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BBA Report

Mycoplasma contamination greatly enhances the apparent transport and concentrative accumulation of formycin B by mammalian cell culture

Peter G.W. Plagemann

Department of Microbiology, Medical School, University of Minnesota, Minneapolis, MN 55455 (U.S.A.)

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S49 mouse leukemia cells exhibit both equilibrative and Na^+-dependent, concentrative formycin B transport. The latter represents only a minor nucleoside transport component and is detectable only when equilibrative nucleoside transport is inhibited by dipyridamole or another transport inhibitor. Thus in uncontaminated S49 cells formycin B accumulated only to slightly above the intracellular-extracellular equilibrium level. In contrast, in suspensions of S49 cells contaminated with mycoplasma, formycin B accumulated in the intracellular water space in unmodified form to 40–50-times the extracellular concentration in a dipyridamole-independent manner during 90 min of incubation at 37°C. The mycoplasma active formycin B transport system was inhibited by all nucleosides tested, including thymidine and deoxycytidine, which are not substrates for the concentrative nucleoside transporter of S49 cells. Mycoplasma contamination was detected by the presence of cell-associated adenosine phosphorylase activity.

With few exceptions, all mammalian cell express equilibrative nucleoside transport with broad nucleoside specificity [1,2]. A second basic type of nucleoside transport, Na^+-dependent, concentrative transport is prevalent in brush border membranes of epithelial cells of the kidney and intestine [3–7]. However, Na^+-dependent, concentrative transport has also been found to be expressed in all mouse cell types that have been investigated [8–15]. It is specific for uridine and purine nucleosides, largely resistant to inhibitors of equilibrative nucleoside transport, such as nitrobenzylthioinosine and dipyridamole, and its K_m for formycin B transport is about two orders of magnitude lower than that for equilibrative formycin transport. In mouse macrophages and lymphocytes concentrative transport represents a major nucleoside transport component [8–11], whereas in different mouse cell lines derived variously from lymphocytes (P388, L1210, S49, LK35.2), macrophages (RAW 309Cr.l) and fibroblasts (L929) it represents only a minor transport component [11,15]. In fact, in the mouse cell lines concentrative nucleoside transport becomes only detectable if equilibrative nucleoside transport is inhibited [11–13]. The concentrative substrate accumulation results from the inhibition of the efflux via equilibrative transport of the nucleoside that is actively transported into the cells [11,12].

Measurements of the transport of natural nucleosides in mammalian cells are generally complicated by the rapid intracellular conversion of the nucleosides to phosphorylated products, which is especially efficient at low micromolar concentrations [1,2]. Thus it is often difficult in influx measurements to distinguish between rates of transport per se and the intracellular trapping of the substrate in phosphorylated products [1,2]. This problem can be avoided by the use of a non-metabolizable nucleoside as transport substrate [2]. We and others have promoted the use of formycin B for this purpose [4,10–13,16]. It is an efficient substrate for both the equilibrative nucleoside transporter and the Na^+-dependent, concentrative nucleoside transporter of mammalian cells but not for any mammalian nucleoside kinase or other metabolic enzymes [4,10,12,16]. Thus its accumulation in cells can be measured unimpeded by metabolic conversions.

In the course of studies of the formycin B transport systems of various mammalian cell lines it became apparent that formycin B accumulation by cell cultures can become greatly distorted by the presence of con-

Correspondence: P. Plagemann, Department of Microbiology, Medical School, University of Minnesota, Minneapolis, MN 55455, U.S.A.

