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Transport of B-Vitamins in Microorganisms

V. Comparative Studies on the ATP-hydrolyzing Activities of Cell Fractions Obtained from Thiamine Sufficient and Thiamine Deficient Cells of *L. fermenti*

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Normal and thiamine deficient cells of *L. fermenti* were disrupted in a French press and subjected to differential centrifugation. The ATP-hydrolyzing activities of the resulting soluble and particulate fractions were studied.

The cell fractions from both kinds of cells contain an adenosine triphosphatase which removes the terminal phosphate group from ATP. The enzyme requires Mg^{2+} for its activity, the optimum Mg^{2+}:ATP ratio being close to 1:2. Ca^{2+} also activates the enzyme(s), although less efficiently than Mg^{2+}; in the presence of Mg^{2+}, however, Ca^{2+} ions are inhibitory. The Mg^{2+}-activated ATPase is further stimulated in the presence of certain concentrations of Na^+ and K^+.

In addition to the ATPase(s), the cell material also contains a pyrophosphatase which is found mainly in the soluble fraction.

A comparison of the specific ATPase activities of a number of preparations obtained from normal and thiamine deficient cells indicates that the ATPase activity of cell fractions is depressed and the distribution of this activity between the particulate and soluble cell fraction is changed in favour of the soluble enzyme when the cells of *L. fermenti* are grown under conditions of thiamine deficiency.

ATPases * are widely distributed in biological tissues, mainly bound to various particle and membrane structures.[1] Whereas the participation of an Ca^{2+}-activated ATPase in the process of muscle contraction is well established,[2] the involvement of ATP-hydrolyzing enzymes in the phenomenon of active transport has been considered only comparatively recently.[4,5,24,25] A membrane bound, Mg^{2+}-activated and K^+—Na^+ stimulated ATPase from erythrocytes is believed to participate in the active transport of K^+ and Na^+ ions.[4,5] Mg^{2+}-activated and K^+-Na^+-stimulated ATPases have also been found

* The expression ATPase will be used here to denote enzyme preparations which hydrolyse adenosine-5′-triphosphate (ATP) irrespective of their substrate specificity.

in mitochondria and microsomes from brain, kidney, liver and crab nerve (for review, *cf.* Ref. 3). The occurrence of Mg^{2+}-activated ATPases in isolated bacterial cell membranes obtained by lysozyme treatment has been demonstrated by Abrams *et al.*[6] using *Streptococcus faecalis* and by Weibull *et al.*[7,8] using *Bacillus megaterium*. The latter workers found, in addition to the membrane bound enzyme(s), comparatively large amounts of both Ca^{2+}- and Mg^{2+}-activated ATPases in the soluble cell fraction. Abrams *et al.*[6] suggested that the ATPase found by them in membranes from *S. faecalis* is involved in bringing about changes in the permeability of the cell towards nutrients to be taken up.

It was reported previously from this laboratory that the uptake of labelled thiamine by non-proliferating cells of *L. fermenti* exhibited several characteristics of active transport and that the accumulation of the vitamin was much higher in the thiamine deficient than in the thiamine sufficient cells.[9-11] It was therefore considered to be of interest to study the ATP hydrolyzing activities of those two types of cells. *L. fermenti* is not susceptible to lysozyme treatment and the preparation of cell membranes from this organism has not yet been described. While attempting to find a method for a similar preparation studies were carried out on the ATPase activities of cell homogenates and cell fractions obtained by differential centrifugation of disrupted cells.

EXPERIMENTAL PROCEDURE

Materials. Adenosine-5′-triphosphate disodium salt (ATP), adenosine-5′-diphosphate sodium salt (ADP), and adenosine-5′-monophosphate sodium salt (AMP) were preparations obtained from Sigma, St. Louis, Mo, USA, sodium pyrophosphate, reagent grade, was purchased from Merck, Darmstadt, Germany. All other chemicals were also of reagent grade. Redistilled water was used throughout the investigation.

The *maintenance, storage and cultivation* of *L. fermenti 36* (ATCC 9833) was carried out as previously described.[10] Thiamine sufficient cells were obtained from a medium containing 1 mg thiamine per litre, and thiamine deficient cells from a similar medium containing 0.02 mg per litre. The cells harvested from a 200 ml volume of the growth medium were washed once with 0.9 % sodium chloride solution, twice with distilled water and then re-suspended in 10 ml distilled water.

