ACTA CHEMICA SCANDINAVICA 17 (1963) 1902–1906

Transport of B-Vitamins in Microorganisms

I. On the Permeability of *Lactobacillus fermenti* to ^{35}S-Thiamine

HALINA Y. NEUJAHR

Royal Institute of Technology, Division of Food Chemistry, Stockholm 70, Sweden

The permeability of *Lactobacillus fermenti* to ^{35}S-thiamine was studied using thiamine-deficient and thiamine-sufficient cells.

Evidence was obtained that (1) Mg^{2+} ions stimulate this permeability process, (2) the uptake of ^{35}S-thiamine is markedly stimulated by glucose, (3) the glucose stimulation is inhibited by iodoacetate, (4) the iodoacetate inhibition can be reversed by ATP, (5) ATP can to a considerable extent replace glucose as a permeability stimulator in thiamine-deficient cells, but is without effect on the sufficient ones.

It is generally recognized that the transport of nutrients into bacterial cells is of major importance for the cell metabolism. The brilliant works of Monod, Cohen and their associates have revealed the existence of specific and adaptive permeation enzymes, *permeases*, which participate in the uptake of carbohydrates by the cell.[1] These workers demonstrated also that the adaptive character of such enzymes represents a means for regulating the carbohydrate metabolism of bacterial cells. Whereas most of the bacterial permeability studies have been directed towards the transport of carbohydrates [1–3] and amino acids,[4,5] very little has, hitherto, been done with respect to vitamins. Since the latter function as metabolic regulators themselves and represent a very differentiated group from a chemical point of view, it is reasonable to assume that a control of the vitamin supply may provide another means for regulating the cell metabolism. The fact that many microorganisms possess the ability to take up and concentrate vitamins against high concentration gradients indicates that active transport may be involved in this phenomenon. Further indications that this may be the case come from the few reported studies on the permeability of certain bacteria to vitamin B_{12},[6] folic acid [7] and biotin.[8,9]

The present investigation deals with the permeability of intact cells of *Lactobacillus fermenti* to ^{35}S-thiamine.

MATERIALS AND METHODS

The ^{35}S-thiamine was a gift from Prof. Dr. O. Wiss of Hoffmann-La Roche. The specific activity of the preparation was 75 μC/mg at the beginning of the investigation and about 30 μC/mg at the end. Adenosine-5′-triphosphate disodium salt (ATP) and sodium iodoacetate were purchased from Fluka.

The stock cultures of *Lactobacillus fermenti 36* (ATCC 9833) were maintained on Micro Assay Culture Agar (Difco) and stored in a refrigerator after growth for 24 h at 37°C. They were transferred to fresh agar media monthly. Working cultures of the organism were grown in two types of media in order to obtain thiamine-sufficient and thiamine-deficient cells respectively.

Medium I. Micro Inoculum Broth (Difco) enriched with B-vitamins, purine and pyrimidine bases and several amino acids including tryptophane and cysteine. The growth of *Lactobacillus fermenti* in this medium was very abundant. The cells harvested from it after 18—24 h of growth at 37°C will be designated as *thiamine-sufficient cells.*

Medium II. A semi-synthetic medium, the composition of which was based on the media recommended for microbiological vitamin assays [10] but with thiamine levels of 10 mμg/ml (Medium IIa) and 1 mμg/ml (Medium IIb), respectively. The thiamine levels recommended for the media supporting a good growth of lactobacilli are 100—1000 mμg/ml.[10] The growth in the thiamine-deficient media was very slow. The preparation of a sufficient amount of cells — *thiamine-deficient cells* — was carried out in two steps. In the first step, 5 ml portions of medium IIa were inoculated with a loop of the stock culture. After growth at 37°C for 48 h, the cells were harvested, washed with saline and transferred to medium IIb. Using a heavy inoculum, satisfactory growth in this latter medium could be obtained after 24 h. The cells grown in media I and IIb were washed three times with saline, resuspended in saline and usually adjusted to a density of 10^9—10^{10} cells/ml (corresponding to 30 % transmission read on Coleman Spectrophotometer, Modell 11). 2 ml of such a suspension was added to 3 ml saline containing variable components. The reaction was started following the addition of ^{35}S-thiamine. The mixture was incubated at 37°C for 20 min if not otherwise stated, with gentle shaking to secure good mixing of the medium with the cells. In certain experiments, nitrogen gas was blown over the tubes in order to prevent an excessive aeration of the medium. All incubations were carried out in duplicate. After the incubation had been completed, the cells were cooled and spun down immediately. The supernatant was withdrawn and the cells washed three times with saline, great care being taken to get a quantitative recovery of the cells from the suspension. The washed cells were frozen overnight, heated for 5 min in boiling water and digested for 2 h at 60°C with 0.3—0.5 ml formamide. The use of the latter reagent was suggested to the author by Dr. George Wolf of the Massachusetts Institute of Technology and proved very satisfactory for the purpose. Aliquots of the digests were mixed with 5 ml ethyl alcohol and 10 ml toluene containing 0.3 % 2,5-diphenyl-oxazole and counted for radioactivity in a Packard Automatic Tri-Carb ® Liquid Scintillation Spectrometer.

