Biochimica et Biophysica Acta 812 (1985) 841–844
Elsevier

BBA 70200

BBA Report

Alternative-substrate inhibition of L-lactate transport via the monocarboxylate-specific carrier system in human erythrocytes

A.W. de Bruijne, H. Vreeburg and J. van Steveninck

Sylvius Laboratories, Department of Medical Biochemistry, Wassenaarseweg 72, 2333 AL Leiden (The Netherlands)

(Received October 17th, 1984)

Key words: Lactate transport; Monocarboxylate carrier; Alternative-substrate inhibition ; (Erythrocyte membrane)

The L-lactate/proton symport system of the red blood cell membrane was studied under conditions of alternative-substrate inhibition by glycolate. At constant pH of the medium glycolate caused competitive inhibition of L-lactate transport. In Lineweaver-Burk plots of $1/v$ against $1/[H]$, on the other hand, glycolate caused an uncompetitive inhibition. These observations indicate, that the monocarboxylate carrier exhibits ordered substrate binding, with the proton binding first.

Monocarboxylate transport across the red cell membrane proceeds by three different pathways: by nonionic diffusion, via the band 3 anion exchange protein and via a specific monocarboxylate carrier system [1–5]. Under physiological conditions the latter system accounts for about 90% of total L-lactate transport [1]. These three pathways can be discriminated by the use of specific inhibitors. Transport via the band 3 anion exchange protein is completely blocked by DIDS, whereas transport via the specific monocarboxylate carrier is highly sensitive to PCMBS [4]. Thus it is possible to quantitate lactate transport via the monocarboxylate carrier by comparing transport in the presence of DIDS and transport in the presence of DIDS and PCMBS.

It has been shown that L-lactate transport by the monocarboxylate carrier proceeds via a substrate/proton symport system [2,4,6]. In symport systems substrate binding may occur either by an ordered or by a random mechanism. As has been pointed out before, this order of substrate binding is very important to the understanding of various aspects of the transport process [6,7]. Recently this notion prompted many studies, designed to elucidate the substrate binding mechanism in various symport systems [6–13].

In a previous paper experimental evidence was presented, indicating an ordered binding mechanism, with H^+ binding first, for the monocarboxylate carrier of human erythrocytes [6]. In these studies the influence of the medium pH on the kinetic parameters was studied. The possibility of random binding could not be excluded completely, however. It could be rationalized that if the translocation velocity of the loaded carrier is much larger than the translocation velocity of the empty carrier, the experimental results might still be compatible with a random binding mechanism [6]. For this reason the binding mechanism of the monocarboxylate carrier system was reinvestigated, utilizing kinetic analysis of alternative-substrate inhibition.

Materials and methods. Preparation of erythrocyte suspensions, pretreatment of the cells with DIDS and PCMBS, manipulation of medium pH and measurements of initial lactate uptake velocities were done as described previously [6].

As substrate analogue for L-lactate glycolate

Abbreviations: DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; PCMBS, *p*-chloromercuriphenylsulfonic acid.

was used. In control experiments it appeared that glycolate was transported via the monocarboxylate carrier system. In inhibition experiments unlabeled glycolate and ^{14}C-labeled L-lactate were added to the red cell suspension simultaneously, followed by measurements of the initial uptake velocity of [^{14}C]lactate as described before [6]. L-[^{14}C]Lactate was obtained from Amersham International, DIDS from Pierce and PCMBS from Sigma.

Theoretical. The general model for substrate/proton symport is shown in Fig. 1. Using this model to discriminate between random and ordered substrate binding, kinetic parameters for initial influx ($S_i = I_i = 0$) can be derived as described previously [8]. At constant inhibitor concentrations Lineweaver-Burk plots can be made of $1/v$ against $1/[S_o]$ (at constant pH) and of $1/v$ against $1/[H_o]$ (at constant substrate concentration). The relevant parameters for these plots are summarized in Table I. It is clear that both with random binding and with ordered binding, proton binding first, an alternative substrate will cause competitive inhibition with respect to the substrate. In both cases a $1/v$ against $1/[S_o]$ plot exhibits an increased slope (K_{app}/V_{app}) in the presence of the inhibitor, with an unchanged V_{app}. The difference between random and ordered binding is reflected in $1/v$ against $1/[H_o]$ plots. In the case of random binding both $1/V_{app}$ and K_{app}/V_{app} are increased in the presence of the inhibitor. With ordered binding, proton binding first, however, the alternative substrate will cause an uncompetitive inhibition: $1/V_{app}$ is increased, whereas the slope (K_{app}/V_{app}) is unaffected.

