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Small neutral molecular carriers for selective carboxylate transport†

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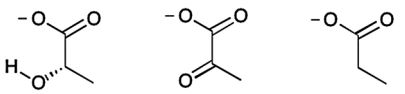
A series of neutral thiourea receptors were found to mediate the antiport of chloride with a range of biologically relevant carboxylate anions across phospholipid bilayers. Simple structural modification of the carriers resulted in a change in the lactate/pyruvate transport selectivity.

The design of molecular carriers for the transport of chloride and bicarbonate across phospholipid bilayers has progressed rapidly in recent years.[1] However, there have been few reports to date of the lipid bilayer transport of carboxylate anions. The transmembrane transport of N-protected amino acids through U-tubes is known, although the receptors that are reported to mediate this process by selectively binding to the carboxylate component are often charged and relatively hydrophilic,[2] which can be an undesirable characteristic for a lipid bilayer anion carrier.[3] The transport of lactate across liquid[4] and polymeric[5] membranes has been reported using simple amines and ammonium salts as mobile carriers, with applications in industrial lactate extraction.

Carboxylates are common in biological systems and their transmembrane transport is crucial for cell function.[6] There are a large number of small molecule carboxylates within the body, which are often extremely similar structurally; as such it is critical that a prospective carboxylate transporter should be inherently selective for a target species over highly similar analogues. For example, pyruvate and lactate differ only in the keto- or hydroxyl-functionality in the 2-position as they are directly interconvertible metabolites. Pyruvic acid is the end product of glycolysis and is the starting point for the Krebs cycle. In both the absence and presence[7] of oxygen, pyruvic acid is converted to lactic acid that can later be re-oxidised to pyruvic acid to feed into the respiratory process. Lactate may be oxidised within the muscle tissue in which it was produced, or transported elsewhere for oxidation.[8] Some organs, including the heart, brain and slow twitch muscle fibres preferentially take up lactate rather than glucose as an energy source.[9] The majority of lactate is transported by monocarboxylate carrier proteins, which can also transport other carboxylates including pyruvate and propionate.[6] A recent study has suggested that disrupting the expression of the neuronal lactate transporter MCT2 leads to amnesia in rats, suggesting that lactate transport in neurones is necessary for long-term memory formation.[10] The production of small molecule carboxylate transporters may useful provide biological tools for the study of such processes.

Our group[11] and others[3,12] have shown that thioureas can function as excellent anion carriers. For example, we have previously reported that thioureas **1** and **2** can facilitate Cl^-/NO_3^- and Cl^-/HCO_3^- exchange,[11d,13] whilst indole substituted thioureas show enhanced transport rates.[11b,13] We decided to investigate whether thioureas could facilitate the selective transmembrane transport of L-lactate, herein referred to as lactate, and pyruvate, in addition to propionate.



Using receptors **1** and **2** as a starting scaffold, we incorporated additional hydrogen bond donors and acceptors into the design in order to allow the formation of hydrogen bonds between the hydroxyl or keto groups of lactate and pyruvate. Receptor **3** contains a methylimidazole group to potentially offer a basic nitrogen hydrogen bond acceptor in a position which may be able to bind to the hydroxyl O–H of lactate but offer no enhancement to pyruvate binding. Receptor **4** contains an amido functionality in the 2-position of the indole scaffold, which could function as either a hydrogen bond donor (NH) or acceptor (C=O) depending on the conformation of the receptor.

Anion binding strength has previously been correlated with transport activity.[3,14] In order to investigate the solution phase binding properties of receptors **3–4**, we performed ^{1}H NMR

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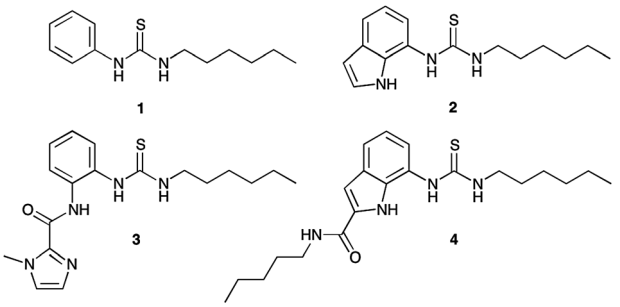

titrations with tetrabutylammonium or tetraethylammonium salts of the anions in DMSO-d_6/H_2O (0.5%). The data was fitted to a 1 : 1 binding model using WinEQN MR2.[15] The binding constants calculated are shown in Table 1 and show that the receptors have low affinity for chloride and do not detectably interact with nitrate under these experimental conditions. Higher affinities are observed with bicarbonate and carboxylates with the highest affinity observed with compound **4** and propionate.

