THE ACTIVITY OF ADENOSINTRIPHOSPHATASE IN MYCOBACTERIUM TUBERCULOSIS IN THE PRESENCE OF ANIMAL SERUM WITH DIFFERENT SPECIES RESISTANCE

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In studies of the biochemical foundation of humoral immunity in animals, resistant to tuberculosis, we had been able to establish that the blood serum of mice is capable of suppressing the assimilation of inorganic phosphate by Mycobacterium tuberculosis. This became manifest in the fact that the organisms did not adsorb mineral phosphorus from the medium when incubated with mouse serum; in other cases an excess of mineral phosphorus could be established. It seems that in the latter case the breakdown of organic phosphorus compounds, already present or newly synthetized in the cells, was the predominant process.

TABLE 1. Composition of the Mixture (in ml)

ATP (2 mg/ml)	$MgCl_2$ (0.15 M)	Borate buffer (pH 7.4)	Bacterial suspension	Animal blood serum
1	0.2	0.1	0.45	0.75

The phenomenon described above was assumed to be due to the fact that the serum of resistant animals activates the enzyme adenosintriphosphatase (ATP-ase) in M. tuberculosis.

METHODS

The following mixture was used for the experiments (Table 1):

To the control samples normal saline was added instead of serum in the same volume and, in addition, controls for the spontaneous breakdown of ATP were set up. The volume of the mixture was restored by addition of 1.2 ml normal saline. Simultaneously zero samples were set up to estimate the original content of mineral phosphate, added in 0.5 ml 30% trichloroacetic acid (final concentration 5%). The whole work was carried out in the cold at 0° C. In the experimental samples the reaction was stopped after incubation in a waterbath for 15 min at 37° C by adding 0.5 ml 30% trichloroacetic acid. The phosphorus was estimated by the method of Fieske-Subbarow. The ATP-ase activity was assessed by the quantity of mineral phosphorus liberated by the enzyme.

RESULTS

As expected, the ATP-ase activity of M. tuberculosis increased in the presence of mouse serum in 70% of the experiments, 2-2½ times compared to the control samples (Fig. 1.), a phenomenon which could never be observed in the presence of guinea pig serum. In the latter case there was, on the contrary, a decrease in the enzymatic activity, which decrease made the difference between the ATP-ase activity, observed in the presence of serum from resistant and susceptible animals respectively, even more striking.

Fig. 2 shows that in the presence of mouse serum (animals, resistant to tuberculosis) the ATP-ase activity of the organisms was in the majority of cases 2-5 times higher than in the presence of guinea pig serum, which animals are susceptible to tuberculosis.

Activation of this enzyme will apparently lead to a more rapid breakdown of ATP and to a quantitative predominance of that process over the ATP synthesis; this will undoubtedly have an effect upon a number of intracellular synthetic reactions (including multiplication).

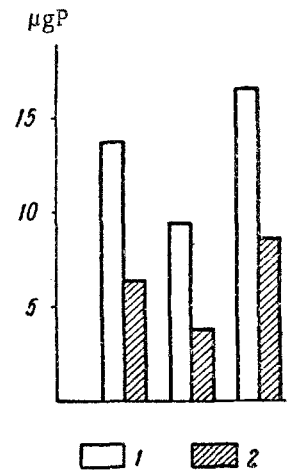

Fig. 1. The influence of mouse serum upon the ATP-ase activity of Mycobacterium tuberculosis. 1) Experiment; 2) control.

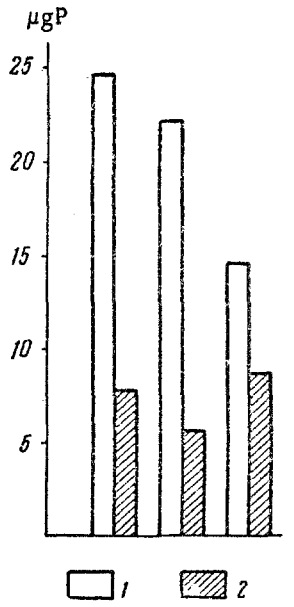

Fig. 2. The influence of mouse serum (1) and guinea pig serum (2) upon the ATP-ase activity of Micobacterium tuberculosis.

