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Transport of B-Vitamins in Microorganisms

IV. The Specificity of the High Accumulation of Labelled Thiamine in Non-proliferating Thiamine Deficient Cells of *L. fermenti*

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Cells of *L. fermenti* harvested from media with normal and growth limiting levels of thiamine, respectively, were exposed to certain labelled nutrients and the radioactivity retained by the carefully washed cells was measured. Only the permeability to thiamine was increased by the thiamine deficiency, whereas the permeability to adenine, aspartic acid, tryptophan and niacinamide was virtually not affected and the permeability to glutamic acid and biotin was considerably decreased.

Comparative studies were carried out on the uptake of labelled thiamine in non-proliferating cells of *L. fermenti* and of certain organisms normally not requiring exogeneously supplied thiamine. The uptake of labelled thiamine in cells of such organisms was much smaller than in *L. fermenti* and, contrarily to the uptake in *L. fermenti*, it was practically not affected by exogeneously supplied glucose. Contrarily to what was observed in the case of *L. fermenti*, the permeability to thiamine of the cells of the other organisms was very little affected by thiamine deficiency in the growth medium.

It was found earlier that the uptake of labelled thiamine by non-proliferating cells of *L. fermenti* was considerably larger in thiamine deficient than in thiamine sufficient cells.[1-3] The uptake process exhibited several features of active transport, *viz.* dependence on an exogeneous energy source,[1,2] pH and temperature optima, stereospecificity,[2] and accumulation of the vitamin against a concentration gradient.[3] One of the purposes of the present investigation was to elucidate whether the observed greater permeability of the thiamine deficient cells as compared to the normal ones was specific with respect to thiamine or a reflection of a non-specifically increased permeability of the cell. For this purpose normal and thiamine deficient non-proliferating cells of *L. fermenti* were incubated with different labelled nutrients and the radioactivity retained by the cells was measured. Comparative studies were also carried out on the permeability to thiamine of certain other microorganisms, normally not requiring exogeneous thiamine for growth.

MATERIALS AND METHODS

The nutrients selected for permeability studies were — in addition to thiamine — niacin, biotin, tryptophan, glutamic and aspartic acids, and adenine. They were all obtained from the Radiochemical Centre, Amersham, England, as the ^{14}C-compounds labelled in the following way: thiamine (thiazole-*2), adenine-*8-sulphate hemihydrate, L-tryptophan (* methylene), L-glutamic acid-*U, L-aspartic acid-*U, niacinamide (*carbonyl), D-biotin (*carbonyl). All chemicals used for the permeability experiments were of reagent grade. Redistilled water was used throughout the investigation.

Organisms, growth media and washed cell suspensions. The organisms employed for the comparative studies on the permeability to thiamine were — in addition to *Lactobacillus fermenti 36* (ATCC 9833) — *Streptococcus faecalis* (ATCC 9790), *Streptococcus faecalis* (ATCC 8043), *Lactobacillus leichmannii 313* (ATCC 7830) *Propionibacterium shermannii*, *Escherichia coli B*, and *Escherichia coli K12*. The organisms were maintained and stored by conventional laboratory methods using Difco Micro Assay Culture Agar for lactic acid bacteria, and Difco Nutrient Agar for *E. coli*. Cell suspensions for the permeability experiments were prepared by growing each organism simultaneously in two media, one normal (N) of a composition given in Ref. 2 and a thiamine level 1 mg per litre, the other of a similar composition, but containing 0.02 mg thiamine per litre, *i.e.* "thiamine deficient" (D). Washed cell suspensions of the different organisms were obtained essentially as previously described for the studies with *L. fermenti*.[2]

The cell suspensions obtained from the normal and thiamine deficient media were always tested simultaneously for their permeability to the nutrient under study. *L. fermenti* was, in addition, grown in media containing unusually high levels of thiamine (200 mg/l). The washed cell suspensions were adjusted to 5 % transmission using a Coleman Spectrophotometer, Model 11. 2 ml of the suspension were added to 3 ml incubation mixture.

