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Electrogenic and nonelectrogenic ion fluxes across lipid and mitochondrial membranes mediated by monensin and monensin ethyl ester

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ABSTRACT

Monensin is a carrier of cations through lipid membranes capable of exchanging sodium (potassium) cations for protons by an electroneutral mechanism, whereas its ethyl ester derivative ethyl-monensin is supposed to transport sodium (potassium) cations in an electrogenic manner. To elucidate mechanistic details of the ionophoric activity, ion fluxes mediated by monensin and ethyl-monensin were measured on planar bilayer lipid membranes, liposomes, and mitochondria. In particular, generation of membrane potential on liposomes was studied via the measurements of rhodamine 6G uptake by fluorescence correlation spectroscopy. In mitochondria, swelling experiments were expounded by the additional measurements of respiration, membrane potential, and matrix pH. It can be concluded that both monensin and ethyl-monensin can perform nonelectrogenic exchange of potassium (sodium) ions for protons and serve as electrogenic potassium ion carriers similar to valinomycin. The results obtained are in line with the predictions based on the crystal structures of the monensin complexes with sodium ions and protons (Huczyński et al., Biochim. Biophys. Acta, 1818 (2012) pp. 2108–2119). The functional activity observed for artificial membranes and mitochondria can be applied to explain the activity of ionophores in living systems. It can also be important for studying the antitumor activity of monensin.

1. Introduction

Monensin (Fig. 1) is extensively used as an anticoccidial drug in poultry and as a growth promoter in ruminants. It belongs to a broad class of antibiotics termed polyether ionophores, i.e. compounds capable of transporting monovalent and divalent cations through cellular membranes [1]. It is widely used in cell biology to dissipate intracellular pH gradients and disturb the functioning of Golgi apparatus [2]. However, its toxicity on mammalian cells is associated with the action on their mitochondria, which exhibit substantial swelling [3]. As an antibiotic, monensin is predominantly effective against Gram-positive bacteria [4,5]. Moreover, it also exhibits antiviral, antifungal, antiparasitic, antimalarial, anti-inflammatory, and tumor cell cytotoxic activity [6–9]. Recently, the antitumor activity of polyether ionophores was substantiated by the finding that they have the ability to kill cancer stem cells and inhibit breast cancer growth and metastasis in mice [10,11]; effects that could be a result of the alteration of the intracellular pH by ionophores [12].

The major mechanism of monensin action as a cation carrier is the electrically silent exchange of sodium (or potassium) cations for protons across the membrane [1,7]. A scheme of this process is displayed in Fig. 2, showing that monensin carboxylate anion (I-COO^-) forms complexes with a monovalent cation M^+ (I-COOM) and a proton (I-COOH) and diffuses across the membrane. The coordination of a metal cation by I-COO^- anion is always accompanied by formation of a pseudo-cyclic structure of monensin anion, which is stabilized by the 'head-to-tail' intramolecular hydrogen bonds formed between the carboxylate anion and hydroxyl groups. In contrast to the other popular polyether ionophore such as nigericin, monensin has a substantial preference for sodium over potassium ions as shown in the binding experiments [13,14] or in the transport experiments [15–17]. However, the experiments with planar lipid bilayers (BLM) [18,19] and liposomes [20,21] showed that monensin was able to transport sodium and potassium cations in an electrogenic manner, that is, generating electrical current on BLM and electrical potential on liposomes. The structural basis for this type of transport can be the structure of the acidic form of monensin with a sodium cation (I-COOH-M^+) present in crystal and in solutions, as has been proved by Huczyński et al. [22]. Diffusion of I-COOH-M^+ complex in one direction and a backward diffusion of

Abbreviations: DPhPC, diphytanoylphosphatidylcholine; DPhytanylPC, diphytanylphosphatidylcholine; BLM, bilayer lipid membrane; FCCP, carbonyl cyanide-*p*-(trifluoromethoxy)phenylhydrazone; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; TMRE, tetramethylrhodamine ethyl ester; Ethyl-monensin, monensin ethyl ester; FCS, fluorescence correlation spectrometry; TPP^+, triphenylphosphonium cation; BCECF, pH probe 2′,7′-biscarboxyethyl-5(6)-carboxyfluorescein; TTFB, tetrachlorotrifluoromethylbenzimidazole

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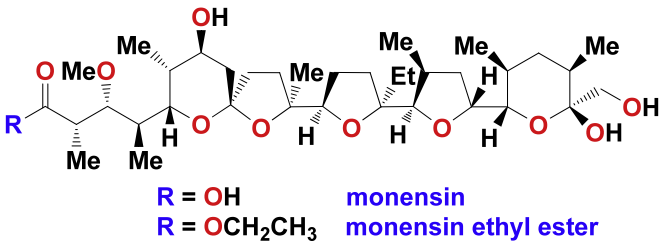

Fig. 1. The structures of monensin and monensin ethyl ester (ethyl-monensin).

I-COOH without the metal cation can be a mechanism of this type of electrogenic ion transport (Fig. 2B). This type of transport is characteristic of another popular ionophore, valinomycin.

The carboxylic group of monensin (COOH) can be chemically modified to obtain ester derivatives blocking COOR group (Fig. 2), which eliminates the possibility of protonation–deprotonation [5]. According to the scheme in Fig. 2A, modification of the carboxylic group of monensin must substantially change the cation transport properties of monensin. However, it has been proved that monensin derivatives show a certain activity against Gram-positive bacteria [5]. The complexation of monovalent and divalent metal cations by monensin esters and the structure of these complexes have been studied using spectroscopic methods and are discussed in detail [5,23–29]. These esters have been found to show especially high affinity to Na^+ and Ca^{2+} cations. The structures of the complexes formed between one of the monensin esters, i.e. monensin 1-naphthylmethyl ester, with sodium and lithium cation have been determined by crystallographic methods [23].

