Minireview

Palmitoylcarnitine, a surface-active metabolite

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Abstract Palmitoylcarnitine is a well-known intermediate in mitochondrial fatty acid oxidation. Less known are its properties as a surfactant, with a capacity to solubilize biological membranes similar to that of many synthetic detergents used in the biochemical laboratory. Some of the physico-chemical properties of palmitoylcarnitine may help to explain the need for coenzyme A-carnitine-coenzyme A acyl exchange during mitochondrial fatty acid import. The amphiphilic character of palmitoylcarnitine may also explain its proposed involvement in the pathogenesis of myocardial ischemia.

Key words: Carnitine; Membrane leakage; Membrane solubilization; Mitochondrial fatty acid oxidation; Carnitine palmitoyl transferase; Myocardial ischemia

1. Introduction and definitions

Palmitoylcarnitine is an important intermediate of fatty acyl degradation in mitochondria, and it is recognized as such in any basic biochemistry textbook. However, its remarkable physico-chemical properties are not so widely known, although they may help to explain some aspects of fatty acid metabolism. In addition, there are recent data suggesting a role for palmitoylcarnitine in pathological processes. All these aspects are reviewed, and to some extent correlated, below.

In our context, the term palmitoylcarnitine is used as an example of the variety of long-chain fatty acylcarnitines that occur in the mitochondrial degradation of fatty acids and among which the palmitoyl (IUPAC hexadecanoyl) derivative occurs frequently.

Surface-active molecules, or surfactants, are those molecules that, when added to a solvent (in our case water or aqueous solution), cause a decrease in the surface tension at the air-solvent interface. Surfactants are amphipathic (amphiphilic) molecules, i.e. molecules having both a polar and a non-polar moiety. More specifically, surface-active molecules are *soluble* amphiphiles, i.e. giving rise to clear, stable solutions. (Not all amphiphiles are soluble, e.g. diacylglycerols, or phospholipids.) The behavior of surfactants in solution is relatively complex. Below a certain concentration, the so-called critical micellar concentration (cmc), the individual molecules in the bulk of the solvent are in equilibrium with those at the air-water interface, the latter oriented with their polar moieties towards the water, and their non-polar parts towards the air. The molecules at the interface may be quantitatively unimportant, but they are the ones giving a surfactant its characteristic property, i.e. the decrease in surface tension. Above the cmc, most of the molecules in the bulk of the solvent associate with eachother giving rise to micelles, and the following equilibrium is established:

micelles ⇄ monomers in solution ⇄ monomers at interface

The net effect of adding a surfactant to a two-phase system is to increase the surface of contact between both phases, or interface. Adding soap to a recipient with water increases the surface of air-water contact through foam formation. In the digestive tract, bile salts increase the fat-water interface by giving rise to lipid-bile salt mixed micelles. In the lung alveoli, the complex mixture known as the 'lung surfactant' increases the tissue-air interface and thus prevents alveolar collapse.

2. Palmitoylcarnitine as a surfactant

The surface-active properties of palmitoylcarnitine (Fig. 1) have been known for long. They have been independently described in the physico-chemical and biological 'worlds' but, as is often the case, the integrated understanding of both groups of results has taken considerably longer. Among the physico-chemical studies, Yalkowsky and Zografi [1,2] described the critical micellar concentrations and other parameters of various long-chain carnitine derivatives. In the following decade, Pande [3] indicated the tendency of palmitoylcarnitine in solution to migrate to the air-water interface. In 1990, Stinson [4] demonstrated that palmitoylcarnitine can exist in various phases (e.g. lamellar, hexagonal) in the presence of water, a typical property of amphiphiles and surfactants. More recently, Requero et al. [5] described the influence of electrolytes and other factors on the critical micellar concentrations and other pertinent properties of acylcarnitines. Finally, Requero et al. [6] have provided direct measurements, using a Langmuir-type balance, of the increase in surface pressure that occurs at the air-water interface when palmitoylcarnitine is injected in the bulk of the solvent. In addition, when the air-water interface is initially covered by a monomolecular layer of phospholipids (e.g. egg phosphatidylcholine), injection of palmitoylcarnitine in the solvent is followed by penetration of the carnitine derivative into the phospholipid monolayer, a lateral stabilisation arising from the interaction between both lipids [6].

