ARENAEMYCIN (PENTALENOLACTONE): A SPECIFIC INHIBITOR OF GLYCOLYSIS

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1. Introduction

In 1970 an acidic, lipophilic antibiotic was isolated from the fermented broth of *Streptomyces arenae* strain Tü 469 which was called arenaemycin [1]. Preliminary results on the structure and the biological activity of three different forms of the antibiotic (arenaemycin C = chlorhydrine; arenaemycin D = diol; arenaemycin E = epoxide) were published [2]. Arenaemycin C inhibits the growth of microorganisms at 0.5–10 μg/ml only when growing on a synthetic medium in the presence of a fermentable sugar. The inhibitory effect can be lowered or abolished by the use of complete medium or by, e.g., pyruvic acid or other carbon compounds which enter carbohydrate catabolism 'below' the pyruvate kinase reaction. These observations suggested that arenaemycin interferes with a glycolytic reaction which is not involved in gluconeogenesis [2].
A recent survey (W. Keller-Schierlein, personal communication) showed by comparison of the chemical data [2–4] that arenaemycin E is identical with pentalenolactone, an antibiotic isolated from the fermented broth of different *Streptomyces* species in the course of screening programs for antimetabolites with antitumor activity [3,4]. The structure and absolute configuration was determined [4] and is shown in fig.1.

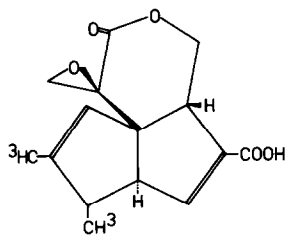

Fig.1. Structure of arenaemycin E/pentalenolactone [4].

The data presented here show that arenaemycin E/pentalenolactone is a potent and selective inhibitor of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12).

2. Material and methods

2.1. *Isolation of arenaemycin E*

Arenaemycin E was isolated by acidification of the fermented broth of *Streptomyces arenae* strain Tü 469 and adsorption of the cell-free filtrate on a XAD-2 resin. After elution with methanol, purification was performed by column chromatography on silica gel in chloroform:methanol (7:3) and on Sephadex LH-20 in methanol.

2.2. *In vivo tests*

The inhibition of growth of *Bacillus subtilis* ATCC 6051 and *Escherichia coli* K 12 W 1485 by arenaemycin E was assayed in 5 ml minimal medium cultures in the presence of 40 mM glucose. Growth rate was followed under sterile conditions in a Klett-photometer at 578 nm.

The uptake of α-[^{14}C]methylglucopyranoside in *E. coli* cells was assayed as in [5].

2.3. *Separation of glycolytic metabolites*

Exponentially growing cultures of *E. coli* were inhibited by 5 μg/ml (18 μM) arenaemycin E. Then 25 μCi [U-^{14}C]glucose was added and 30 min after addition of radioactive substrate ice-cold perchloric acid was added to 12% final conc. After removal of

cell debris the supernatant was neutralized with 30% KOH. After centrifugation the supernatant was chromatographed on Dowex 1X8 as in [6].

2.4. *Enzyme assays*

For assays in crude extracts of *E. coli* and *B. subtilis* the cells were harvested in the middle of the logarithmic phase and disrupted by sonification. The activity of glyceraldehyde-3-phosphate dehydrogenase was assayed in 0.1 M imidazole buffer pH 7.4, containing 250 μM freshly prepared glyceraldehyde-3-phosphate, 5 mM sodium arsenate and 2.5 mM NAD. The other enzymes were assayed as in [7]. In inhibition experiments the assay mixture without substrate was incubated with arenaemycin E for 15 min. All enzymes and coenzymes were products of Boehringer, Mannheim. Silica gel, Dowex 1X8, 200–400 mesh and XAD-2 were purchased from Serva, Heidelberg; Sephadex LH-20 from Pharmacia, Uppsala; and the radioactive compounds from Amersham, Braunschweig. The other chemicals were of analytical grade and from commercial sources.

3. Results

3.1. *The inhibitory effect of arenaemycin E and its reversibility*

Since we had isolated the epoxide of arenaemycin, we first repeated the in vivo experiments [1,2] which were performed with the epichlorhydrine. There was no difference in the inhibitory action despite lysis of *B. subtilis* after incubation with the antibiotic. Because the cross-feeding by metabolites of the destroyed cells lowered the inhibitory effect, the following experiments were all performed with *E. coli* as test organism. To investigate the reversibility of growth inhibition, arenaemycin E-treated cells of *E. coli* were, after several washings, resuspended in minimal medium without the antibiotic. In fig.2 the rapid onset of growth as well as the completely unchanged growth rate clearly indicates that the effect of arenaemycin E is reversible.