Enzyme preparations. The thick cell suspensions were disrupted in a French press (X-Press, Biox, Sweden) and the homogenate was diluted with water to 20 ml. After withdrawal of samples for the determinations of ATPase activity in the whole homogenate the remainder was centrifuged at 74 000 *g* for 30 min in a Spinco Ultracentrifuge, Model L 50. The supernatants were retained and the residues were washed once with a volume of water corresponding to half of the original homogenate volume.

The washings were combined with the supernatants. The two enzyme preparations obtained, *viz.* the combined supernatants and the residues will in the following be referred to as the soluble fraction (S) and the particulate fraction (P). The enzyme preparations could be stored at + 4°C for several days, with very small decrease in the ATPase activity. In certain experiments the preparations were frozen and stored at −20°C, in certain others they were freeze-dried. The ATPase activity of the soluble fraction from both types of cells remained practically unaffected by these treatments whereas the activity of the particulate fraction obtained from normal cells decreased by about 25 % upon freezing and thawing (once) and by about 50 % upon freeze-drying. The loss of activity upon freezing and freeze-drying was even larger using the corresponding enzyme preparation from thiamine deficient cells. These data are not reported. The protein content of the enzyme preparations was determined by the method of Lowry *et al.*[12] utilizing the procedure for insoluble proteins with a corresponding standard curve (1 M NaOH) for the whole homogenate and the particulate enzyme and both procedures (H_2O resp. 1 M NaOH)

for the soluble enzyme. The agreement between the two procedures was usually very good. All operations involving the enzymic preparations were carried out at temperatures around 0°C.

Enzyme assays. If not otherwise stated the reaction mixture contained Tris-HCl buffer 0.1 M pH 7.5, ATP disodium salt 5×10^{-3} M, $MgSO_4$ 2.5×10^{-3} M, cell protein 100—500 μg, total volume 2 ml. The ATP solution was prepared in 0.2 M Tris, original pH 9-10, 12.5 mg $ATPNa_2$ per ml. This gave a pH only slighty higher than the required pH 7.5. The final pH adjustment was carried out with dilute HCl. The ATP solution was kept on ice until it was brought to the temperature of the reaction which was then initiated by the addition of the ATP solution (0.5 ml). After completed incubation (20 min in most experiments) at 37°C, 1 ml ice-cold 1 M perchloric acid was added while the rack with the tubes was placed in an ice-water bath. After centrifugation aliquot portions of 0.5, 1.0, or 2.0 ml were removed for the determination of the amount of liberated phosphate which was estimated by the method of Martin and Doty [13] as modified by Ernster and Lindberg.[14] Each determination was carried out in duplicate and each experiment was repeated at least once.

The enzyme preparations to be compared were always tested simultaneously. The protein level was kept constant within each experiment and in most experiments it was kept at the level of 100 μg per tube (50 μg per ml).

The phosphate values obtained were always corrected for the spontaneous decomposition of the substrate during the incubation and for the content of P_i in the enzyme preparations and in the reagents. Enzyme and substrate blanks in duplicate were thus included in each incubation series. When varying levels of ATP were tested appropriate blanks for every level of ATP were included. At the comparatively low protein content employed (50 μg per ml incubation mixture) the enzyme blanks were insignificant. The spontaneous hydrolysis of ATP represented usually less than 20 % of the total enzymic decomposition of this compound.

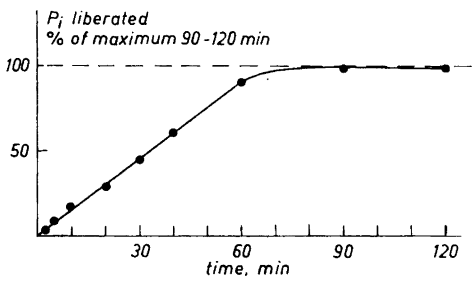

Fig. 1. Liberation of inorganic phosphate (P_i) from ATP by disrupted normal cells of *L. fermenti* at varying time intervals. The incubation mixture contained Tris-HCl buffer 0.1 M pH 7.5, ATP disodium salt 5×10^{-3} M, $MgSO_4$ 2.5×10^{-3} M, cell protein 170 μg/ml, total volume 2 ml. Incubation at 37°C.