Detergents (or, more appropriately, surfactants) are commonly used in biological experimentation for the breaking down and solubilization of membranes, to the point that, from the biological point of view, surfactants are empirically characterized by their ability to solubilize membranes, rather

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Abbreviations: CoA, coenzyme A; cmc, critical micellar concentration; R_e, effective surfactant/lipid molar ratio

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than after their affinity for interfaces. Perhaps the earliest realization of palmitoylcarnitine as a surfactant, from the point of view of biochemical experimentation, can be attributed to Schwartz and co-workers [7], who studied the effect of this amphiphile on the Na, K-ATPase and Ca-ATPase activities from heart sarcolemma and sarcoplasmic reticulum respectively. These authors correctly explained the concentration-dependent effects of palmitoylcarnitine by assuming that this molecule acts 'as a naturally occurring detergent', and they invoked in support of their idea the physico-chemical studies by Yalkowsky and Zografi [1,2]. The abilities of various long-chain acylcarnitines to destabilize and/or solubilize sonicated phosphatidylcholine liposomes and sarcoplasmic reticulum vesicles were explored in a systematic way by Haeyaert et al. [8].

The proposal by Lichtenberg [9,10] of the use of 'effective surfactant/lipid ratios' R_e as parameters in solubilization studies has been very useful in rationalising experimental results in this field. In calculating these effective ratios only the detergent molecules in the membrane phase are taken into account, but not those in the bulk of the solvent (the amount at the air-water interface being negligible). Effective surfactant/lipid ratios R_e are independent from membrane concentration. R_e^{SAT} is the effective ratio at which the bilayer is detergent-saturated, so that solubilization starts; R_e^{SOL} is the effective ratio at which solubilization is completed. For reasons that are not fully understood at present, R_e^{SAT} and R_e^{SOL} have rather constant values of ~ 1 and of ~ 2–3 respectively, for most if not all the biochemically used surfactants. Requero et al. [11] have characterized in detail the binding and solubilization of large unilamellar vesicles of phosphatidylcholine by palmitoylcarnitine; they conclude that this amphiphile has the properties of the commonly used biochemical detergents. The critical micellar concentration of palmitoylcarnitine is in the 10^{-5} M range under a variety of conditions, well below the corresponding values for detergents such as Triton X-100 (3.7×10^{-4} M), CHAPS (3.5×10^{-3} M), or Hecameg (1.65×10^{-2} M) [5]. A low critical micellar concentration is generally indicative of extensive intermolecular hydrophobic interactions. In the presence of membranes, it should lead to a high degree of partition into the lipid phase, as is indeed the case for palmitoylcarnitine according to Requero et al. [11]. Their binding experiments indicate that when palmitoylcarnitine is added to a liposome suspension, virtually all of the surfactant becomes membrane-bound, the concentration of free palmitoylcarnitine in water approaching the critical micellar concentration. Also in agreement with the above, the membrane/water partition equilibrium constant K (34 mM^{-1}) is much higher than those of, e.g. Triton X-100 (1.9 mM^{-1} [12]) or Hecameg (0.08 mM^{-1} [13]). The R_e^{SAT} and R_e^{SOL} values for palmitoylcarnitine in egg phosphatidylcholine bilayers are respectively of 0.78 and 1.97 [11]. As was mentioned above, these values are similar to the corresponding ones for biochemically used surfactants, e.g. Triton X-100 (0.71 and 3.0 [12]) or Hecameg (1.4 and 2.6 [13]). In summary, palmitoylcarnitine is, from the physico-chemical point of view, a surfactant with solubilising properties equivalent to many natural or synthetic detergents that are commonly in use in biological laboratories.

It should be noted at this stage that, while being also an amphiphile, palmitoyl-CoA does not share the membrane lytic properties of palmitoylcarnitine. Palmitoyl-CoA inserts its hydrophobic moiety in the bilayer, but without producing membrane leakage or lysis [11,14]. The importance of this observation will become obvious in the next section.

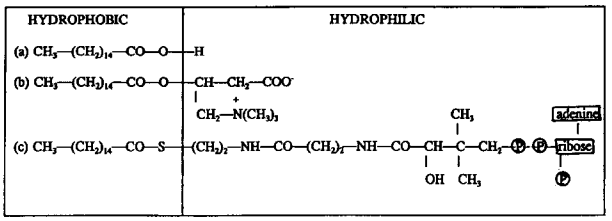

Fig. 1. Chemical structures of long-chain fatty acyl compounds. (a) Palmitic (IUPAC hexadecanoic) acid. (b) Palmitoylcarnitine. (c) Palmitoyl-coenzyme A.