Results. Influx of [^{14}C]lactate via the monocarboxylate carrier was calculated from the differences of fluxes into DIDS-treated cells and into (DIDS + PCMBS)-treated cells, as described previously [6]. Fig. 2 shows the results of such influx measurements at constant pH, in the absence and in the presence of glycolate. As expected, glycolate caused competitive inhibition under these circumstances.

In further experiments lactate influx was measured at a constant lactate concentration, at varying medium pH. In all experiments glycolate caused an uncompetitive inhibition of lactate in-

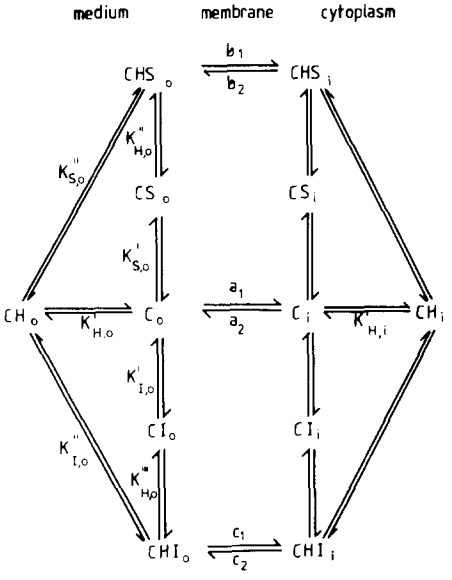

Fig. 1. Kinetic model of transport via the monocarboxylate carrier in red blood cells under conditions of alternative-substrate inhibition. $C_o \ldots CHS_o$, CHI_o, $C_i \ldots CHS_i$, CHI_i, ligand-binding sites facing, respectively, the outside and inside of the cell. H, proton; S, lactate; I, glycolate; K, dissociation constant; $a_1 \ldots c_2$, translocation velocity constants.

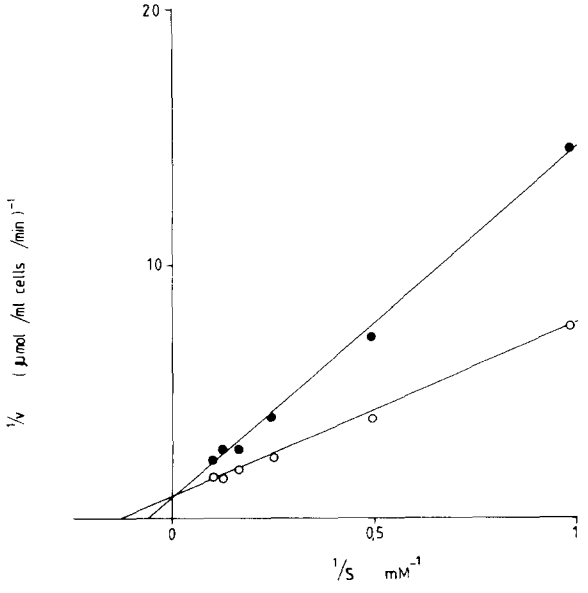

Fig. 2. Lineweaver-Burk plot of lactate transport via the monocarboxylate carrier at pH 6.8, in the absence of glycolate (○——○) and in the presence of 10 mM glycolate (●——●).