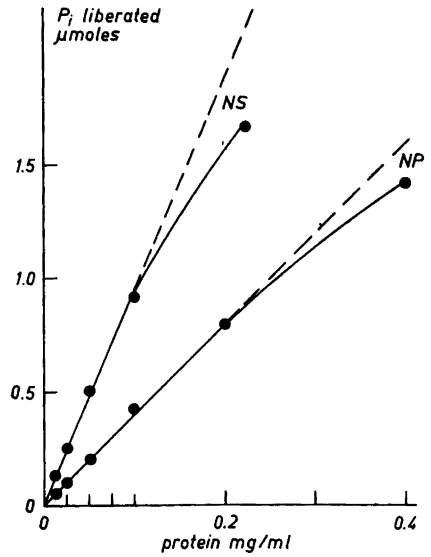

Fig. 2. The relationship between the content of cell protein in the incubation mixture and the amount of liberated P_i. Incubation for 20 min as in Fig. 1 except for the protein content. NS, soluble fraction from normal cells; NP, the corresponding particulate fraction.

The products of the reaction were determined by descending paper chromatography for 18 h at 22°C in isobutyric acid:ammonia:H_2O = 50:1:20.[15] The spots were localized by means of UV-illumination.

In the first part of the investigation selected kinetic studies were carried out using enzyme preparations from the normal cells and were compared on certain points with the preparations obtained from the thiamine deficient cells. Later, the ATPase activities of a number of normal and deficient preparations from different crops of cells were determined in such a way that one normal culture was always grown simultaneously with one thiamine deficient culture. Selected studies were also carried out on the influence of Ca^{2+}, Na^+, and K^+ on the enzymic activities.

RESULTS

The liberation of inorganic phosphate (P_i) at varying time intervals upon incubation of the cell homogenate (disrupted normal cells) with ATP is shown in Fig. 1. It can be seen that the reaction velocity increases linearly with time during the first 60 min. For subsequent studies an incubation time of 20 min was selected as representing the initial reaction velocity.

The ATP-hydrolyzing activities of the soluble and particulate cell fractions were proportional to the amounts of the corresponding cell proteins in the ranges 25—100 and 25—200 μg/ml, respectively (*cf.* Fig. 2).

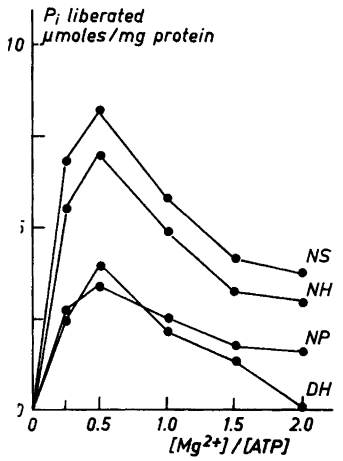

Fig. 3. The relationship between the Mg^{2+}: ATP ratio in the incubation mixture and the ATPase activity of cell homogenates and cell fractions from *L. fermenti*. The incubation mixture contained Tris-HCl buffer 0.1 M pH 7.5, ATP disodium salt 5×10^{-3} M, $MgSO_4$ $0-10^{-2}$ M, cell protein 50 μg/ml, total volume 2 ml. Incubation for 20 min at 37°C. NH, whole cell homogenate from normal cells; NS, the corresponding soluble preparation; NP, the corresponding particulate preparation; DH, whole cell homogenate from thiamine deficient cells.

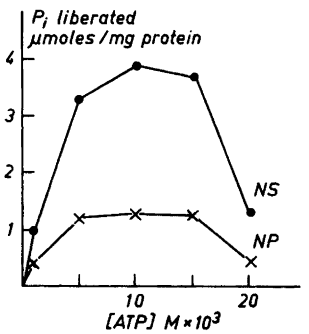

Fig. 4. The relationship between the concentration of ATP and the ATPase activity of cell fractions from *L. fermenti*. Symbols and incubation conditions as in Fig. 3 except for the concentration of ATP which was varied between $0-20 \times 10^{-3}$ M and that of $MgSO_4$ which was kept constant at 2.5×10^{-3} M.

Results of studies on the effect of Mg^{2+} on the P_i-releasing activities are given in Fig. 3 for the whole cell homogenate from normal cells (NH), the corresponding soluble and particulate fractions (NS and NP, respectively) as well as the whole cell homogenate from thiamine deficient cells (DH). The ATP-concentration in all these experiments was kept constant at 5×10^{-3} M. It can be seen in Fig. 3 that, in all four cases, Mg^{2+} was required for the P_i releasing activities, the optimal ratio between the Mg^{2+} and ATP-concentrations being approximately 1:2. This ratio was selected as standard procedure for most subsequent experiments.