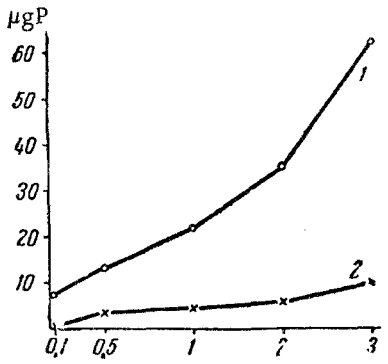

Fig. 3. The ATP-ase activity of M. tuberculosis in relation to the ATP-concentration of the incubation medium.

In an earlier study [3] we found that the inhibition of the incorporation of inorganic phosphorus by M. tuberculosis, observed in the presence of the mouse serum factor is accompanied by an intensification of the cell respiration; this is possibly due to the ATP deficiency inflicted upon the cells. The intensification of the oxydative processes is useless, however, as the extremely active ATP-ase prevents the accumulation of macro-ergic compounds.

It is a fact deserving attention that the inhibiting effect of mouse serum upon phosphorylation, described in an earlier study, and its stimulating effect upon the ATP-ase activity of M. tuberculosis can be observed in a similar percentage of cases: both effects can be observed in 70-73% of cases, a fact which once again confirms the view that the above two processes are correlated. The absence of the effect in question from the serum of 27-30% of mice is apparently due to individual features and sexual differences: it is known that male white mice are more resistant to experimental tuberculosis than female white mice [4].

These findings show that the serum of animals resistant to tuberculosis undoubtedly exerts an influence upon the metabolism of M. tuberculosis; the results can, however, be correctly interpreted only if the localization of the

enzyme in question is precisely established. The findings can be interpreted in a different manner depending on whether the bacterial ATP-ase in question represents an exo-enzyme, ecto-enzyme [1, 2] or an endo-enzyme.

To solve this question we carried out special studies on certain properties of bacterial ATP-ase.

Exo-enzymes, as is well known, are activated by an increase (within a certain range) in the substrate concentration. The following concentrations of ATP were used by us: 0.1, 0.5, 1.2, and 3 mg/ml. It appeared that within a range between 0.5 and 3 mg/ml the activity of bacterial ATP-ase barely changed (Fig. 3, 2). Consequently the ATP-ase in question was not an ecto-enzyme. It must be emphasized, however, that in the presence of mouse serum a well marked correlation between the enzyme activity and the substrate concentration could be observed (Fig. 3, 1). The cause of this phenomenon is obscure and the question is at present under investigation.

TABLE 2. The Activity of ATP-ase (in mg of P Liberated) in M. tuberculosis

In the presence of versene	In the presence of NaF	Without addition
9.8	1.7	7.7
7.7	0.4	6.5
4.5	0.5	3.5
7.1	0.0	5.5

As we said above Mg^{++}, required for the formation of the enzyme metal-substrate complex, activates bacterial ATP-ase. If the magnesium is bound, the enzyme can not react with the substrate and no breakdown of the substrate takes place. Versene (ethylenediaminotetraacetate) is a substance capable of binding bivalent metals, including Mg^{++}. Versene is a powerful inhibitor of ecto- ATP-ase, whereas potassium and sodium fluorides inhibit the activity of endo-ATP-ase.

Table 2 shows that versene did not inhibit the ATP-ase activity in any of the experiments, whereas fluorides caused a marked inhibition in the enzymatic activity of the cells.

Our findings enable us to state that the ATP-ase studied by us represents an endo-enzyme which directly participates in the phosphorus metabolism of M. tuberculosis.

The facts discussed above warrant the conclusion that the serum of animals with a natural resistance to tuberculosis, contains an unspecific factor, connected with the manifestations of immunity to tuberculosis; this factor affects the energetic metabolism of M. tuberculosis by acting upon the bacterial ATP-ase.

SUMMARY

A study was made of the effect produced by the serum obtained from the animals resistant to tuberculosis on the adenosintriphosphatase activity of bacillus tuberculosis. In the presence of the serum of mice relatively resistant to tuberculosis the activity of the enzyme has exhibited a marked increase (2-2.5 fold), whereas guinea pigs' serum (sensitive) produced a reduction of the enzymatic activity. The enzyme studied proved to be endoadenosintriphosphatase. In the author's opinion, activation of microbial adenosintriphosphatase should affect unfavourably the balance of the macro-ergic compounds in the microbial cells.

LITERATURE CITED

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