TABLE I

KINETIC PARAMETERS FOR SUBSTRATE/PROTON SYMPORT IN THE PRESENCE OF AN ALTERNATIVE SUBSTRATE, DURING INITIAL INFLUX

(1) Random; $1/v$ against $1/[S_o]$

$$\frac{1}{V_{app}} = \frac{1}{b_1 C_T}\left(\frac{K'_{H,o}K''_{s,o}}{H_o K'_{s,o}} + 1 + \frac{b_1}{a_2} + \frac{b_1 H_i}{a_2 K'_{H,i}}\right)$$

$$\frac{K_{app}}{V_{app}} = \frac{1}{b_1 C_T}\left\{\frac{K'_{H,o}K''_{s,o}}{H_o} + K''_{s,o} + \frac{a_1 K'_{H,o}K''_{s,o}}{a_2 H_o} + \frac{a_1 H_i K'_{H,o}K''_{s,o}}{a_2 H_o K'_{H,i}} + I_o\left(\frac{K'_{H,o}K''_{s,o}}{H_o K'_{I,o}} + \frac{K''_{s,o}}{K''_{I,o}} + \frac{c_1 K''_{s,o}}{a_2 K''_{I,o}} + \frac{c_1 H_i K''_{s,o}}{a_2 K'_{H,i} K''_{I,o}}\right)\right\}$$

(2) Ordered (H^+ first); $1/v$ against $1/[S_o]$

$$\frac{1}{V_{app}} = \frac{1}{b_1 C_T}\left(1 + \frac{b_1}{a_2} + \frac{b_1 H_i}{a_2 K'_{H,i}}\right)$$

$$\frac{K_{app}}{V_{app}} = \frac{1}{b_1 C_T}\left\{\frac{K'_{H,o}K''_{s,o}}{H_o} + K''_{s,o} + \frac{a_1 K'_{H,o}K''_{s,o}}{a_2 H_o} + \frac{a_1 H_i K'_{H,o}K''_{s,o}}{a_2 H_o K'_{H,i}} + I_o\left(\frac{K''_{s,o}}{K''_{I,o}} + \frac{c_1 K''_{s,o}}{a_2 K''_{I,o}} + \frac{c_1 H_i K''_{s,o}}{a_2 K'_{H,i} K''_{I,o}}\right)\right\}$$

(3) Random; $1/v$ against $1/[H_o]$

$$\frac{1}{V_{app}} = \frac{1}{b_1 C_T}\left\{\frac{K''_{s,o}}{S_o} + 1 + \frac{b_1}{a_2} + \frac{b_1 H_i}{a_2 K'_{H,i}} + I_o\left(\frac{K''_{s,o}}{S_o K''_{I,o}} + \frac{c_1 K''_{s,o}}{a_2 S_o K''_{I,o}} + \frac{c_1 H_i K''_{s,o}}{a_2 S_o K'_{H,i} K''_{I,o}}\right)\right\}$$

$$\frac{K_{app}}{V_{app}} = \frac{1}{b_1 C_T}\left(\frac{K'_{H,o}K''_{s,o}}{S_o} + \frac{K'_{H,o}K''_{s,o}}{K'_{s,o}} + \frac{a_1 K'_{H,o}K''_{s,o}}{a_2 S_o} + \frac{a_1 H_i K'_{H,o}K''_{s,o}}{a_2 S_o K'_{H,i}} + \frac{I_o K'_{H,o}K''_{s,o}}{S_o K'_{I,o}}\right)$$

(4) Ordered (H^+ first); $1/v$ against $1/[H_o]$

$$\frac{1}{V_{app}} = \frac{1}{b_1 C_T}\left\{\frac{K''_{s,o}}{S_o} + 1 + \frac{b_1}{a_2} + \frac{b_1 H_i}{a_2 K'_{H,i}} + I_o\left(\frac{K''_{s,o}}{S_o K''_{I,o}} + \frac{c_1 K''_{s,o}}{a_2 S_o K''_{I,o}} + \frac{c_1 H_i K''_{s,o}}{a_2 S_o K'_{H,i} K''_{I,o}}\right)\right\}$$

$$\frac{K_{app}}{V_{app}} = \frac{1}{b_1 C_T}\left(\frac{K'_{H,o}K''_{s,o}}{S_o} + \frac{a_1 K'_{H,o}K''_{s,o}}{a_2 S_o} + \frac{a_1 H_i K'_{H,o}K''_{s,o}}{a_2 S_o K'_{H,i}}\right)$$

flux via the monocarboxylate carrier (Fig. 3).