The surfactant properties of long-chain acylcarnitines have been observed in a variety of membranous systems. Palmitoylcarnitine was shown to induce alterations in membrane fluidity of erythrocytes [15]. The observed enhancement of hydrophobic drug absorption through epithelia in the presence of palmitoylcarnitine [16] can probably be ascribed to the membrane permeabilizing effect of the amphiphile at sublytic concentrations [11]. Palmitoylcarnitine decreases the electrical resistance of phospholipid model membranes and, in rat liver mitochondria, it induces energy-dependent swelling of the mitochondrial matrix [17]. Both palmitoyl-CoA and palmitoylcarnitine dissipate the transmembrane potential of rat heart mitochondria [18], the effects of the former being prevented by ruthenium red, cyclosporin A or Mg^{2+}. The fall of membrane potential induced by palmitoylcarnitine cannot be prevented by any of those agents, suggesting that only palmitoylcarnitine is exerting its surfactant properties at the level of the membrane bilayer. Paradoxically, Piper et al. [45] have shown that long-chain acylcarnitines prevent osmotic lysis of red blood cells at low concentrations; similar 'protective' effects have been found for other surfactants at low concentrations in various cell and model membranes. Other examples of membrane activity of palmitoylcarnitine are related to pathological conditions, and will be discussed in the next sections.

3. Palmitoylcarnitine in mitochondrial fatty acid import

In the former section we have dealt with palmitoylcarnitine as a molecule. Now we shall think of it as of a metabolite. It is well known that palmitoylcarnitine is an essential intermediate in fatty acid transport across the mitochondrial inner membrane. Mitochondrial β-oxidation of long-chain fatty acids takes place in the matrix. Fatty acyl-CoAs are generated on the outer mitochondrial membrane and converted into fatty acylcarnitines (e.g. palmitoylcarnitine) by carnitine palmitoyltransferase I, an enzyme located on the inner side of the outer mitochondrial membrane (Fig. 2). Palmitoyl-carnitine passes through a carnitine-acylcarnitine translocase to the inner part of the inner mitochondrial membrane, where a second coenzyme exchange takes place, opposite to the former one: carnitine palmitoyltransferase II converts fatty acylcarnitines into fatty acyl-CoAs, that can now undergo β-oxidation in the mitochondrial matrix. More details on the carnitine palmitoyltransferases may be found in [19,20]. Long-chain carnitine acyltransferases are also found in peroxisomes, and in endoplasmic reticulum, that also exchange carnitine for coenzyme A and vice versa [20].

The reasons by which this double coenzyme exchange accompanies fatty acid membrane transport are unclear. The traditional explanation was that carnitine esters would traverse the bilayer more easily than the corresponding derivatives of the bulky coenzyme A (Fig. 1). This hypothesis was proposed well before the discovery of the carnitine-acylcarnitine translocase [21,22] but has found its way even to present-day biochemistry textbooks. In fact, fatty acylcarnitines hardly if at all diffuse ('flip-flop') across phospholipid bilayers [11,23,26,44]. It is true that, for a relatively simple translocator (M_r 33 000, [24]) acylcarnitines may be easier to deal with than acyl-CoAs, but this explanation does not appear very satisfactory, particularly when we know that macromolecules much larger than acyl-CoAs are being actually imported into the mitochondria. One possible difficulty offered by coenzyme A, but not by carnitine derivatives, is the establishment of attractive interactions between the long and complex coenzyme A moiety and the membrane surface, that would hinder the translocation. Recent infrared spectroscopic evidence suggests that a significant interaction may occur between some coenzyme A group and the carbonyl groups of the phospholipid [14]. Such interactions would constitute another factor favoring the coenzyme A-carnitine exchange.

Another view on the need for a double coenzyme exchange at the mitochondrial membrane level is that carnitine would be used to buffer the availability of coenzyme A in the matrix

Table 1
Concentrations of carnitine, coenzyme A, and their derivatives, in various tissues, cells and subcellular fractions