The amide NH resonance of receptor **4** underwent only a very small downfield shift (<0.5 ppm) during the titration with lactate, implying that it is not directly involved in the binding (see Fig. S28 in the ESI†). Bates *et al.* have proposed a carboxylate binding mode for receptors of this type as shown in Fig. 1, in which the amide NH faces away from the binding cleft.[16] By extension, such a binding mode could facilitate hydrogen bond formation with the amide carbonyl as an acceptor. An interaction of this type has also been previously reported.[17] However, no evidence of this interaction could be observed by NMR techniques. In contrast, the amide NH resonance was observed to undergo a small but detectable downfield shift during the titrations with pyruvate and propionate, which implies some hydrogen bond formation and possibly a change in receptor conformation (see Fig. S29 in the ESI†).

In order to assess the carboxylate transport activity of these receptors we adapted the pulse assay which has previously been reported. We prepared a sample of POPC vesicles containing NaCl, which were suspended in Na_2SO_4. A sample of the receptor in a DMSO (2 mol% w.r.t. lipid) was added, and any resulting chloride efflux was monitored using a chloride selective electrode (ISE). After 2 minutes, a spike of the sodium salt of the anion of interest was added such that the external concentration of this salt was 40 mM. In this way, by studying the efflux of chloride after the pulse of the anion salt it is possible to indirectly monitor the antiport of the anion of interest. For comparison to previously reported receptors, we also included nitrate and bicarbonate as antiport substrates. At the end of the experiment, the vesicles were lysed with detergent to calibrate 100% chloride efflux. The results are shown in Fig. 2–4 and Fig. S32 in the ESI.†

The relative lipophilicity of receptors **1–4** was investigated by using HPLC to examine their retention time on a reverse phase column (see the ESI† Table S1).[18] These results indicate that receptor **3** is less lipophilic than parent compounds **1**, while receptor **4** is more lipophilic than receptor **2**. This parameter does not appear to be a determining factor in the relative transport activity of these receptors; however, given the structural variation across the series this is perhaps unsurprising

Prior to the spike of anion at 120 s, no transport is observed, confirming an anion antiport mechanism. No chloride efflux was observed in the absence of a receptor (Fig. S31 in the ESI†). For every receptor the most efficient antiport process was the exchange of chloride with propionate, due to the hydrophobicity of this anion. Receptor **1** showed only very poor transport properties (Fig. S32 in the ESI†).

Receptor **2** is an excellent anion antiporter,[13] and is the most efficient antiporter for every transport process in this study (Fig. 2). Close to 100% of the intra-vesicular chloride was

Table 1 Stability constants (K_a) of 1 : 1 complexes in DMSO-d_6/H_2O 0.5% at 25 °C, calculated following the resonance of the aromatic adjacent thiourea NH. Errors are estimated to be <15%[a,b]

Anion	1	2	3	4
Chloride	14[b]	17[b]	12	12
Nitrate	<10[b]	<10[b]	<10	<10
Bicarbonate	262[b]	414[b]	—[c]	389
Propionate	434	771	362	1735
Lactate	66	61	38	180
Pyruvate	42	28	42	90

[a] Anions were added as the tetrabutylammonium salt except for bicarbonate, which was added as the tetraethylammonium salt. [b] Binding constants for **1** and **2** with chloride, nitrate and bicarbonate have been previously reported.[13] [c] Binding constant could not be calculated due to broadening of the NH resonances.

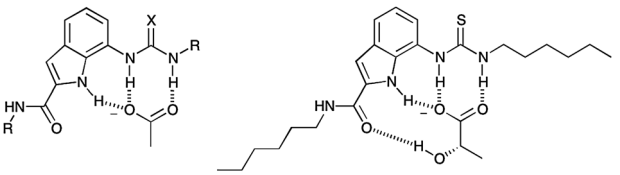

Fig. 1 The acetate binding mode proposed by Bates *et al.* and a possible binding mode of receptor **4** with lactate.[16]