Fig. 4 shows the relationship between the concentration of ATP and the ATPase activity of cell fractions when the concentration of Mg^{2+} is kept constant at 2.5×10^{-3} M. The results in Fig. 4 indicate once more the existence of a stoichiometric Mg^{2+}-requirement for the P_i-releasing activity, but indicate also that, under the conditions employed, a concentration of 5×10^{-3} M ATP is lower than a saturating concentration of the substrate, so far as the soluble enzyme is concerned (NS). At ATP concentrations higher than 15×10^{-3} M the ATPase activity markedly decreases.

Fig. 5 shows the corresponding relationship between the concentration of ATP and the reaction velocity when the Mg^{2+}:ATP ratio is kept constant at 1:2. It can be seen that in this case the inhibition of the enzyme activity at ATP concentrations higher than 15×10^{-3} M becomes very small. It also appears from Fig. 5 that the optimal concentration of ATP, at the Mg^{2+}:ATP ratio of 1:2, is found to be between 5×10^{-3} and 15×10^{-3} M. The soluble (NS) and the particulate cell fraction (NP), both derived from normal cells, gave essentially similar curves. Because such high concentrations of ATP usually gave high ATP-blanks and also in order to facilitate the comparison of the present results with those reported by other authors the concentrations of ATP 5×10^{-3} M and that of Mg^{2+} 2.5×10^{-3} M were selected as standard procedure for subsequent experiments.

Table 1. The effect of certain cations on the ATPase activity of cell fractions from *L. fermenti*. Incubation conditions as in Fig. 2 except Mg^{2+}.

Cation $M \times 10^3$	P_i liberated by cell fraction Soluble %	Particulate %
None	3	0
Mg^{2+} 2.5	100	100
Ca^{2+} 2.5	44	38
Mg^{2+} 2.5 + Ca^{2+} 2.5	68	62
Mg^{2+} 2.5 + K^+ 10 + Na^+ 100	140–160 *	150–250 *

* Stimulation by K^+–Na^+ was obtained in five out of six experiments carried out at the appropriate concentrations of these ions.

The results of studies on the influence of certain other cations on the ATP-hydrolyzing activities of cell fractions as compared to the influence of Mg^{2+} are summarized in Table 1. It can be seen that Ca^{2+} has an activating effect on the ATPase activity of both the soluble and the particulate cell fraction and that this effect is about 40 % of the corresponding effect of Mg^{2+}. However, when the two ions are present together at equimolar concentrations (2.5×10^{-3} M) the activating effect of Mg^{2+} on the ATPase activity of both fractions is only 60 —70 % of the effect in the absence of Ca^{2+}. The inhibition by Ca^{2+} of the Mg^{2+}-activation was studied further in the case of the soluble enzyme using varying concentrations of the latter ion. The results are shown in Fig. 6. It can be seen that

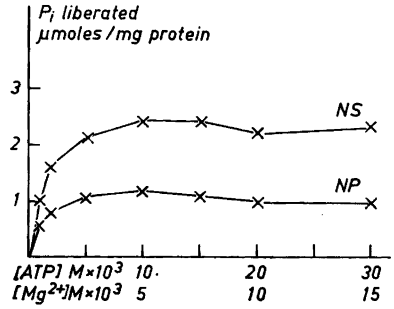

Fig. 5. The relationship between the concentration of ATP and the ATPase activity of fractions from normal cells of *L. fermenti* when the Mg^{2+}:ATP ratio is kept constant at 1:2. The concentration of ATP was varied between $0-30 \times 10^{-3}$ M. Other incubation conditions and symbols as in Fig. 3.

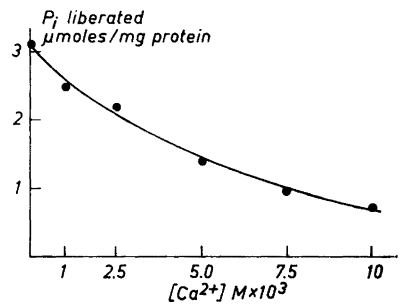

Fig. 6. The influence of Ca^{2+} on the Mg^{2+}-activated ATPase of the soluble cell fraction from *L. fermenti*. Incubation conditions as in Fig. 3 using 2.5×10^{-3} M $MgSO_4$.

increasing concentrations of Ca^{2+} gradually decrease the activating effect of Mg^{2+}. At a concentration of Ca^{2+} four times that of Mg^{2+} the activating effect of the latter ions is only about 25 % of what it is in the absence of Ca^{2+}.

