SHORT COMMUNICATION

Specificity of Exochelins for Iron Transport in Three Species of Mycobacteria

By MARIS C. STEPHENSON AND COLIN RATLEDGE*

Department of Biochemistry, University of Hull, Hull HU6 7RX

(Received 6 September 1979)

Iron chelated to the water-soluble extracellular iron-binding compound (exochelin) from *Mycobacterium smegmatis* was taken up by that organism by an energy-coupled transport system which was not found in *M. bovis* BCG or *M. intracellulare*. The uptake of iron from the chloroform-soluble exochelins produced by the latter two species was by an inhibitor-insensitive system which was also found in *M. smegmatis*.

INTRODUCTION

Stephenson & Ratledge (1979) have shown that uptake of iron into *Mycobacterium smegmatis* is mediated by exochelin MS, which is a water-soluble extracellular chelator found in filtrates of cultures of the same organism grown under conditions of iron-deficiency (Macham *et al.*, 1977). This uptake is an energy-coupled process readily inhibited by electron-transport inhibitors, uncouplers of oxidative phosphorylation, reagents for thiol groups and by anaerobiosis and low temperature. Mycobactin, a lipid-soluble intracellular iron chelator, is not involved in this process. Two types of exochelin have so far been recognized: the one found in *M. smegmatis* cannot be extracted into any organic solvent; the other type is extractable from the medium as its ferric complex into chloroform and has been isolated from *M. bovis* BCG (Macham *et al.*, 1975), *M. tuberculosis* H37Rv (H. J. Morgan, personal communication), *M. avium* (McCready & Ratledge, 1977) and several other species of mycobacteria (J. M. Beaumont, personal communication). The former type reversed the bacteriostatic effect of serum on the growth of *M. smegmatis* but not of *M. bovis* BCG, whereas the latter type of exochelin was effective with both species of mycobacterium in this test (Macham *et al.*, 1975). We have therefore compared the processes of iron transport mediated by these two types of exochelin. *Mycobacterium intracellulare* was included in these studies as it produced an exochelin of the *M. bovis* (MB) type and also grew reasonably quickly.

METHODS

Organisms and growth. *Mycobacterium smegmatis* NCIB 8548, *M. bovis* var. BCG (batch P691 from Glaxo Laboratories, Greenford; this is a different strain from that used previously by Macham *et al.*, 1975) and *M. intracellulare* (received and previously classed as *M. brunense*) NCTC 10574 were grown at 37 °C on a glycerol/asparagine/mineral salts medium with controlled concentrations of iron under shaken or stationary conditions as previously described (Ratledge & Hall, 1971).

Isolation of exochelins. Exochelins were isolated as their ferri-complexes. Exochelin MS was isolated as previously described (Stephenson & Ratledge, 1979) but was not fractionated into its individual components. Exochelin MB and exochelin MI were isolated as described by Macham *et al.* (1975) from, respectively, *M. bovis*, which was grown with 0.04 µg iron ml⁻¹ without shaking for 7 to 8 weeks, and *M. intracellulare*, which was grown with 0.10 µg iron ml⁻¹ under shaken conditions for 21 d.

Preparation of ⁵⁵Fe-exochelins. ⁵⁵Fe-Exochelin MS was prepared as described by Macham *et al.* (1977).

^{55}Fe -Exochelin MB and MI were prepared by mixing the isolated ferri-complex in methanol (2 ml) with a solution of EDTA in water (2 ml) (EDTA was in about a 10-fold excess but the absolute concentration was not important). After stirring at 18 °C for 16 h, approx. 5 ml water was added and the solution was extracted with 2×10 ml chloroform. The solvent extract was evaporated under vacuum, the residue was dissolved in ethanol (2 ml) and $^{55}\text{FeCl}_3$ [of the required concentration and specific activity; about 1 μCi (37 kBq) per μg Fe] was added. After 2 h at 18 °C, the solution was evaporated to dryness, the residue was dissolved in chloroform and washed twice with water to remove unchelated $^{55}\text{FeCl}_3$ and the chloroform was evaporated under vacuum. The residue, ^{55}Fe -exochelin, was dissolved in methanol.

Uptake studies. Initial rates of uptake were determined in cell suspensions of *M. smegmatis*, grown with shaking for 3 or 4 d, *M. intracellulare*, grown with shaking for 21 d, and *M. bovis*, grown without shaking for 42 to 60 d. Protocols for the preparation of the suspensions, for following the uptake of ^{55}Fe -exochelins over the first 3 min, for administering inhibitors, for counting the radioactivity and for processing the results were as previously described (Stephenson & Ratledge, 1979).