RESULTS AND DISCUSSION

It was noticed in a series of preliminary experiments that ascorbic acid and magnesium ions favor the uptake of thiamine. These components were therefore included in the incubation medium if not otherwise stated. The effect of Mg^{2+} is demonstrated in Table 1. It was found in a great number of experiments that thiamine-deficient cells take up more thiamine than thiamine-sufficient cells. However, the difference in the thiamine uptake between these two kinds of cells was dependent not only on the degree of thiamine deficiency as measured by the level of thiamine in the medium but also on the general physiological status of the cells, *viz.* their age, number of transfers into liquid media, size of inoculum, *etc.* Glucose stimulated the uptake of

Table 1. Effect of Mg^{2+} on the ^{35}S-thiamine uptake by *Lactobacillus fermenti*.

Addition to the incubation mixture	cpm ^{35}S taken up by thiamine sufficient cells	deficient cells
None	60	558
Mg^{2+} 4.5 mM	121	900
Glucose 5.4 mM	721	1360
Glucose 5.4 mM + Mg^{2+} 4.5 mM	867	4045

Incubation mixture: 50 mg ascorbic acid; potassium phosphate buffer 0.01 M, pH 5.5; 10^{-2} μmoles ^{35}S-thiamine; 10^{11} cells; saline to 5 ml. Incubation: 20 min at 37°C with gentle shaking.

thiamine by both kinds of cells very markedly. The results of some representative experiments are shown in Table 2 (*cf.* also Table 1). It can

Table 2. Glucose stimulation of the permeability of *Lactobacillus fermenti* to ^{35}S-thiamine.

Addition to the incubation mixture	cpm ^{35}S taken up by thiamine sufficient cells				deficient cells			
	Expt. No.							
	1	2	3	4	1	2	3	4
None	189	186	119	107	180	301	333	1973
Glucose 1 %	737	867	1330	1012	809	5099	4948	17382
Stimulation by glucose, fold	3.9	4.7	12.2	9.5	4.5	17	15	8.9

Incubation mixture: 50 mg ascorbic acid; 22 μmoles $MgCl_2$; potassium phosphate buffer 0.01 M, pH 5.5; 10^{-2} μmoles ^{35}S thiamine; 10^{11} cells; saline to 5 ml. Incubation: 20 min at 37°C with gentle shaking.

further be seen in Table 1 that Mg^{2+} ions stimulate the uptake of ^{35}S-thiamine not only in the presence of added glucose but also in its absence. The Mg^{2+} effect in the latter case reflects probably the utilization of endogeneous glucose sources. The effect of Mg^{2+} in the presence of added glucose is much more pronounced in the thiamine-deficient than in the thiamine-sufficient cells.

The observation that glucose stimulates the uptake of thiamine is similar to that made by other workers for the uptake of amino acids,[4,5] sugars,[2] various carbohydrates,[1] folic acid [7] and biotin.[8] This phenomenon has been interpreted by the respective authors as an indication of a need of an external energy source for the transport of these substances. None of the cited studies provides evidence for the stimulation, by Mg^{2+} ions, of the vitamin uptake processes. Wood and Hitchings in their studies on folic acid found that Mg^{2+} ions are not essential for the uptake of this vitamin by bacteria.[7]

Lichstein and Ferguson [8] in their studies on biotin demonstrated that the stimulating effect of glucose is inhibited by iodoacetate, an agent which inhibits glycolysis and thus the generation of energy from glucose. These workers used unlabelled biotin and determined the extent of uptake by microbiological tests. The present investigation provides similar results for the uptake of ^{35}S-thiamine (see Table 3). At the same time, more specific evidence is presented for the

Table 3. Energy requirement for the uptake of ^{35}S-thiamine by *Lactobacillus fermenti.*

Addition to the incubation mixture	cpm ^{35}S taken up by thiamine sufficient cells		deficient cells		
	Expt. No.				
	1	2	1	2	3
None	89	75	112	511	327
Glucose	824	811	3274	4803	3987
Glucose + iodoacetate	328[a]	91[b]	105[b]	649[b]	352[b]
Glucose + iodoacetate + ATP	769[a,c]	1002[b,d]	355[b,d]	—	—
ATP	94[c]	72[d]	—	1285[d]	1109[d]