A further stimulation of the Mg^{2+}-activated ATPase of both the soluble and the particulate cell fractions was obtained in the presence of K^+ 0.01 M and Na^+ 0.1 M, both ions being present. Such stimulation was obtained in five out of six cases tested. Its extent seemed to be somewhat higher with the particulate fraction than with the soluble one, *viz.* 150—250 % in the aforementioned as compared to 140—160 % in the last mentioned case (*cf.* Table 1). Varying the concentrations of K^+ over the range 5×10^{-3} to 20×10^{-3} M and simultaneously those of Na^+ over the range 10^{-1} to 2×10^{-1} M did not give any maxima. Above these ranges increasing concentrations of the respective cations caused a gradually increasing inhibition of the enzyme activity. Some inhibiting effect (20—40 %) by the cardiac glycoside ouabain on the K^+—Na^+-stimulation was observed in two cases out of four tested.

Table 2. The P_i releasing activities of cell fractions from *L. fermenti* using various substrates. Preparations from normal (N) and thiamine deficient (D) cells. Soluble (S) and particulate (P) fractions. Incubation conditions as in Fig. 3 using 2.5×10^{-3} M $MgSO_4$.

Substrate 5×10^{-3} M	P_i liberated by		
	N		D
	S	P	S
	μmoles/mg protein/min		
ATP	0.32	0.20	0.21
ADP	0.04	0.02	0.02
AMP	0.00	0.00	0.00
PP_i	0.80	0.12	0.61
PP_i corrected *	0.40	0.06	0.30

* with respect to the reaction $PP_i \rightarrow 2P_i$

The P_i-releasing activities of the soluble and particulate fractions from normal and of the soluble fraction from thiamine deficient cells using various substrates are shown in Table 2. It can be seen that all three kinds of preparation released about 8—10 times less P_i in the presence of ADP than they did with ATP as substrate and that the hydrolysis of AMP was, in all three cases, insignificant. Both soluble enzyme preparations were very active towards inorganic pyrophosphate releasing about 20—30 % more P_i from this substrate than from ATP, whereas the pyrophosphatase activity of the particulate fraction was only about 30 % of the corresponding ATPase activity. The soluble enzyme from the thiamine deficient cells was less active towards all the substrates studied than was the enzyme from the normal cells, but it exhibited essentially the same relative affinity for the different substrates as did the enzyme from the normal cells.

Results of chromatographic determinations indicated that the only product of the reaction with ATP, using both the soluble and the particulate enzyme preparations, was ADP.

The results of determinations of the specific ATPase activity of a number of preparations of the soluble and the particulate cell fractions obtained from different crops of normal and thiamine deficient cells are summarized in Table 3. It can be seen that there were great variations within each group which gave very high standard deviations from the average values. These are listed below, expressed as μmoles P_i/mg protein/min. The soluble fractions from normal and thiamine deficient cells: 0.27 ± 0.13 and 0.11 ± 0.08, respectively; the particulate fractions from normal and thiamine deficient cells : 0.14 ± 0.02 and 0.03 ± 0.09, respectively. Owing to the high standard deviations it was considered to be rather meaningless to carry out a significance analysis with respect to the difference between the average values, obtained with normal and "deficient" preparations. It can be seen in Table 3, however, that the soluble preparations from normal cells (N_s) were on the average 0.16 units more active than the corresponding preparations from deficient cells (D_s). Only one of the 18 D_s-values listed was higher than the simultaneously tested N_s-value. The specific activity of the particulate preparations

Table 3. The specific ATPase activities of soluble (s) and particulate (p) cell fractions obtained from normal (N) and thiamine deficient (D) cells of *L. fermenti*.