	Source	Metabolite	Concentration	Reference
1	Human red blood cells	Free carnitine	12–29 μM	[38]
		Acetylcarnitine	13–30 μM	[38]
2	Human muscle	Total carnitine	4 mM	[38]
3	Human mononuclear phagocytes	Free carnitine	0.20 μM[a]	[39]
		Fatty acylcarnitine	0.40 μM[a]	[39]
		Total carnitine	8 μM[a]	[39]
4a	Rat heart (non-perfused)			
	Cytosol	Acid-soluble carnitine[b]	2.5 mM	[40]
		Fatty acylcarnitine	0.025 mM	[40]
		Total carnitine	2.65 mM	[40]
	Mitochondrial matrix	Acid-soluble carnitine	2.03 mM	[40]
		Fatty acylcarnitine	0	[40]
		Total carnitine	2.03 mM	[40]
4b	Rat heart (control, perfused)			
	Cytosol	Acid-soluble carnitine	2.18 mM	[40]
		Fatty acycarnitine	0.38 mM	[40]
		Total carnitine	2.56 mM	[40]
	Mitochondrial matrix	Acid-soluble carnitine	1.62 mM	[40]
		Fatty acylcarnitine	0.22 mM	[40]
		Total carnitine	1.84 mM	[40]
4c	Rat heart (ischemic, perfused)			
	Cytosol	Acid-soluble carnitine	0.43 mM	[40]
		Fatty acylcarnitine	1.95 mM	[40]
		Total carnitine	2.37 mM	[40]
	Mitochondrial matrix	Acid-soluble carnitine	2.93 mM	[40]
		Fatty acylcarnitine	1.88 mM	[40]
		Total carnitine	4.81 mM	[40]
4d	Rat heart (control, perfused)			
	Cytosol	Total coenzyme A	0.014 mM	[40]
	Mitochondrial matrix	Fatty acyl-CoA	0.52 mM	[40]
		Total coenzyme A	2.10 mM	[40]
5	Rat plasma	Fatty acylcarnitine	3–18 μM	[41]
		Free carnitine	28 μM[a]	[42]
		Fatty acylcarnitine	1.7 μM[a]	[42]
		Total carnitine	33.5 μM[a]	[42]
6	Rat liver	Free carnitine	183.7 μM[a]	[42]
		Fatty acylcarnitine	24.2 μM[a]	[42]
		Total carnitine	251.9 μM[a]	[42]
7	Rat liver	Total coenzyme A	136.4 μM[a]	[42]
8	Rat skeletal muscle	Free carnitine	630.3 μM[a]	[42]
		Fatty acylcarnitine	85.8 μM[a]	[42]
		Total carnitine	977.9 μM[a]	[42]
9a	Dog myocytes (control)			
	Sarcolemma	Fatty acylcarnitine	70 μM[a]	[43]
	Mitochondria	Fatty acylcarnitine	467 μM[a]	[43]
	Cytosol	Fatty acylcarnitine	427 μM[a]	[43]
9b	Dog myocytes (hypoxia)			
	Sarcolemma	Fatty acylcarnitine	6967 μM[a]	[43]
	Mitochondria	Fatty acylcarnitine	773 μM[a]	[43]
	Cytosol	Fatty acylcarnitine	357 μM[a]	[43]

[a] These values are only approximate, since they were originally published in units not directly convertible into IS units (e.g. nmol per 10^9 cells).
[b] Acid-soluble carnitine refers to free plus short-chain acid esterified carnitine.

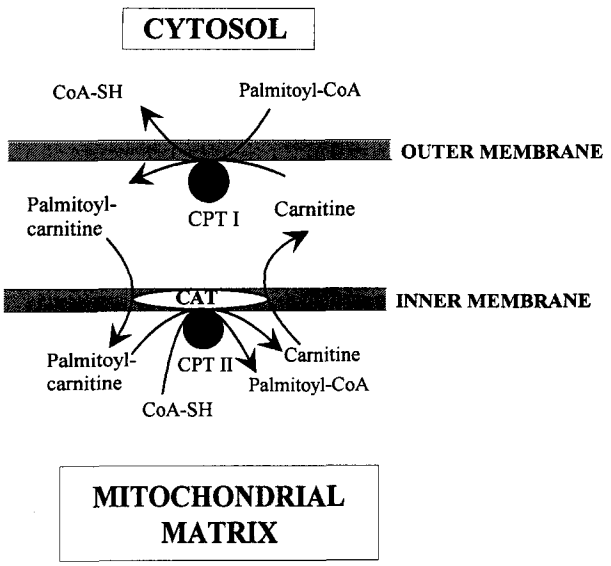

Fig. 2. A schematic view of the fatty acid import mechanism in mitochondria. CPT, creatine palmitoyltransferase. CAT, carnitine-acylcarnitine translocase.