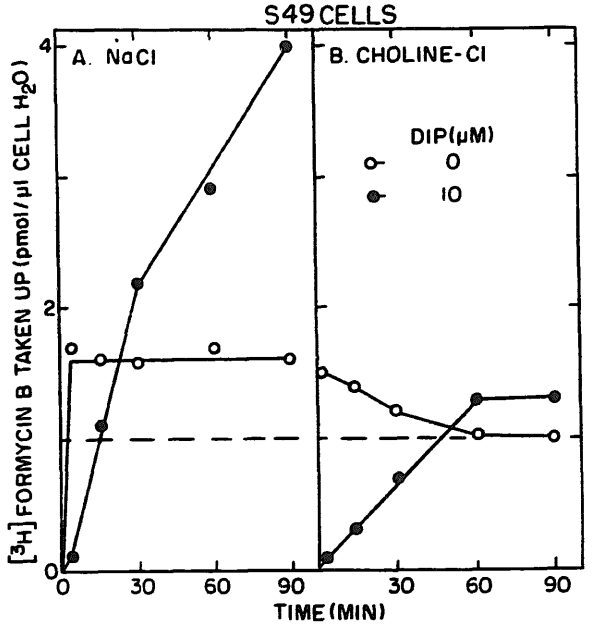

Fig. 1. Effect of dipyridamole on the long-term uptake of formycin B by control (A) and Na^+-depleted (B) S49 cells. S49 cells were propagated in suspension culture as described previously [20]. Cells were collected from late exponential phase cultures by centrifugation at about $400 \times g$. In A, the cells were suspended to $2 \cdot 10^7$ cells/ml in a serum-free basal medium (RPMI). In B, the cells were washed in 0.145 M choline chloride containing 5 mM Tris-HCl (pH 7.4) and then suspended in the same [12,13]. One half of each suspension was supplemented as indicated with 10 μM dipyridamole and then all suspensions received 1 μM [^{3}H] formycin B (Moravek Biochemicals, Brea, CA; supplemented with unlabeled formycin B to 210 cpm/pmol). At the indicates times of incubation at 37°C, the cells from 0.5-ml samples of suspension were collected by centrifugation through an oil layer and analyzed for radioactivity as described previously [10,16]. Radioactivity/cell pellet was corrected for that attributable to extracellular space in the cell pellet as estimated with [^{14}C]inulin [21]. Intracellular H_2O space was measured with ^3H_2O [21]. All values are averages of duplicate samples. The broken lines indicate the intracellular concentration of formycin B equal to that in the medium.

taminating mycoplasmas. This effect is a potential serious problem in nucleoside transport studies because mycoplasma contamination of cell lines is not an uncommon occurrence [17,18] and may remain undetected for long periods of time if cultures are only infrequently assayed for their presence. Contamination may remain undetected because it is not readily apparent by visual observation of the cultures and may not have significant effects on the growth and other properties of the cells. Detection requires specific assays [17,18].

The effect of mycoplasma on the uptake of formycin B by S49 mouse leukemia cells is illustrated by a comparison of the data in Figs. 1 and 2. When uncontaminated S49 cells were incubated with 1 μM [^{3}H]formycin B it equilibrated with the intracellular water space in less than 2 min (Fig. 1A; also see Ref. 13). Facilitated formycin-B transport was strongly inhibited by 10 μM dipyridamole (Fig 1A). However, as discussed already, in the presence of dipyridamole formycin B ultimately accumulated concentratively in the cells because of the operation of the Na^+-dependent concentrative transporter and the inhibition of the efflux of the entering formycin B via equilibrative formycin B transport. That the concentrative accumulation of formycin B was mediated by the Na^+-dependent carrier is demonstrated by the fact that it was abolished in a medium lacking Na^+ (Fig. 1B; see Refs. 10–13).

In contrast to the uncontaminated cultures, suspensions of S49 cells contaminated with mycoplasma (S49-