Preparation No.	P_i liberated by cell fraction			
	Soluble		Particulate	
	N_s	D_s	N_p	D_p
	μmoles/min/mg protein			
1	0.22	0.16	0.10	0.05
2	0.15	0.00	0.08	0.00
3	0.16	0.00	0.08	0.00
4	0.39	0.32	0.20	0.08
5	0.12	0.09	0.07	0.02
6	0.17	0.14	0.09	0.05
7	0.16	0.00	0.08	0.00
8	0.59	0.16	0.22	0.04
9	0.40	0.09	0.21	0.02
10	0.31	0.07	0.16	0.02
11	0.15	0.16	0.07	0.04
12	0.42	0.17	0.25	0.04
13	0.36	0.13	0.19	0.04
14	0.14	0.12	0.07	0.03
15	0.16	0.07	0.03	0.00
16	0.42	0.17	0.22	0.05
17	0.17	0.00	0.09	0.00
18	0.30	0.18	—	—
19	0.27	—	0.15	—
20	0.42	—	0.22	—
Average	0.27	0.11	0.14	0.03
Standard deviation	0.13	0.08	0.02	0.09

obtained from normal cells (N_p) was on the average 0.11 units higher than that of particles from deficient cells (D_p). All N_p-values were higher than the simultaneously tested D_p-values. The average ratios N_s/N_p and D_s/D_p were 2.0 and 4.0, respectively. The significance of the difference between these two ratios was tested by means of standard regression analysis according to models:
$N_p = a_N + b_N \times N_s$ and $D_p = a_D + b_D \times D_s$. The constants found were: $b_N = 0.468 \pm 0.046$; $a_N = 0.012 \pm 0.006$; $b_D = 0.253 \pm 0.043$; $a_D = -0.008 \pm 0.003$. The observed difference is thus significant.

DISCUSSION

Generally speaking, the characteristics of the ATP-hydrolyzing activity of *L. fermenti* agree on many points with previously reported data on microbial ATPases.[6-8,17,18] The ATPases found in *S. faecalis*,[6] *B. megatherium*[7,8] *B. stearothermophilus*,[17] and *S. pyogenes*[18] are all activated by Mg^{2+} and so is (are) the ATPase(s) found in *L. fermenti* (*cf.* Fig. 3). There also seems to exist in all these cases a stoichiometric requirement for Mg^{2+} for a maximum ATPase activity. Somewhat varying results are reported, however, with respect to

the amount of this requirement. The optimum Mg^{2+}:ATP ratio close to 1:2 found in this investigation for all the kinds of preparation is similar to that obtained by Weibull *et al.*[7,8] for their soluble and membrane bound ATPases from *B. megatherium* and to that obtained by Marsh and Militzer[17] with *B. stearothermophilus*, but differs from the optimal value (1:1) reported by Sokawa[18] with *S. pyogenes* and by Abrams *et al.*[6] with *S. faecalis*. The last-mentioned authors found the Mg^{2+}:ATP ratio of 1:1 to be optimal and suggested that, at least at the level tested, the true substrate for their enzyme may be MgATP instead of ATP analogously to what had been suggested for, *e.g.*, ATPases from rat liver mitochondria.[19] It appears, however, from the studies of Cooper[20] on a particulate ATPase from mitochondria that the optimal Mg^{2+}:ATP ratio may vary with the ATP-concentration.

The observation that high concentrations of either Mg^{2+} or of ATP, markedly depress the ATPase activity of *L. fermenti* (*cf.* Figs 3 and 4) is similar to the observations reported for the other microbial ATPases. Analogously to the system from *B. megatherium*,[7,8] but contrary to the systems from *B. stearothermophilus*[17] and *S. faecalis*[6] this despression becomes very small when the Mg^{2+}:ATP ratio is kept constant at 1:2.

The pH 7.5 of the reaction mixture was selected because most of the previously reported studies on ATPases were carried out at pH values close to 7.[6-8,16,20] Further, Weibull *et al.*[7,8] in their studies on the ATPase in ghosts from *B. megatherium* did not find any pronounced pH optima for the enzyme activity. The incubation temperature of 37°C was selected in order to facilitate the comparison of the ATPase of *L. fermenti* with the membrane bound ATPase of *S. faecalis* described by Abrams *et al.*[6], who employed 38°C and pH 7.5 in their determinations.

The preparation of membrane fragments by ultrasonic disruption has been described in the case of human erythrocytes.[21]. Such fragments contained a Mg^{2+}-activated and K^+—Na^+-stimulated ATPase. It seems probable that the particulate fraction obtained in the present investigation by mechanical disruption of the cells contained fragments of both the cell envelope and of the cell membrane in addition to cytoplasmic particles. A stimulation by K^+—Na^+ of the Mg^{2+}-activated ATPase was obtained with both the soluble and the particulate fraction. This effect was possibly more pronounced in the particulate as compared to the soluble fraction. However, not enough experiments were carried out to permit any final conclusions to be made on this point (*cf.* Table 1). As pointed out below, some of the soluble enzyme might have been derived from the particles during their preparation.