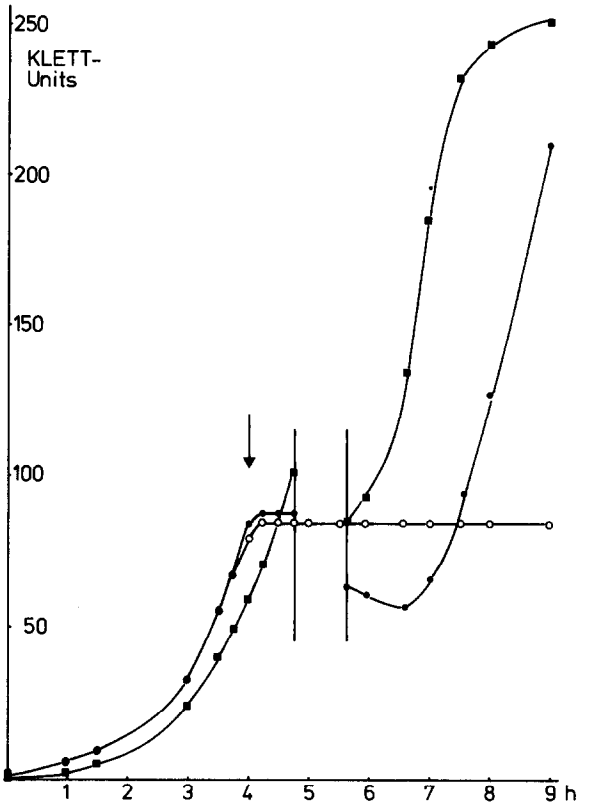

Fig.2. Inhibition of growth of *E. coli* by arenaemycin E. The arrow indicates the addition of 18 μM arenaemycin E. Population 1 (–○–) remained during the experiment in the medium with arenaemycin E. Population 2 (–●–) was harvested 60 min after addition of the antibiotic and resuspended in fresh medium. Population 3 (–■–) is a uninhibited control, resuspended in fresh medium like population 2.

3.2. *Effect of arenaemycin E on the sugar uptake system*

The effect of the antibiotic on the uptake of glucose has been tested using the nonfermentable glucose analogue α-methylglucopyranoside, which is known to be transported by the glucose uptake system [8]. The concentration dependency of arenaemycin action on the sugar transport was determined during a period of linear uptake of α-methylglucopyranoside (data not shown). At different concentrations between 0.064 mM and 1.28 mM arenaemycin E showed no significant effect on the accumulation of labeled α-methylglucopyranoside into the cells.

3.3. *Effect of arenaemycin E on the levels of glycolytic metabolites*

A separation of ^{14}C-labeled glycolytic metabolites

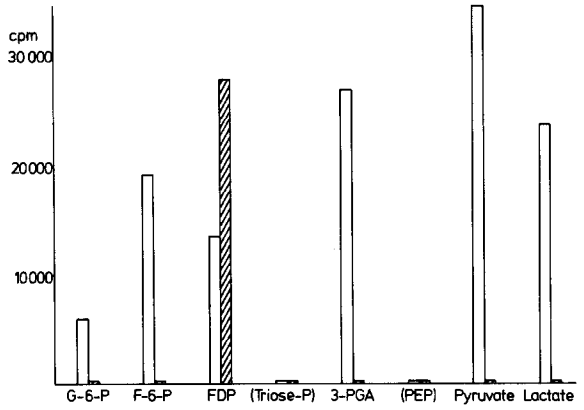

Fig.3. Levels of glycolytic metabolites in extracts of *E. coli*. The figure shows the total radioactivity/metabolite from inhibited (▪) and uninhibited (□) cells after chromatographic separation.

from arenaemycin E-treated and -untreated *E. coli* cells demonstrated that in the presence of arenaemycin E a remarkable increase in the level of fructose-1,6-bisphosphate and a decrease in the levels of other metabolites occurs (fig.3). If the incubations were terminated after 15 min (data not shown), the effect on the level of fructose-1,6-bisphosphate was less striking, but the levels of glucose-6-phosphate and fructose-6-phosphate were elevated, too. Despite the instability of some glycolytic metabolites (triose phosphates, phosphoenolpyruvate) during chromatography, the increase of fructose-1,6-bisphosphate indicates that arenaemycin E acts on a glycolytic reaction 'below' the phosphofructokinase reaction.