Uptake experiments were carried out in 16 × 110 mm pyrex centrifuge tubes essentially as previously described.[2] The incubation mixture contained in all cases: Tris-HCl buffer 0.02 M pH 6.8, potassium chloride 0.02 M, magnesium chloride 0.02 M, ascorbic acid 0.006 M, sodium chloride 0.15 M, washed cells 18^8-10^9, labelled nutrient 2×10^{-6} M, except in the cases of tryptophan and biotin, both of which, owing to the low specific activity of the preparations available, had to be supplied at higher levels, viz. tryptophan 2×10^{-4} M, biotin 2×10^{-5} M, total volume 5 ml. In certain experiments the incubation mixture contained, in addition, glucose 0.06 M or glucose 0.06 M and iodoacetate 0.02 M.

The reaction was initiated by adding the labelled nutrient in 0.1 ml H_2O. Incubation time was in all cases 20 min at 37°C employing gentle shaking to secure good mixing of the cells with the medium.

The reaction was stopped by immersing the rack with tubes in an ice-water bath. This was followed immediately by centrifugation and washings.[2] The washed cells were frozen. Each incubation was carried out in duplicate and each experiment was repeated at least once using cultures grown on different days. Each experiment comprised the study on the uptake of one nutrient only. With this arrangement a maximum of twelve tubes was used in each experiment, and this permitted a reasonable standardization of the experimental conditions. The radioactivity retained by the carefully washed and frozen cells was determined by the liquid scintillation method after a digestion procedure described elsewhere.[2,4] The apparatus used was a Packard Automatic Tri-Carb ® Liquid Scintillation Spectrometer Model 3000. The overall counting efficiency under the conditions used was 56 %. The background varied between 50—60 cpm and the probable counting error was < 5 %.

RESULTS AND DISCUSSION

The results of studies on the permeability to several nutrients of normal and thiamine deficient cells of *L. fermenti* are summarized in Table 1. The left part of the table gives uptake values obtained in the absence of an exogeneous energy source (a), in the presence of glucose (b), and in the presence of glucose

Table 1. The uptake of certain labelled compounds by normal (N) and thiamine deficient (D) non-proliferating cells of *L. fermenti*. Incubation mixture: Tris-HCl buffer 0.02 M pH 6.8, KCl 0.02 M, $MgCl_2$ 0.02 M, ascorbic acid 0.006 M, NaCl 0.15 M, cells 10^8-10^9, total volume 5 ml. Incubation time 20 min at 37°C. Glucose 0.06 M, IA-iodoacetate 0.02 M.

Compound supplied $M \times 10^6$	Type of cells	Further additions to the incubation mixture (cpm) None a	Glucose b	Glucose + IA c	(Ratio) D/N * d (a)	e (b)	f (b−a)
Adenine							
2	N	211	52313	1035			
	D	3370	64681	595	16.0	1.2	1.1
Glutamic acid							
2	N	757	117819	82			
	D	799	47206	52	1.0	0.4	0.4
Aspartic acid							
2	N	11588	30861	0			
	D	22810	38342	34	2.0	1.2	0.8
Tryptophan							
200	N	596	1960	86			
	D	321	1771	19	0.5	0.9	1.0
Niacinamide							
2	N	0	10616	5930			
	D	3	12851	4260		1.2	1.1
Biotin							
20	N	207	4641	51			
	D	115	251	62	0.5	0.05	0.03
Thiamine							
2	N	1580	6200	210			
	D	5200	25400	520	3.3	4.1	5.5

* average of three independent experiments

and iodoacetate (c). These values refer to single series of representative experiments. In the right part of the table average values obtained in three independent experiments were used to calculate the ratios between the uptake in the thiamine deficient cells and the corresponding uptake in the normal cells. The ratios in columns d and e are based on the values obtained in the absence (a) and the presence (b) of glucose, respectively, whereas the ratios in column f have been calculated after subtraction of the values obtained in the absence from those obtained in the presence of exogeneous glucose.

It can be seen in Table 1 that, analogously to what was observed in the case of thiamine (*cf.* also Refs. 1 and 2), in all the cases studied there was a substantial increase in the corresponding uptakes in the presence of exogeneous glucose and the uptakes were inhibited by iodoacetate, an inhibitor of glycolysis. The extent of the glucose stimulation varied with different nutrients, being

Table 2. The uptake of ^{14}C-thiamine by non-proliferating cells of certain organisms grown in normal medium (N) and in medium deficient in thiamine (D). H — unusually high thiamine content in medium. Incubation mixture and other conditions as in Table 1. ^{14}C-Thiamine 2×10^{-6} M. IA — iodoacetate 0.02 M.