According to the results of the present investigation Ca^{2+} activated both the soluble and the particulate enzyme preparation from *L. fermenti* to an extent that was approximately 40 % of the activation by Mg^{2+} at an equimolar concentration (2.5×10^{-3} M). These results are somewhat different from the results reported for other bacterial ATPases inasmuch the ATPase from *B. megatherium*[7] is activated almost as efficiently by Ca^{2+} as it is by Mg^{2+} whereas the enzyme from *B. stearothermophilus*[17] is not activated by Ca^{2+} at all. The conditions of enzyme assays in these systems were, however, different and also different from the conditions employed in the present investigation. On the other hand, the inhibiting effect of Ca^{2+} on the Mg^{2+}-

activation, observed with the *L. fermenti* enzyme has also been reported for enzymes present in the two aforementioned organisms. It would seem that Ca^{2+} and Mg^{2+} compete for the same binding site(s) on the enzyme (or enzyme-substrate complex) rather than there being two different enzymes, one activated by Ca^{2+} and the other by Mg^{2+}. In the latter case the activating effect of the two ions would be additive as pointed out by Weibull *et al.*[7]

Preliminary kinetic analysis of the *L. fermenti* enzyme(s) employing double reciprocal plots of the reaction velocity *versus* the reciprocal concentration of $(ATP)_2Mg$ or ATPMg in the absence and in the presence of varying Ca^{2+} concentrations did not indicate competitive inhibition between the two kinds of ions. Not enough experiments were carried out, however, to permit a final conclusion on this point. It should also be kept in mind that the true substrate for the reaction is not known with certainty.

The selectivity of the *L. fermenti* enzyme(s) with respect to ATP as compared to ADP as well as the results of chromatographic determinations indicate that the enzyme is a "true ATPase", removing the terminal phosphate of ATP. In addition to the ATP-hydrolyzing activity the cell fractions also exhibited inorganic pyrophosphatase activity, which was, in the soluble fraction, higher than the ATPase activity. The particulate fraction from *L. fermenti* exhibited much lower pyrophosphatase activity than the soluble fraction. These results are analogous to the results reported for *B. megatherium*.[7]

As was pointed out in the preceding section (*cf.* Table 3), the specific ATPase activities of the particulate fractions were lower than those of the soluble ones. This is at variance with the results reported for the membrane-bound bacterial ATPases from *S. faecalis* [6] and *B. megatherium*,[7,8] which exhibited much higher specific activities than the corresponding soluble enzymes. However, the results of the present studies are not directly comparable with those quoted above, because of the entirely different fractionation procedures employed. According to a recent report by Abrams [22] the membrane bound ATPase from *S. faecalis* can be released into solution if the membranes are repeatedly washed with water instead of 10^{-3} M $MgSO_4$ as was done previously.[6] The cell homogenates and cell fractions in the present investigation were prepared in distilled water in order to avoid interference of buffers and ions with the subsequent enzyme assay. This might have caused partial release of the enzyme from the particles. On the other hand, there was a striking constancy of the ratio between the ATPase activity of the soluble fraction and the activity of the particulate fraction as observed with a large number of preparations (*cf.* Table 3) despite the great variations in the respective ATPase activities of preparations obtained from different cell crops. This makes it less probable that the distribution obtained was to some extent an artefact.

Voelz [23] has studied the sites of ATPase activity in bacteria by means of electron microscopy and utilizing the method of lead deposition (precipitation of the liberated P_i by lead nitrate). This author found a diversity of activity sites including the cytoplasm, the cell wall, and the cytoplasmic membrane, and suggested that the occurrence of ATPase in these areas may vary depending on the stage of their development.

The results of this investigation indicate that the ATPase activity of cell fractions of *L. fermenti* is depressed and the distribution of this activity between cell fractions is changed in favour of the soluble enzyme when the cells are grown under conditions of thiamine deficiency. However, it is difficult to find some specific correlation between these differences and the earlier observed differences between the normal and thiamine deficient cells with respect to thiamine uptake,[10] With view to the above quoted report by Voelz [23] the difference between the normal and the thiamine deficient cells with respect to the amount and the distribution of the ATPase activity between the soluble and the particulate cell fraction may reflect a difference in the general physiological state of these two types of cells.

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