Discussion. Kinetic analysis of symport systems yields equations with complicated, composite constants, which can not be measured experimentally. As a consequence, interpretation of experimental data will not always be unequivocal. For instance, a previous analysis of the influence of medium pH on L-lactate transport in erythrocytes via the monocarboxylate carrier indicated ordered binding, proton binding first [6]. However, it could be shown that if $K''_H/[H_o] \ll 1 + b/a(1 + [H_i]/K'_H)$, the results would still be reconcilable with random binding [6]. Therefore it appeared appropriate, to reinvestigate the binding order of the monocarboxylate carrier system, utilizing the completely different approach of alternative substrate inhibition.

The results shown in Fig. 3 confirm ordered binding, proton binding first: as shown in the theoretical section, uncompetitive inhibition at varying medium pH can only be expected with this binding order.

This type of kinetic analysis is important for the understanding of the transport process at the molecular level [6,7]. As shown previously, the monocarboxylate transport system behaves kineti-

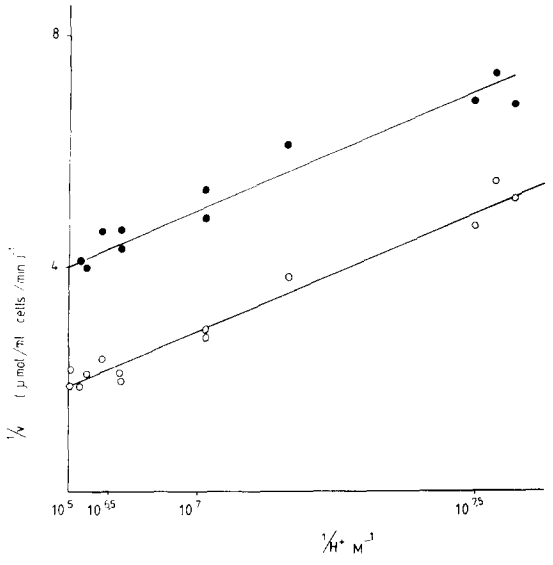

Fig. 3. Lineweaver-Burk plot of lactate transport via the monocarboxylate carrier at varying medium pH. Lactate concentration: 4 mM. ○———○: uptake in the absence of glycolate; ●———●: uptake in the presence of 10 mM glycolate.

cally as a mobile carrier, signifying that the carrier molecule has a single set of binding sites, exposed alternately to the outside and to the inside of the membrane, via a conformational change in the carrier molecule [8].

Also the conclusion of ordered substrate binding has important implications at the molecular level. In the case of random binding more or less independent binding sites for proton and substrate should be assumed. Translation will be triggered by binding of the second ligand, presumably via a conformational change of the carrier. With ordered binding, on the other hand, two possibilities should be considered. Assuming two binding sites, for proton and lactate, respectively, binding of the proton may induce a conformational change, resulting in a major increase of the affinity of the lactate binding site for its substrate. A second possibility would be that proton binding to an uncharged group actually creates the binding site for the negatively charged lactate ion [6]. Further investigations will be required to discriminate between these possibilities.

References

1 Deuticke, B., Rickert, I. and Beyer, E. (1978) Biochim. Biophys. Acta 507, 137–155
2 Dubinsky, W.P. and Racker, E. (1978) J. Membrane Biol. 44, 25–36
3 Deuticke, B., Beyer, E. and Forst, B. (1982) Biochim. Biophys. Acta 684, 96–110
4 Deuticke, B. (1982) J. Membrane Biol. 70, 89–103
5 Jennings, M.L. and Adams-Lackey, M. (1982) J. Biol. Chem. 257, 12866–12871
6 De Bruijne, A.W., Vreeburg, H. and Van Steveninck, J. (1983) Biochim. Biophys. Acta 732, 562–568
7 Page, M.G.P. and West, I.C. (1982) Biochem. J. 204, 681–688
8 Van den Broek, P.J.A. and Van Steveninck, J. (1982) Biochim. Biophys. Acta 693, 213–220
9 Cupoletti, J. and Segel, I.H. (1975) Biochemistry 14, 4712–4718
10 Bentaboulet, M. and Kepes, A. (1981) Eur. J. Biochem. 117, 233–238
11 Legrum, B. Fasold, H. and Passow, H. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 1573–1590
12 Milanick, M.A. and Gunn, R.B. (1982) J. Gen. Physiol. 79, 87–113
13 Van den Broek, P.J.A. and Van Steveninck, J. (1980) Biochim. Biophys. Acta 602, 419–432