Organism	Conditions of growth	Further additions to the incubation mixture			(Ratio) D/N (in the presence of glucose) *
		None	Glucose	Glucose + IA	
		cpm			
L. fermenti 36					
	N	1500	6400	150	
	D	4250	26322	280	4.0
	H	60	396	20	
L. leichmannii 313					
	N		1065		
	D		1110		1.0
S. faecalis 9790					
	N	414	181	49	
	D	193	155	44	0.9
S. faecalis 8047					
	N	170	509	122	
	D	308	669	64	1.3
Pr. shermannii					
	N	1366	1855	67	
	D	450	1134	324	0.7
E. coli B					
	N	494	330	65	
	D	348	292	69	0.9
E. coli K12					
	N	195	178	30	
	D	137	315	30	1.5

* average of three independent experiments.

most pronounced with niacinamide and glutamic acid, and least pronounced in the case of aspartic acid.

Further, it can be seen in Table 1 that the relative uptake in normal and thiamine deficient cells varied with the different nutrients studied. In the absence of exogeneous glucose (columns a and d) there is virtually no difference between the normal and deficient cells with respect to the uptake of glutamic acid whereas the uptake of adenine is markedly larger in the deficient cells as compared with the normal ones. Also the uptake of thiamine was, in accordance with previous findings, considerably larger in the deficient than in the normal cells (D/N = 3.3) although the difference was less pronounced than in the case of adenine (D/N = 16). The uptake of aspartic acid in the deficient cells was twice as large as in the normal ones, whereas the corresponding uptakes of tryptophan and biotin were only half of the uptakes in normal cells.

When glucose was supplied in the incubation mixture the differences between the normal and deficient cells with respect to the uptake of most nutrients studied became less pronounced. It can be seen in Table 1 (columns b and e) that there was virtually no difference between the normal and thiamine deficient cells in the case of adenine, aspartic acid, tryptophan, and niacinamide, whereas the uptake of glutamic acid in the deficient cells was 40 % and the corresponding uptake of biotin was only 5 % of the uptake in normal cells.

If the values obtained in the absence of exogeneous glucose are considered as a kind of rough measure of a "passive diffusion" then the difference between these values and the corresponding values obtained in the presence of glucose will represent the "active accumulation process". It can be seen in Table 1 (column f) that, in this case, there was essentially no difference between the normal and deficient cells with respect to the uptake of adenine, aspartic acid, tryptophan and niacinamide, whereas such "active" uptake of biotin was pronouncedly lower and the corresponding uptake of glutamic acid considerably lower in the deficient cells as compared to the normal ones.

Contrarily to what was observed with the above-mentioned nutrients and in accordance with previous observations the uptake of thiamine was always considerably larger in the thiamine deficient cells as compared with the normal cells, the D:N ratio being 3.3:1 in the absence and 4.1:1 in the presence of exogeneous glucose (*cf.* Table 1). The difference was even more pronounced when only this part of the accumulation process, which was brought about by the exogeneously supplied glucose, is taken into consideration, the D:N ratio in this case was 5.5:1 (Table 1, column f).

The results of comparative studies on the uptake of labelled thiamine in non-proliferating cells of *L. fermenti* and of certain organisms normally not requiring exogeneously supplied thiamine are summarized in Table 2. It can be seen that the uptake of the labelled vitamin in such non-requiring organisms is much smaller than in *L. fermenti*. It is somewhat higher in *L. leichmannii* and *Pr. shermannii* than in the other organisms studied. There is practically no glucose effect, but iodoacetate inhibits the accumulation to levels considerably below those observed in the absence of glucose. Contrarily to what was observed in the case of *L. fermenti*, cells of the other organisms grown in thiamine deficient media (D) did not exhibit any pronouncedly higher accumulation capacity than the corresponding cells obtained from normal media (N). The value D/N calculated for uptakes in the presence of glucose varied between 0.7 (*Pr. shermannii*) and 1.5 (*E. coli* K12).

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