[25]. Coenzyme A is important in a variety of metabolic pathways: as an acetyl carrier, in the synthesis of acetylcholine, ketone bodies and fatty acids, and as a substrate for enzymes of the Krebs cycle. On the contrary, the carnitine pool is a metabolic dead end. Also, carnitine would be available in larger amounts than coenzyme A (Table 1). Thus the relatively unreactive acylcarnitines would be stored as a reservoir of activated acyl groups, which would be transferred to coenzyme A according to the needs of the cell [25,26]. This would be correct from the point of view of chemical reactivity, considering that the thioester bond of coenzyme A derivatives is far less stable than the O-ester group of palmitoylcarnitine. However, as was pointed out in the former section, palmitoyl-CoA is virtually inert with respect to bilayer destabilization, while palmitoylcarnitine is a powerful surfactant, so that storage of free palmitoylcarnitine beyond a certain limit (that can be estimated at around the critical micellar concentration, $\sim 10^{-5}$ M) is not compatible with the maintenance of a tight membrane. Thus our studies on the relative membrane lytic properties of acyl-CoAs and acylcarnitines [11] are hardly compatible with the idea of a significant pool of acylcarnitines. In fact, although the overall carnitine concentration in the cell is rather high, most of it is in the non-esterified form (Table 1). Apparent K_m values for palmitoylcarnitine in the carnitine palmitoyltransferases have been estimated at $1-5\times10^{-5}$ M [27,28]. These are just above the critical micellar concentration of palmitoylcarnitine, so that these enzymes would prevent the build-up of dangerous concentrations of that metabolite, against the concept of large pools of acylcarnitine in membranes. It should also be noted, in this respect, that a significant fraction of acylcarnitine may exist in the cell in protein-bound form, so that it would be relatively harmless to membranes.

Genetic deficiencies have been described [29] for each of the three proteins involved in fatty acid import, namely carnitine palmitoyltransferase I, carnitine-acylcarnitine translocase and carnitine palmitoyltransferase II. Of all three, the neonatal form of deficiency of carnitine palmitoyltransferase II is particularly lethal, the infants dying of cardiac arrest at the age of 3–5 days. A possible pathogenic factor in the latter disease could be the accumulation of palmitoylcarnitine in the mitochondrial membrane producing leakage, i.e. mitochondrial uncoupling, and subsequent impairment of all the energy-requiring processes in the cell.

4. Palmitoylcarnitine and myocardial ischemia

Palmitoylcarnitine may be involved in pathological processes unrelated to the machinery of mitochondrial fatty acid import that we have just reviewed. In particular, increased levels of acylcarnitines have been implicated in the pathology of cardiac ischemia for the last two decades. Wood et al. [30] demonstrated that palmitoylcarnitine was a potent inhibitor of isolated cardiac Na,K-ATPase, and, as mentioned above, Adams et al. [7] demonstrated that the palmitoylcarnitine effect on sarcolemmal and sarcoplasmic reticulum membranes was due to the surfactant properties of that amphiphile. These observations have become more relevant in the light of recent results: palmitoylcarnitine concentrations in the heart sarcolemma (plasma membrane) have been reported to increase in cells subjected to hypoxia for 10 min (Table 1) [31,40,43]. More recently, Wu and Corr [32] have shown that palmitoylcarnitine modifies sodium currents and elicits a transient inward current in isolated rabbit ventricular cells. According to the latter authors, the primary effect of the amphiphile would be to induce a Na^+ inward current, which would in turn elicit an increase in intracellular Ca^{2+} via Na^+/Ca^{2+} exchanger, leading to the development of delayed after-depolarizations and triggered activity. Wu and Corr [32] pointed out that similar 'Na^+-channel openings' have been observed with lysophosphatidyl-choline [33]. The data by Requero et al. [11] on vesicle leakage induced by palmitoylcarnitine are in agreement with those observations and may explain the role of fatty acylcarnitines in acute myocardial ischemia.

The amphipathic properties of palmitoylcarnitine are used by Pappano and co-workers to explain in a different way at least some of the pathologic effects of palmitoylcarnitine and particularly the inhibition of the Na,K-ATPase. According to them, palmitoylcarnitine incorporates into the membranes and its insertion leads to a reduction of the surface negative charge [34]. In turn, reduction of negative charge in the environment of Na,K-ATPase molecules would mimic the hyperpolarization-induced reduction of Na efflux and Na/K current seen in squid giant axon [35]. In favor of the surface charge hypothesis is the fact that it allows for effects at low amphiphile concentrations, not requiring membrane disruption. A related similar effect of palmitoylcarnitine at low concentrations is the increased Ca^{2+} release from skeletal muscle sarcoplasmic reticulum [36]. The mechanism appears in this case to be one of direct interaction with the channel (ryanodine receptor), involving particularly the hydrocarbon chain moiety of the amphiphile, since long-chain coenzyme A derivatives appear to have a similar effect. Interestingly, carnitine and its short-chain esters appear to have a protective effect in perfused hearts subjected to ischemia or to oxidative stress [37]. The mechanisms involved are obscure, but the protective effects do not appear to be directly related to fatty acid metabolism.

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