RESULTS AND DISCUSSION

From the initial rates of uptake of ^{55}Fe from the various exochelins into suspensions of the three species of mycobacteria and the sensitivity of these processes to three types of inhibitor (NaN_3 as an electron transport inhibitor, carbonyl cyanide *m*-chlorophenylhydrazone as an uncoupler and HgCl_2 as a thiol group reagent) (Table 1), the following conclusions can be drawn. (i) *Mycobacterium smegmatis*, which produces a water-soluble exochelin, transports iron by a specific energy-coupled transport system (see also Stephenson &

Table 1. *Specificity and sensitivity to inhibitors of iron uptake from ferriexochelin by three species of mycobacteria*

Cell suspension	^{55}Fe -Exochelin* (0.6 μM)	Inhibitor†	Initial rate of ^{55}Fe uptake‡	
			[nmol min ⁻¹ (g dry wt) ⁻¹]	(% of control)
<i>M. smegmatis</i>	MS	None	18.2	100
		NaN_3	0.36	2
		CCCP	0.55	3
		HgCl_2	1.27	7
	MI	None	7.0	100
		NaN_3	6.7	95
		CCCP	6.5	93
		HgCl_2	6.3	90
	MB	None	6.3	100
		NaN_3	5.9	94
		CCCP	5.5	87
		HgCl_2	5.7	90
<i>M. intracellulare</i>	MI	None	3.4	100
		NaN_3	3.5	103
		CCCP	3.1	91
		HgCl_2	3.7	109
	MS	None	0.075	—
<i>M. bovis</i> BCG	MB	None	3.0	100
		NaN_3	2.9	98
	MS	None	0.072	—
	MI	None	3.4	100
		NaN_3	3.0	88

* MS, From *M. smegmatis* (water-soluble chelator); MI, from *M. intracellulare* (chloroform-soluble chelator); MB, from *M. bovis* (chloroform-soluble chelator).

† NaN_3 at 30 mM; CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) and HgCl_2 at 0.1 mM.

‡ Slight variations in rate occurred with different batches of cell suspensions.

Ratledge, 1979). (ii) Mycobacteria which produce a chloroform-soluble exochelin (*M. intracellulare* and *M. bovis*) do not possess the specific transport system to enable them to take up iron from a water-soluble exochelin. (iii) The uptake of iron from the chloroform-soluble exochelins (which are also water-soluble) appears to be by a non-specific system which is insensitive to inhibitors. All three mycobacteria possess the ability to take up iron in this fashion from the chloroform-soluble exochelins tested.

On the basis of this very limited survey of three different species of mycobacteria, we can deduce that quite distinct processes for iron acquisition exist within this genus. Already we have demonstrated different processes within *M. smegmatis* itself (Stephenson & Ratledge, 1979), one of which is the energy-coupled uptake of iron from exochelin MS when at its physiological concentration (about 1 μ M). Clearly, the different types of exochelin (water-soluble and chloroform-soluble types) which occur in the mycobacteria have different modes of uptake and it is not surprising that the inhibitor-insensitive process, probably being a facilitated diffusion type of uptake, is found in all three mycobacteria whereas the specific energy-coupled process is only found with the organism which produces the water-soluble chelator. Whether this difference has any ecological significance remains to be seen.

Miss J. M. Beaumont is thanked for her technical assistance. This work was supported by a research grant from the Science Research Council.

REFERENCES

- MACHAM, L. P., RATLEDGE, C. & NOCTON, J. C. (1975). Extracellular iron acquisition by mycobacteria: role of exochelins and evidence against the participation of mycobactin. *Infection and Immunity* **12**, 1242-1251.
- MACHAM, L. P., STEPHENSON, M. C. & RATLEDGE, C. (1977). Iron transport in *Mycobacterium smegmatis*: the isolation, purification and function of exochelin MS. *Journal of General Microbiology* **101**, 41-49.
- MCCREADY, K. A. & RATLEDGE, C. (1977). Mycobactins from *Mycobacterium avium*. *International Journal of Systematic Bacteriology* **27**, 288-289.
- RATLEDGE, C. & HALL, M. J. (1971). Influence of metal ions on the formation of mycobactin and salicylic acid in *Mycobacterium smegmatis* grown in static culture. *Journal of Bacteriology* **108**, 312-319.
- STEPHENSON, M. C. & RATLEDGE, C. (1979). Iron transport in *Mycobacterium smegmatis*: uptake of iron from ferriexochelin. *Journal of General Microbiology* **110**, 193-202.