

Inhibition of ammonium (methylammonium) transport in *Klebsiella pneumoniae* by glutamine and glutamine analogues

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Received 9 July 1982

Ammonium transport

Methionine sulfoximine

6-Diazo-5-oxo-norleucine

Regulation

Klebsiella pneumoniae

1. INTRODUCTION

Ammonium-transport systems are widely distributed among bacteria [1–8]. In several strains the synthesis of these transport systems is regulated by the nitrogen source [5–7]. Nothing is known, however, about the regulation of the activity of the transport systems. By employing the glutamine analogues methionine sulfone (MSF), methionine sulfoximine (MSX) and 6-diazo-5-oxo-norleucine (DON), which are well known as inhibitors of the enzymes glutamine synthetase (EC 6.3.1.2) and glutamate synthase (EC 1.4.1.13), we found evidence for a regulation of ammonium transport by the intracellular glutamine level.

2. MATERIALS AND METHODS

Klebsiella pneumoniae M5a1 (a gift from Professor R.H. Burris, University of Wisconsin) was grown aerobically in batches with 20 mM histidine as the nitrogen source and assayed for methylammonium uptake as in [5]. Glutamine synthetase (ATP-dependent formation of γ -glutamyl hydroxamate from glutamate) and glutamate synthase were assayed according to established methods [9]. DON and MSX were from Sigma (München); MSF was from Riedel-De Haen (Hannover).

3. RESULTS

Ammonium transport in *K. pneumoniae* is inhibited weakly by glutamine, and strongly by MSF, MSX and DON (table 1). Inhibition by the analogues

was very slow at 15°C (requiring > 15 min for completion) but was completed in < 2 min at 30°C.

The same inhibitors also act on glutamine synthetase and glutamate synthase [10,11], but with generally less efficiency (table 1). MSX and DON inhibited the ammonium transport irreversibly, since 2 washes with buffer did not relieve their effect. In contrast, the inhibition by MSF disappeared after washing the cells. Similar patterns of reversibility and irreversibility have been observed for glutamine synthetase and glutamate synthase [10–12].

The addition of glutamine counteracted the inhibition of transport by the glutamine analogues,

Table 1

Effect of glutamine (Gln) and glutamine analogues on ammonium transport, glutamine synthetase and glutamate synthase in *K. pneumoniae*

Reaction	Conc. (μ M) at which 50% inhibition occurs			
	Gln	MSF	MSX	DON
Ammonium transport	~ 4000	45	40	2
Glutamine synthetase	—	500	60	>> 2000
Glutamate synthetase	—	70	500	9

Assays were done at 30°C after incubation with the inhibitors for 2 min

Table 2

Protective effect of glutamine (Gln) on the inhibition of ammonium transport by glutamine analogues

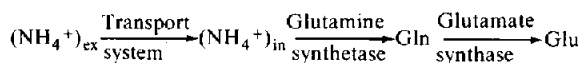
Compound	Conc. (mM)	Remaining act. (%)
None	—	100
Gln	5	41
Don	0.012	0
Gln/DON	5/0.012	35
DON/Gln	0.012/5	0
Gln	1.2	86
MSF	0.2	0
Gln/MSF	1.2/0.2	49
MSF/Gln	0.2/1.2	0
MSX	0.2	0
Gln/MSX	1.2/0.2	62
MSX/Gln	0.2/1.2	0

The cells were incubated for 2 min with the compound indicated first; then the second compound was added; after a further 2 min the assay was started

but only when glutamine was added prior to the latter compounds (table 2). Addition of glutamine after incubation with the analogues had no effect.

4. DISCUSSION

Under the growth conditions employed, ammonium assimilation in *K. pneumoniae* proceeds via the pathway [11,13]:



The glutamine analogues MSX, MSF and DON have been described as potent inhibitors of glutamine synthetase [10] and glutamate synthase [11]. Here, we show that all 3 compounds also block ammonium uptake. Inhibition of 50% of the different enzymes was obtained at different concentrations of the inhibitors (table 2). Although the intracellular inhibitor concentrations are unknown, the inhibition patterns revealed in table 1 suggest a direct binding of the glutamine analogues to the ammonium transport system. This is

corroborated by the finding that glutamine (which was checked to be ammonium-free) also exhibited an (albeit weak) inhibitory effect on the ammonium transport. Some suggestion about the sidedness of the site can be reached from table 2, which describes the protection by glutamine (glutamate had no effect) of the inhibition of ammonium transport by glutamine analogues. The finding that the reversible inhibitor MSF is also efficient only when added before glutamine, is best explained by the assumption that, as in *Salmonella typhimurium* [14], the glutamine analogues enter the cell via the glutamine transport system. Prior addition of glutamine then largely prohibits entry into the cell and the access to the binding site at the ammonium transport system, which is thus concluded to be located at the inner side of the cytoplasmic membrane.

From these results, we infer that the ammonium transport system in *K. pneumoniae* contains a regulatory site for glutamine. Such a control mechanism would be biologically significant, since the intracellular glutamine concentration responds fast and strongly to the extracellular ammonium level [13].

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

- [1] Stevenson, R. and Silver, S. (1977) Biochem. Biophys. Res. Commun. 75, 1133–1139.
- [2] Kleiner, D. (1981) Biochim. Biophys. Acta 639, 41–52.
- [3] Gordon, J.K. and Moore, R.A. (1981) J. Bacteriol. 148, 435–442.
- [4] Barnes, E.M. jr and Zimniak, P. (1981) J. Bacteriol. 146, 512–516.
- [5] Kleiner, D. (1982) Biochim. Biophys. Acta in press.
- [6] Alef, K. and Kleiner, D. (1982) Arch. Microbiol. in press.
- [7] Hartmann, A. and Kleiner, D. (1982) FEMS Microbiol. Lett. 15, 65–67.
- [8] Wiegel, J. and Kleiner, D. (1982) FEMS Microbiol. Lett. 15, 61–63.

- [9] Kleiner, D. (1979) Arch. Microbiol. 120, 263–270.
- [10] Brenchley, J.E. (1973) J. Bacteriol. 114, 666–673.
- [11] Nagatani, H., Shimizu, M. and Valentine, R.C. (1971) Arch. Mikrobiol. 79, 164–175.
- [12] Meister, A. (1974) in: The Enzymes (Boyer, P.D. ed) vol. 10, pp. 699–754, Academic Press, London, New York.
- [13] Kleiner, D. (1976) Arch. Microbiol. 111, 85–91.
- [14] Ayling, P.D. (1981) J. Bacteriol. 148, 514–520.