3.4. *Inhibition of glycolytic enzymes by arenaemycin E*

In crude extracts of *E. coli* as well as *B. subtilis* the glycolytic enzymes 'below' the phosphofructokinase reaction were assayed in the presence of the in vivo minimal inhibitory concentration of arenaemycin (18 μM). Aldolase, enolase and pyruvate kinase were not affected while glyceraldehyde-3-phosphate dehydrogenase was totally inhibited by the antibiotic. Triosephosphate isomerase and phosphoglycerate kinase have not been assayed as in these enzymatic tests, glyceraldehyde-3-phosphate dehydrogenase is an auxiliary enzyme.

3.5. *Selectivity of arenaemycin E in vitro*

A series of commercially available enzymes of the intermediary metabolism were assayed in the presence of 18 μM arenaemycin E. This concentration produces total inhibition of glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle. Alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, and hexokinase from yeast, lactate dehydrogenase from pig muscle, malate dehydrogenase from pig heart, glutamate dehydrogenase from bovine liver, glutamate–pyruvate transaminase and fructose-1,6-bisphosphatase from rabbit muscle remained completely unaffected by the antibiotic. Enolase and pyruvate kinase from rabbit muscle were only slightly (1–5% inhibition) affected by arenaemycin E.

3.6 *Concentration dependency of arenaemycin action in vitro*

The inhibitory effect of arenaemycin E was investigated using commercially available preparations of glyceraldehyde-3-phosphate dehydrogenase from yeast and rabbit muscle. The concentration dependency of inhibition shows that the enzyme from yeast is less sensitive to the antibiotic compared with the enzyme from rabbit muscle (fig.4). In every case the arenaemycin concentration needed for total enzyme inhibition is much lower than those used in the previous in vivo experiments [1,2].

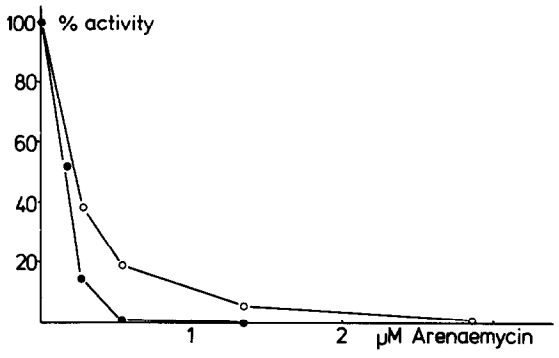

Fig.4. Inhibition of glyceraldehyde-3-phosphate dehydrogenase by arenaemycin E. The enzymes from yeast (open circles) and from rabbit muscle (closed circles) were assayed. Enzyme concentration was 8.6×10^{-8} M.

4. Discussion

The previous investigations on arenaemycin action in vivo [1,2] suggested that this antibiotic acts by inhibition of the glycolytic pathway. This study shows that arenaemycin E inhibits the enzyme glyceraldehyde-3-phosphate dehydrogenase from pro- and eucaryotic organisms. The uptake of glucose, the other glycolytic reactions and a variety of enzymes of the intermediary metabolism remained unaffected by the minimal inhibitory concentration of arenaemycin E in vivo. At this low concentration of the antibiotic growth of microorganisms is possible with pyruvate as sole carbon source. A possible explanation might be a different sensitivity of glyceraldehyde-3-phosphate dehydrogenase to arenaemycin depending upon the actual substrate concentration. Preliminary kinetic results point in that direction. A highly reactive moiety in the molecule of arenaemycin E/pentalenolactone is the epoxide. Glyceraldehyde-3-phosphate dehydrogenase is known to react with epoxides [9,10], but in the case of arenaemycin the reaction of the epoxide moiety with the enzymes seems not to be responsible for inhibition for the following reasons:

1. The inhibition of glyceraldehyde-3-phosphate dehydrogenase by arenaemycin E cannot be prevented by thiols.
2. Arenaemycin E has no effect on alcohol dehydrogenase as described for arene oxides [10].
3. The in vivo experiments [1,2] were performed with the epichlorhydrine and show the same inhibition characteristics as our experiments with the epoxide.

In general, arenaemycin seems to be a potent and selective inhibitor of glycolysis. It therefore might be a helpful tool in studies on energy metabolism, in studies on the glucose effect of metabolic regulation, and possibly in studies with tumor cells which often show a high rate of glycolysis.

Acknowledgements

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