One of the most commonly studied esters of monensin is its methyl ester (methyl-monensin), which was shown to elicit electrogenic sodium transport through liposomes better than the parental monensin [21]. This type of valinomycin-like action can be described in the scheme in Fig. 2C displaying the formation of cationic I-COOR-M^+ complex [29,30], its transmembrane diffusion, metal ion dissociation, and a backward diffusion of the neutral I-COOR molecule. Methyl-monensin was used for the design of a sodium-selective electrode [31], although sodium electrodes based on the parental monensin have also been described [14].

In the present work, we compared the ion carrier properties of monensin and chemically synthesized monensin ethyl ester (ethyl-monensin) with an emphasis on the determination of the electrogenic or nonelectrogenic nature of the ion fluxes. We used artificial membranes (BLMs and liposomes), as well as natural membranes (mitochondria). Importantly, mitochondria are very useful systems to study electrogenic ion fluxes because, in these systems, the actions of electrogenic carrier (valinomycin) and the nonelectrogenic one (nigericin) are qualitatively different [7,32]. In particular, valinomycin decreases the membrane potential of mitochondria while nigericin increases it. This is a result of the dependence of the activity of the respiratory enzymes on the proton-motive force which consists of two components, electrical membrane potential and pH gradient. Nigericin decreases pH gradient and the proton pumps restore the proton-motive force by the increase in the membrane potential. It was shown in the present paper that monensin and ethyl-monensin exhibited both electrogenic and nonelectrogenic types of activity, i.e. monensin can transport potassium ions electrogenically, while ethyl-monensin can perform electrically silent exchange of potassium ions for protons.

2. Materials and methods

Palmitoyloleoylphosphatidylcholine (POPC), diphytanoylphosphatidylcholine (DPhPC), diphytanylphosphatidylcholine (DPhytanylPC),

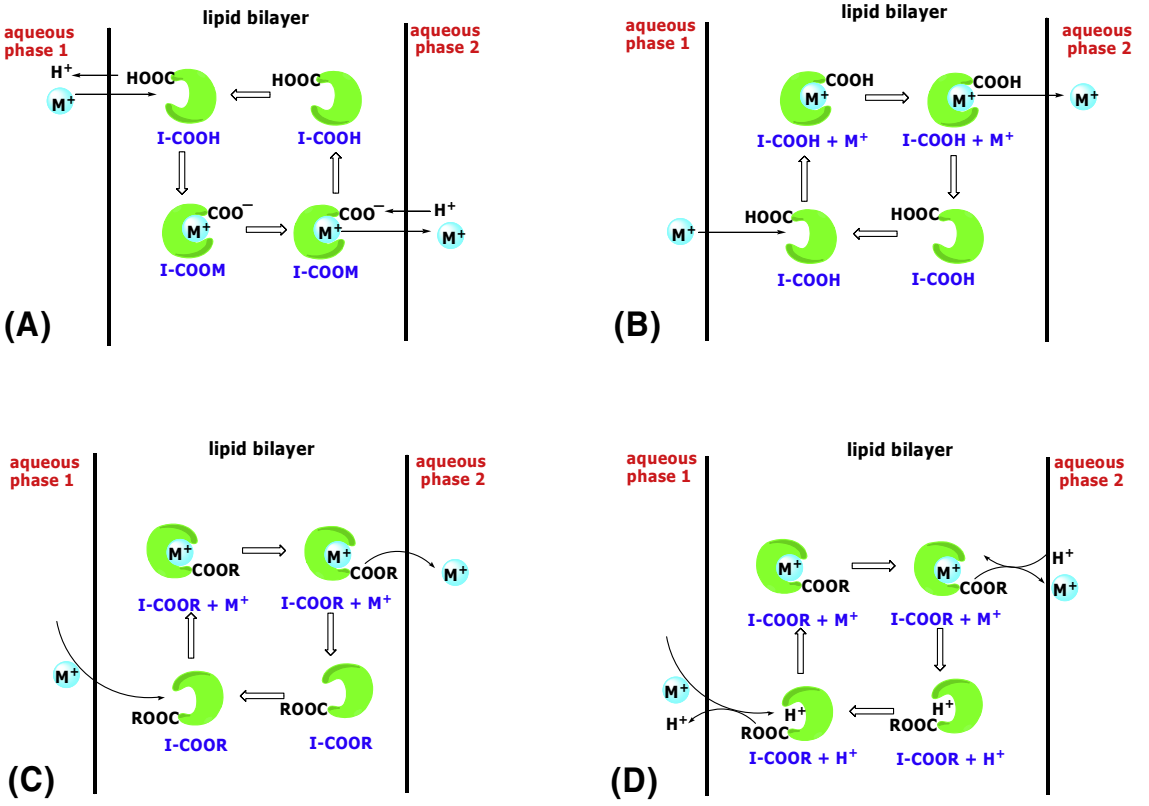

Fig. 2. Four possible ways of cation transport by monensin and ethyl-monensin. In the electroneutral transport (A), the metal cation is incorporated into the monensin skeleton of the pseudocyclic structure of ionophore, and electroneutral I-COOM complex diffuse to another interface, while the neutral ionophore acid molecule I-COOH returns. In electrogenic transport (B), the metal cation is bound and transported by ionophore acid molecule I-COOH forming I-COOH + M^+ complex. In the electrogenic (C) or nonelectrogenic transport (D), the cation fluxes is carried out by monensin with modified carboxylic group (I-COOR) such as ethyl-monenin.