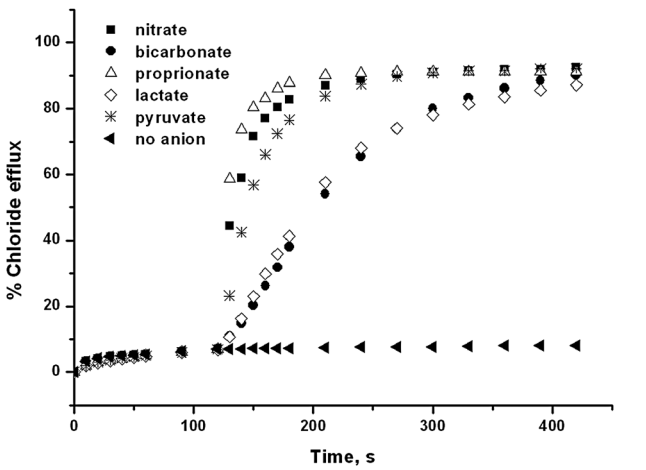

Fig. 2 Chloride efflux mediated by receptor **2** (2 mol% w.r.t. lipid) from POPC vesicles containing NaCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were suspended in Na_2SO_4 buffered to pH 7.2 with sodium phosphate salts. At t = 120 s, a pulse of sodium salt was added such that the final concentration was 40 mM.

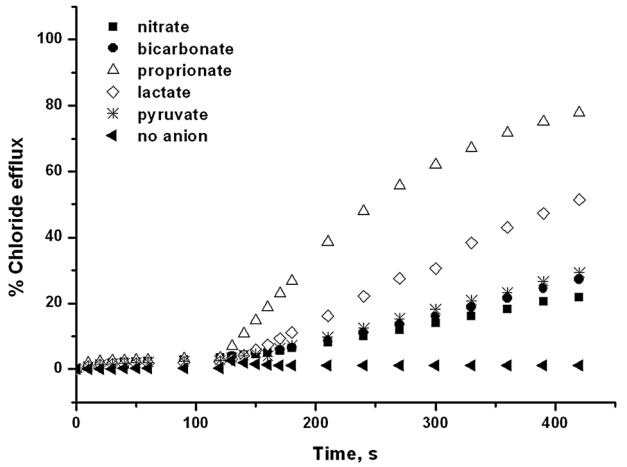

Fig. 3 Chloride efflux mediated by receptor **3** (2 mol% w.r.t. lipid) from POPC vesicles containing NaCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were suspended in Na_2SO_4 buffered to pH 7.2 with sodium phosphate salts. At t = 120 s, a pulse of sodium salt was added such that the final concentration was 40 mM.

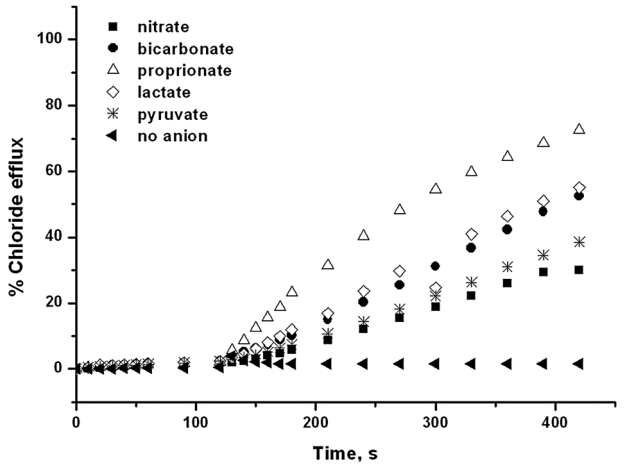

Fig. 4 Chloride efflux mediated by receptor **4** (2 mol% w.r.t. lipid) from POPC vesicles containing NaCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were suspended in Na_2SO_4 buffered to pH 7.2 with sodium phosphate salts. At t = 120 s, a pulse of sodium salt was added such that the final concentration was 40 mM.

released by the end of each experiment regardless of the external anion. Analysis of the initial transport rate indicates that pyruvate is transported faster than lactate.

Interestingly, receptors **3** and **4** were found to transport lactate more efficiently than pyruvate (Fig. 3 and 4), a reversal of the trend observed for receptor **2**. This suggests that the synthetic modification of the thiourea core has resulted in greater selectivity for lactate transport. This may be due to hydrogen bond formation between the hydroxyl group of lactate with the methylimidazole group of receptor **3** and the amide C=O of receptor **4**, providing stronger binding and better shielding of the anion from the apolar interior of the bilayer. This work emphasises the importance of considering the selectivity of a receptor for both components of an anion exchange process.

In summary, we have reported that a series of thioureas can mediate the selective transport of monocarboxylates across lipid bilayers, and that simple structural modification of the receptor scaffold results in modulation of anion selectivity. We are currently working to expand these motifs for the capture and transport of more complex species, and these results will be published in due course.

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