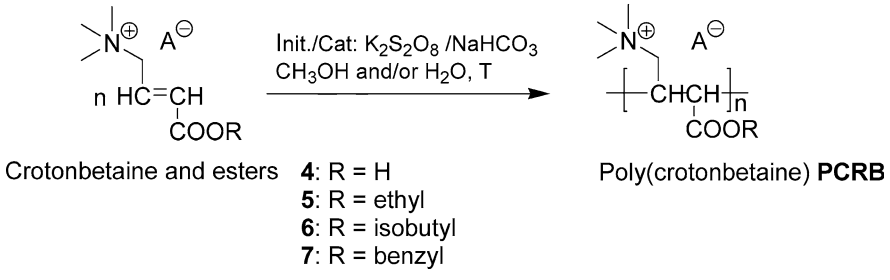

Fig. 6 Synthesis of poly(crotonbetaine), *PCRB*. PCRB includes oligo- and poly(crotonbetaine)

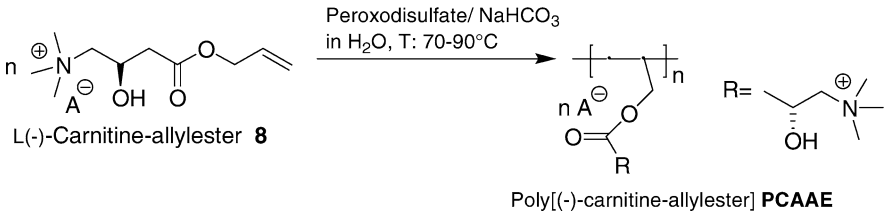

Fig. 7 Synthesis of poly(carnitine allylester), *PCAAE*

is readily soluble in water and methanol and insoluble in acetone. The synthesized poly(carnitine) shows a specific rotation of –9.72 to –9.21 (c=1, H_2O; polarimetry, Na-D lamp, 20°C). During polymerization, an inversion at the asymmetric C-atom can be observed and therefore the polyester exists in the natural L-form of carnitine (Kamm 2004; Kamm et al. 2004; Fig. 5)

Oligo-crotonbetaines

These products were isolated as colorless viscous substances. They show a ready solubility in water and methanol and insolubility in acetone. Oligomer molecular weights of 1,300–1,600 were determined by gel permeation chromatography (vs polyethylene glycol standard in water/salt; detection by refractive index). Patent applications have been lodged for this new class of polycrotonbetaine substances (Kamm et al. 2000b, 2001b)

Poly(carnitine allylester)

This product was isolated as a white amorphous powder. The polymer shows a glass transition temperature of 93°C (differential scanning calorimetry), molecular weights of 5,000–13,000 and polydispersities of 1.91–2.45 (gel permeation chromatography vs polyethylene glycol standard in water/methanol, detection by refractive index). The polymer is readily soluble in water, hardly soluble in methanol and insoluble in acetone. The polymer was comprehensively characterized via nuclear magnetic resonance spectroscopy, infrared spectroscopy and elemental analysis. The specific rotation (Na-D lamp, 20°C) was –16.2 to –15.7 (c=1, H_2O; Kamm et al. 2004; Fig. 7).

Applications of carnitine polymers

The synthesized cationic structures poly(carnitine), poly (crotonbetaine) and poly(carnitine allylester) were tested by Biopos e.V. and Biorefinery.de GmbH, with a view to large-scale industrial applications in cosmetics, pharmacy and medicine. The main goal is the substitution of petrochemical cationic compounds, e.g. polydiallyldimethylammoniumchloride in these application areas by oligomer and polymer structures generated from biobased own-body molecules. One possible application is the preparation of polymer precipitation structures, such as microcapsules, membranes and polymer-modified microemulsions (Ulrich et al. 2001).

Currently, there is a need to substitute petrochemically based polyelectrolytes and there is a demand for new defined polyelectrolytes of specific molecular weight and high charge density. However, there is a general problem in the case of introducing trialkylammonium structures into natural polysaccharides such as cellulose and starch for the generation of stable cationic polyelectrolytes: due to the fact that the alkylation is performed in a chemical way, there is a danger of the potential presence of free alkyl compounds that excludes their application in the fields of medicine and pharmacy (Dautzenberg et al. 1994). Thus, polymers based on carnitine with a stable trimethylammonium group could represent a path-breaking substance group.

Conclusions and outlook

L-Carnitine is a biotechnologically accessible substance that is currently produced by Lonza AG at a scale of 140 t year^{-1}. Due to its structure, L-carnitine is an appropriate starting material for the production of polymerizable cyclic and olefinic structures. It is shown that the poly (carnitine), poly(crotonbetaine), and poly(carnitine allylester) families can be synthesized by chemical conversion of L-carnitin. In the next step, syntheses have to be elaborated and optimized to be able to produce cationic polymer structures in high yields and in a sufficient amount. Interesting fields of application are pharmacy and cosmet ics, where petrochemical polymers could be substituted by biobased polymers. The synthesized structures can be used for the preparation of polymer precipitation structures, such as microcapsules, membranes and polymer-modified microemulsions.

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