# A NEW SODIUM-TRANSPORT SYSTEM ENERGIZED BY THE DECARBOXYLATION OF OXALOACETATE

Peter DIMROTH

*Institut für Physiologische Chemie der Technischen Universität München, Biedersteiner Straße 29, D-8000 München 40, FRG*

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## 1. Introduction

Oxaloacetate decarboxylase (EC 4.1.1.3) from *Klebsiella aerogenes* [1] and methylmalonyl-CoA decarboxylase (EC 4.1.1.41) from *Micrococcus lactilyticus* [2] have been shown to be avidin-sensitive and were therefore classified as biotin enzymes. It has further been assumed that these decarboxylases, like other biotin enzymes, perform a transfer reaction to synthesize the carboxy-biotin intermediate and that this intermediate is subsequently decomposed to $CO_2$ and the free biotin enzyme [3]. This reaction mechanism has been established for oxaloacetate decarboxylase [4,5]. Interestingly, the second partial reaction was specifically dependent on the presence of $Na^+$ [5] and is therefore responsible for the unusual $Na^+$ requirement of the decarboxylase in [1].

This remarkable $Na^+$ requirement and the localization of oxaloacetate decarboxylase in the membrane are reminiscent to the well-known $Na^+$- and $K^+$-activated adenosintriphosphatase and have prompted us to hypothesize that an additional function of the decarboxylase could be the transport of $Na^+$ through the membrane [4]. In this way the chemical energy liberated upon decarboxylation of oxaloacetate ($\Delta G \simeq 7$ kcal/mol) would be utilized to drive the transport of $Na^+$ just as ATP energy is used to drive $Na^+$ and $K^+$ transport by the adenosintriphosphatase. The results described here agree with the above hypothesis. Inverted vesicles containing oxaloacetate decarboxylase were prepared from *K. aerogenes* and were found to accumulate $Na^+$ in response to the addition of oxaloacetate. Furthermore, oxaloacetate decarboxylation and sodium transport were simultanously inhibited by avidin treatment.

## 2. Materials and methods

*Klebsiella aerogenes* was grown anaerobically on citrate [6]. Inverted vesicles were prepared as in [7], but in 50 mM K-phosphate buffer (pH 7.5). Oxaloacetate decarboxylase activity was determined as in [4]. Unless indicated otherwise, sodium transport was determined by incubating inverted vesicles containing 6 U oxaloacetate decarboxylase (7 mg protein) at 20°C in 0.55 ml total vol. 50 mM K-phosphate buffer (pH 7.5), with 0.18 mM $^{22}NaCl$ (714 000 cpm) and 1.8 mM Li-oxaloacetate. The vesicles after appropriate incubation periods were separated from low $M_r$ substances by passage over a Sephadex G-50 column (1 × 23 cm) at 20°C in 50 mM K-phosphate (pH 7.5). The radioactivity of the fractions was determined by liquid scintillation counting.

## 3. Results

To investigate the hypothesis that oxaloacetate decarboxylase might have an additional function in performing sodium transport [4], vesicles had to be prepared with a defined orientation of the membrane and the accumulation of $Na^+$ had to be measured in accordance to the presence of oxaloacetate. It was expected that the physiological function of the enzyme would be an inside-out directed transport of $Na^+$. Therefore, the orientation of the membrane vesicles used for transport studies had to be inverted as compared to the membrane of intact cells to achieve accumulation of the transport substrate.

Inverted membrane vesicles prepared by rupture of *K. aerogenes* with a French press [7] contained high

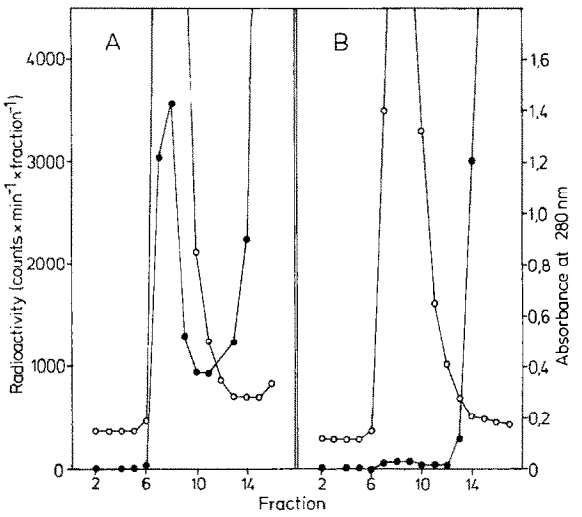


Fig.1. Incorporation of $^{22}Na^+$ into vesicles in the presence and absence of oxaloacetate. The vesicles were incubated with $^{22}NaCl$ in presence (A) and absence (B) of oxaloacetate and isolated after 1 min by Sephadex chromatography. (○) Absorbance at 280 nm; (●) radioactivity. For details see section 2.

amounts of oxaloacetate decarboxylase activity (~1 U/mg protein). The membrane-bound enzyme was absolutely dependent on the presence of $Na^+$ and was completely inhibited by incubation with avidin. Sodium transport was determined by incubating the vesicles with $^{22}NaCl$ and counting the amount of radioactivity entrapped within these vesicles after their separation from free $Na^+$ on a Sephadex column. The results shown in fig.1 indicate that almost no $^{22}Na^+$ was transported into the vesicles, when these were incubated for 1 min with $^{22}NaCl$ in the absence of oxaloacetate. However, in its presence $^{22}Na^+$ accumulated inside these vesicles to a considerable extent. The amount of $^{22}Na^+$ accumulation during a 1 min incubation period increased with increasing oxaloacetate concentrations (between 0.036 and 1.8 mM; table 1). However, the apparent accumulation of $^{22}Na^+$ was always lower, if longer incubation periods (2–15 min) were applied. Since at the above concentrations all oxaloacetate was decarboxylated in <1 min, the cessation of $Na^+$ transport within this period is in accord with the assumption that this transport requires oxaloacetate decarboxylation.