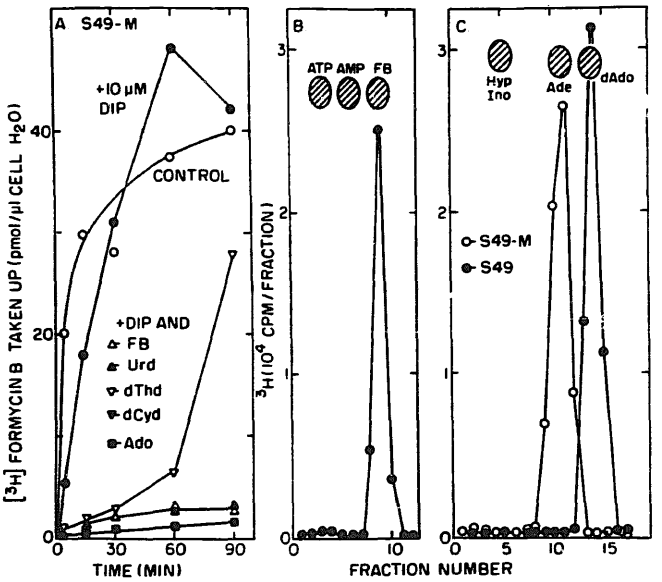

Fig. 2. Uptake of formycin B by a culture of S49 cells contaminated with mycoplasma (S49-M) and demonstration of adenosine phosphorylase activity in this culture. (A) The uptake of 1 μM [^{3}H]-formycin B (180 cpm/pmol) was measured in samples of a suspension of $2 \cdot 10^7$ S49-M cells/ml of RPMI (control, ○———○) as described in the legend to Fig. 1. Where indicated, the suspension was preincubated with 10 μM dipyridamole (●———●) or pretreated with dipyridamole and then supplemented with 100 μM unlabeled formycin B (FB, △———△), uridine (Urd, ▲———▲), thymidine (dThd, △———△), deoxycytidine (dCyd, ▼———▼) or adenosine (Ado, ■———■) simultaneously with the [^{3}H]formycin B. All values are averages of duplicate suspension samples. (B) A trichloroacetic acid extract was prepared from dipyridamole-treated S49-M cells incubated with [^{3}H]formycin B in A for 90 min and analyzed by ascending paper chromatography as described previously [10,16]. The lane of migration of the developed and dried paper was cut into 1-cm fractions, which were analyzed for radioactivity. (C) 10 ml of late exponential phase cultures of S49 and S49-M cells (about $2 \cdot 10^6$ cells/ml) were centrifuged at $10000 \times g$ for 30 min. The pellets were suspended in 0.2 ml of lysing buffer containing deoxycoformycin and assayed for adenosine phosphorylase activity using [^{3}H]deoxyadenosine by a procedure modified from that described previously [19]; 25 μl of each lysate were mixed with 25 μl of 100 μM [^{3}H]deoxyadenosine in 100 mM phosphate buffer (pH 7.4). After 30 min of incubation at 37°C, the mixture was directly spotted onto chromatographic paper and the paper developed with solvent 40 (39 ml butanol, 22 ml ethylacetate, 22 ml ammonium hydroxide, and 17 ml methanol) for the separation of adenine and deoxyadenosine. The lanes of migration of the developed and dried paper were cut into 1-cm fractions, which were analyzed for radioactivity.

M) concentratively accumulated 1 μM [^{3}H]formycin B to about 40-times the intracellular-extracellular equilibrium level in the absence of dipyridamole (Fig. 2A). This finding has been confirmed on two occasions. Furthermore, the concentrative accumulation of formycin B in the contaminated cultures was little affected by dipyridamole, but was inhibited by all nucleosides tested, including thymidine and deoxycytidine (Fig. 2A), which are not substrates for the Na^+-dependent, concentrative nucleoside transporter of these and other mouse cells [12,13].