Incubation mixture contained: 50 mg ascorbic acid; 22 μmoles $MgCl_2$; potassium phosphate buffer 0.01 M, pH 5,5; 10^{-2} μmoles ^{35}S-thiamine; 10^{11} cells; saline to 5 ml. Glucose addition 1 %; iodoacetate *a*) 100 μmoles, *b*) 500 μmoles; ATP *c*) 50 μmoles; *d*) 100 μmoles.

requirement of energy in this permeability process. It can be seen in Table 3 that the glucose stimulation of the thiamine uptake is partly or completely inhibited by iodoacetate with both thiamine-sufficient and thiamine-deficient cells and that the inhibition is partly or completely released by the addition of ATP. It is further demonstrated that ATP to a considerable extent can replace glucose as a permeability stimulator in the deficient cells, but is without effect on the sufficient ones. It is of interest to cite in this connection some recent reports on the presence of ATPase(s) in bacterial cell membranes.[11–13] The presence of similar enzymes has also been demonstrated in isolated red cell membranes,[14] in nerve cells [15] and at the surface of intact mammalian cells.[16] It has been suggested by Abrams *et al.*[11] that the removal of ATP by ATPase may decrease the permeability of the cell to carbohydrates, the action of ATPase being thus the regulating factor in carbohydrate uptake.

Assuming that such an interpretation has a general validity for the uptake of different nutrients then the difference in ATP effects on the thiamine-deficient and thiamine-sufficient cells could be explained by a (presumed) difference in the ATPase level in such cells.

Another aspect of the glucose stimulation of thiamine uptake is the established close interrelationship between thiamine and glucose metabolism. Thus, while glucose has been demonstrated to stimulate the thiamine uptake through its energy generating ability, the supply of thiamine may in turn influence the rate of glucose assimilation and/or uptake. In this respect, the permeability to thiamine may essentially differ from the permeability

to other B-vitamins which are not directly involved in glucose degradation. Studies on this and related problems are in progress.

The results of this investigation provide evidence that an external source of energy is required for the uptake of thiamine and that the energy thus supplied can be utilized in the form of ATP. The latter observation gives a rather interesting perspective on the possibility of physiological control of the uptake of vitamins which — owing to their catalytic character hitherto have been considered to be overproduced and/or present in excess in bacterial cells.

The author is greatly indebted to Professor Dr. O. Wiss of Hoffmann-La Roche for the generous gift of ^{35}S-thiamine and to Dr. George Wolf of the Massachusetts Institute of Technology for the valuable suggestion to use formamide for the digestion of bacterial cells. Thanks are also due to Dr. S. Ullberg and Dr. B. Evaldson of the Stockholm Veterinary College for permission to use their equipment for radioactivity measurements and help in counting the numerous samples. The skilful technical assistance of Mrs. Margareta Bodin and the financial support of *Statens Medicinska Forskningsråd* are greatly appreciated.

REFERENCES

1. Cohen, G. N. and Monod, J. *Bacteriol. Rev.* **21** (1957) 169.
2. Burger, M., Hejmova, L. and Kleinzeller, A. *Biochem. J.* **71** (1959) 233.
3. Abrams, A. *J. Biol. Chem.* **235** (1960) 1281.
4. Gale, E. F. *J. Gen. Microbiol.* **1** (1947) 53.
5. Holden, J. T. and Holman, J. *J. Biol. Chem.* **234** (1959) 865.
6. Oginsky, E. L. *Arch. Biochem. Biophys.* **36** (1952) 71.
7. Wood, R. C. and Hitchings, G. H. *J. Biol. Chem.* **234** (1959) 2381.
8. Lichtstein, H. C. and Ferguson, R. B. *J. Biol. Chem.* **233** (1958) 243.
9. Lichtstein, H. C. and Waller, J. R. *J. Bacteriol.* **81** (1961) 65.
10. Barton-Wright, E. C. *The Microbiological Assay of the Vitamin B-Complex and Amino Acids*, Pitman & Sons, London 1952.
11. Abrams, A., McNamara, P. and Johnson, F. B. *J. Biol. Chem.* **235** (1960) 3659.
12. Weibull, C., Greenwalt, J. W. and Löw, H. *J. Biol. Chem.* **237** (1962) 847.
13. Greenwalt, J. W., Weibull, C. and Löw, H. *J. Biol. Chem.* **237** (1962) 853.
14. Clarkson, E. M. and Maizels, M. *J. Physiol.* **116** (1952) 112.
15. Libet, B. *Federation Proc.* **7** (1948) 92.
16. Marsh, J. B. and Hangaard, N. *Biochim. Biophys. Acta* **23** (1957) 204.

Received April 9, 1963.