THE CHLORAMPHENICOL RESISTANCE OF A CHLORAMPHENICOL-DEGRADING SOIL BACTERIUM

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1. Introduction

In 1966 Lingens and Oltmanns [1] isolated a bacterium capable of growing on the antibiotic chloramphenicol. The organism preliminary called CB 6 probably Flavobacterium spec. degrades CM step by step in a definite manner [2]. This finding suggests that the degrading enzymes are in some way specific for CM* and its degradation products. It was shown that CM induces the synthesis of the degrading enzymes. Thus the question rises in which way CB 6 is able to synthesize degrading enzymes, as the antibiotic usually binds to the 70S ribosome and inhibits protein synthesis.

2. Materials and methods

The bacterium was cultivated in the following media: (1) YEPD-medium: 10 g peptone, 10 g yeast-extract, 20 g glucose, 1 litre deionised water (2) PB-medium: 1.5 g beef-extract, 1.3 g yeast-extract, 5 g peptone, 1 g glucose, 3.5 g NaCl, 3.86 g K_2HPO_4, 1.32 g KH_2PO_4, 1 litre deionised water.

Characterization of CM and his acetylated derivatives was done by thin-layer chromatography on silica gel plates as described by Shaw [4]. Quantitative measurements of these compounds were performed by determination of the u.v. absorption of the spots on silica gel sheets (Merck, Darmstadt) using a Zeiss-Chromatogramm-Spektralphotometer at 275 nm. Optical densities of bacterial cultures were measured in an Eppendorf-Spektralphotometer at 400–600 nm.

*Abbreviations: CM, chloramphenicol; 1-Ac-CM, 1-acetoxy-chloramphenicol; 3-Ac-CM, 3-acetoxy-chloramphenicol.

3. Results and discussion

CB 6 needs complex media for satisfaying growth. The doubling time can be reduced by addition of peptone, yeast-extract, or beef-extract to the culture-liquid. The investigations were performed in rich media (YEPD-medium) and poor complex media (PB-medium). Addition of CM to the media causes a delay in growth. In rich media the delay increases exponentially with the concentrations of the added antibiotic. This delay is followed by an increase in optical density independent of the CM-dose. In poor complex media the doubling time depends on the CM added. With increasing concentration of the antibiotic the growth rate as well as the finally reached cell density is reduced (fig.1).

This behaviour during CM-treatment indicates that in rich media the antibiotic looses its effectiveness during the delay in growth whereas in poor media the effect of CM remains.

The determination of the CM-level present in growing cultures reveales a rapid decrease of the active antibiotic and simultaneously an increase in a product with the characteristics of 3-acetoxy-chloramphenicol (fig.2). Simultaneously a small amount of a product appears with the characteristics of 1-acetoxy-chloramphenicol. The u.v. spectra and the chromatographic behaviour of the isolated compounds were

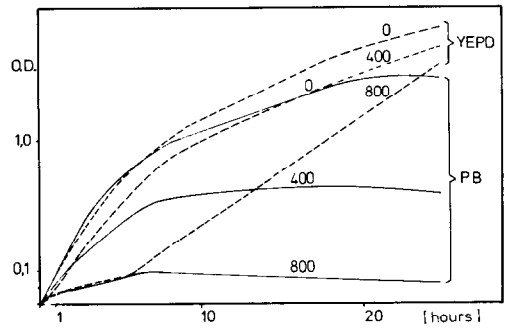

Fig.1. Growth of CB 6 in rich (YEPD) and poor (PB) complex media and the influence of added chloramphenicol (concentration given in mg/litre by the figures) on the growth rate.

identical with those published by Shaw [4]. The rate of synthesis of these products is independent of the medium used (fig.2).

Preincubation of the cells with CM does not accelerate the synthesis of inactivation products. The acetylation of the drug by induced or non-induced cells starts with the same and highest rate immediately after the addition of CM. Acetylating exo-enzymes could not be detected.

During prolonged incubation the acetoxy-derivatives of CM disappear in rich media and the original growth rate is established again. In poor media CM can be detected even after weeks of incubation. After a partial degradation of the antibiotic the bacteria lyse and CM is regenerated from its monoacetoxy-derivatives

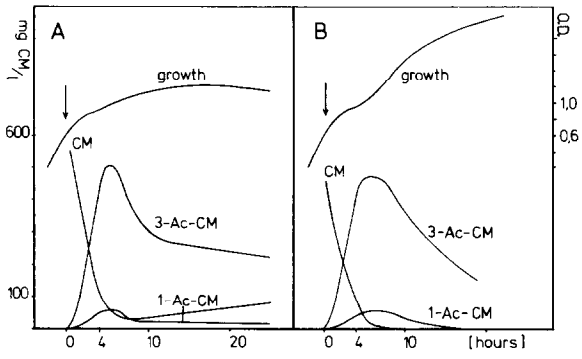

Fig.2. Growth rate of CB 6 correlated with the synthesis of chloramphenicol-inactivation products, (A) in poor, (B) in rich complex medium. Addition of chloramphenicol is indicated by the arrows.

(fig.2). In neither medium 1.3-diacetoxy-chloramphenicol could be detected.

Isolation of pure 3-acetoxy-chloramphenicol by extracting silica gel plates with methanol or ethylacetate and rechromatography in the same system shows the 3-acetoxy-compound together with a small amount of the 1-acetoxy-derivative. Also 1-acetoxy-chloramphenicol yields as main product the 3-acetoxy-compound and little of the 1-acetoxy-derivative after rechromatography. Presumably the monoacetoxy-derivatives of CM are in a nonenzymatic equilibrium 3-acetoxy-chloramphenicol being the main product as was already supposed by Suzuki and Okamoto [5]. This and the finding that the acetylated CM-derivatives are formed simultaneously let assume that the presence of 1-acetoxy-chloramphenicol in the culture medium is not due to an enzymatic secondary reaction, but is formed spontaneously from 3-acetoxy-chloramphenicol.

There are striking differences of the CM-inactivation in media with different nutritional effect. After an initial acetylation the antibiotic readily disappears from the rich medium by degradation of the CM-molecule, while in poor medium the antibiotic cannot be degraded completely. It is noteworthy that growth of CB 6 in poor media cannot regain its initial rate after acetylation of CM. Obviously acetoxy-chloramphenicol still possesses a certain level of antibiotic activity towards CB 6.

1.3-Diacetoxy-chloramphenicol could not be detected in the culture media. One may assume that the degradation of the CM-moiety in rich medium is a more specific reaction than the slowly proceeding second acetylation of acetoxy-chloramphenicol. It is obvious that de novo synthesis of CM-degrading enzymes can only happen in rich medium. Presumably CB 6 needs certain compounds present in this medium to have sufficient energy for protein synthesis while maintaining the pool of inactivated CM. For the mechanism of CM-resistance in CB 6 we propose the following model: The antibiotic activity of CM is decreased by a reversible monoacetylation thus far, that de novo protein synthesis can start. The newly synthesized degrading enzymes attack the CM present in the equilibrium

$$\begin{array}{l} \text{CM} \quad + \text{acetyl-CoA} \rightleftharpoons \text{acetoxy-CM} + \text{CoA} \\ \downarrow \\ \text{Degradation} \end{array}$$

and degrade it. The degradation however can not be the only means rendering CB 6 resistant, because the organism regains growth even at inhibitory CM concentrations of the drug. The existence of a certain 'permeability barrier' or a reduced sensitivity of CB 6 towards the antibiotic has to be assumed.

Acknowledgements

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