This concept was further supported by the results of $Na^+$ uptake experiments with vesicles which had been treated with avidin in order to abolish oxaloacetate decarboxylase activity. With these vesicles the oxaloacetate-dependent $Na^+$ uptake was negligible in comparison to its accumulation by untreated vesicles or vesicles which had been treated with an avidin–biotin complex (table 2). The amount of $Na^+$ accumulation in the latter two cases was about the same and this excludes the interference of avidin with the transport system by a mechanism which would be different from the blockage of the biotin prosthetic group of the decarboxylase. The simultaneous inhibition of oxaloacetate decarboxylation and $Na^+$ transport by avidin could indicate the direct involvement of oxaloacetate decarboxylase in both processes, but would also be compatible with a $Na^+$ transport requiring the products of oxaloacetate decarboxylation, i.e., pyruvate and bicarbonate. The latter possibility was excluded, since pyruvate and bicarbonate could not substitute for oxaloacetate in performing $Na^+$ accumulation to a considerable extent (see table 2E).

Table 1
Dependence of oxaloacetate concentration and incubation time on sodium transport (nmol $^{22}Na^+$ incorporated into vesicles)

| Additions and omissions | Incubation periods: | |
|---|---|---|
| | 1 min | 15 min |
| Oxaloacetate omitted | 0.028 | 0.17 |
| Oxaloacetate 0.036 mM | 0.60 | 0.45 |
| Oxaloacetate 0.18 mM | 1.0 | 0.78 |
| Oxaloacetate 1.8 mM | 1.33 | 0.57 |

## 4. Discussion

Inverted vesicles from citrate-fermenting cells of *K. aerogenes* performed the accumulation of $Na^+$ in response to the decarboxylation of oxaloacetate by the membrane-bound and $Na^+$-activated oxaloacetate decarboxylase. There was almost no $Na^+$ uptake in the absence of oxaloacetate and, after blocking the vesicular oxaloacetate decarboxylase with avidin, the oxaloacetate-dependent $Na^+$ uptake was unimportant in comparison to that of the uninhibited vesicles. The residual $Na^+$ uptake could be due to a small amount of oxaloacetate decarboxylase which survived the avidin treatment (not detectable by the optical assay) or to an independent oxaloacetate requiring $Na^+$ transport, e.g., oxaloacetate–$Na^+$ symport.

Table 2
Inhibition of sodium transport by avidin treatment

| Expt. | nmol $^{22}Na^+$ incorporated into vesicles |
|---|---|
| A | 4.4 |
| B | 0.8 |
| C | 4.7 |
| D | 0.2 |
| E | 0.4 |

(A) Vesicles containing 6.4 U oxaloacetate decarboxylase (6 mg protein) were incubated for 1 min at 25°C in 0.63 ml 50 mM K-phosphate buffer (pH 7.5) with 0.48 mM $^{22}NaCl$ (714 000 cpm) and 0.8 mM Li-oxaloacetate; the vesicles were subsequently isolated and counted for radioactivity as in section 2. (B) and (C) were performed likewise but with vesicles (the same amount as in (A)) preincubated for 15 min with 0.6 mg avidin (B), or with an avidin–biotin complex (0.6 mg avidin + 1 mg biotin) (C); (D) control without oxaloacetate; (E) control without oxaloacetate and with addition of K-pyruvate and $KHCO_3$ (0.8 mM each)

With this vesicle preparation it was not possible to derive a stoichiometry between oxaloacetate decarboxylation and $Na^+$ transport. With different amounts of oxaloacetate and $Na^+$ and the same amount of vesicles decarboxylation always went to completion indicating that the system is at least partially uncoupled. This uncoupling could result from the presence of oxaloacetate decarboxylase in non-vesicular membrane fragments or leaky vesicles or from opposite orientation of the decarboxylase in the same vesicles or other reasons. The results are not unexpected, however, since partial uncoupling has been noted in studies of oxidative phosphorylation [7] and $Ca^{2+}$ transport [8], where similarly prepared bacterial membrane vesicles have been used.

This is the first report of a system where the chemical energy liberated upon decarboxylation of a carbonic acid is used to drive transport and this is also the first report of the participation of a biotin enzyme in a transport. The biotin prosthetic group during decarboxylation of oxaloacetate switches between the free and the carboxylated state [4,5]. $Na^+$ is specifically required for the decarboxylation of the carboxybiotin intermediate and therefore this reaction probably participates in the translocation of $Na^+$ through the membrane. Another bacterial biotin enzyme related to oxaloacetate decarboxylase is methylmalonyl-CoA decarboxylase, which is probably also bound to the membrane [2]. The similarity with oxaloacetate decarboxylase suggests that methylmalonyl-CoA decarboxylase might also function in a cation transport. The enzyme apparently does not require a certain metal ion for activity, but could catalyse the transport of protons.

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