The presence of mycoplasma in the culture was clearly indicated by a high level of adenosine phosphorylase activity as measured with 2′-deoxyadenosine as a substrate [19]. Deoxyadenosine was complete converted to adenine during 30 min of incubation with a cell lysate prepared from these cultures (S49-M), whereas no conversion was observed with a similarly prepared preparation from an uncontaminated culture (S49; Fig. 2C). Mycoplasma contamination had only a minimal, if any, effect on the growth of the S49 cells (data not shown). The mycoplasma contaminant has not been identified but in a previous study a culture of P388 cells in this laboratory was found to be contaminated with *Mycoplasma orale* [19]. The results indicate the mycoplasmas possess a highly active, broadly substrate-specific nucleoside transport system. Since for transport measurements the S49-M cells were collected by low speed centrifugation from exponential phase cultures and suspended in fresh serum-free medium, the mycoplasmas must have remained associated with the S49 cells during sedimentation, probably being cytadsorbed [17]. Furthermore, since the uptake assay involves separating the cells after incubation with [^{3}H]formycin B from the medium by a 2-s centrifugation through an oil layer in an Eppendorf centrifuge, the mycoplasmas must sediment through the oil layer under these conditions or they are carried along with the mammalian cells. Chromatographic analysis of acid extracts of 90-min labeled S49-M cells indicate the exclusive presence of unmodified [^{3}H]formycin B (Fig. 2B). This finding combined with the fact that the mycoplasmas contributed only minimally to the total intracellular water space of the S49 cell suspension indicates that the nucleoside transport system of the mycoplasma must be a concentrative one. The results indicate that in nucleoside transport studies with mammalian cells caution is required to avoid mycoplasma contamination and that the absence of mycoplasma must be ascertained experimentally. The adenosine phosphorylase assay described in the present study (see legend to Fig. 2) is simple and highly sensitive and it has the advantage that it seems to detect all mycoplasmas (see Ref. 19).

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References

1 Plagemann, P.G.W. and Wohlhueter, R.M. (1980) Curr. Top. Membr. Transp. 14, 255–329.
2 Plagemann, P.G.W. and Wohlhueter, R.M. (1988) Biochim. Biophys. Acta 947, 405–443.
3 LeHir, M. and Dubach, U.C. (1985) Pflügers Arch. 404, 238–243.
4 Jacobs, E.S. and Paterson, A.R.P. (1986) Biochem. Biophys. Res. Commun. 14, 1028–1036.
5 Vijayalakshmi, D. and Belt, J. (1988) J. Biol. Chem. 263, 19419–19423.
6 Lee, C.W., Cheeseman, C.J. and Jarvis, S.M. (1988) Biochim. Biophys. Acta 942, 139–149.
7 Jarvis, S.M. (1989) Biochim. Biophys. Acta 979, 132–138.
8 Darnowski, J.W., Holdridge, C. and Handschuhmacher, R.E. (1987) Cancer Res. 47, 2614–2619.
9 Plagemann, P.G.W. and Woffendin, C. (1989) Biochim. Biophys. Acta 981, 315–325.
10 Plagemann, P.G.W., Aran, J.M. and Woffendin, C. (1990) Biochim. Biophys. Acta 1022, 93–102.
11 Plagemann, P.G.W. and Aran, J.M. (1990) Biochim. Biophys. Acta 1025, 32–42.
12 Plagemann, P.G.W. and Aran, J.M. (1990) Biochim. Biophys. Acta 10281, 289–298.
13 Plagemann, P.G.W. (1991) J. Cell. Biochem. in press.
14 Crawford, C.R., Ng, C.Y.C., Noel, J.D. and Belt, J.A. (1990) J. Biol. Chem. 265, 9732–9936.
15 Dagino, L. and Paterson, A.R.P. (1990) Cancer Res. 50, 6549–6553.
16 Plagemann, P.G.W. and Woffendin, C. (1989) Biochim. Biophys. Acta 1010, 7–15.
17 McGarrity, G.J., Sarama, J. and Vanahan, V. (1985) ASM News 51, 170–183.
18 Mowles, J.M. (1990) Methods Mol. Biol. 51, 65–74.
19 Plagemann, P.G.W. and Woffendin, C. (1990) J. Cell. Biochem. 43, 161–172.
20 Plagemann, P.G.W. and Woffendin, C. (1987) Mol. Cell. Biol. 7, 160–166.
21 Wohlhueter, R.M. and Plagemann, P.G.W. (1989) Methods Enzymol. 173, 714–732.