STUDIES ON THE PROTOPLAST-BURSTING FACTOR FROM *BACILLUS AMYLOLIQUEFACIENS*

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1. Introduction

Previous studies in this laboratory [1] have shown that *Bacillus amyloliquefaciens* secretes a heat stable factor into the external medium which has antibiotic activity towards many gram positive organisms and which is capable of rapidly lysing protoplasts of *Bacillus amyloliquefaciens*. This factor is a peptide-lipid, the structure of which appears to be identical to that for 'Surfactin' isolated from *Bacillus subtilis* supernatant by Arima et al. [2]. The structure shown in scheme 1 was proposed by Kakinuma et al. [3], and was the basis for building a space filling model.

It has now been shown that the biological activity depends upon the integrity of the lactone bond and that hydrolysis or reversion to the closed ring form can be simply attained by treatment with alkaline or acidic conditions.

2. Materials and methods

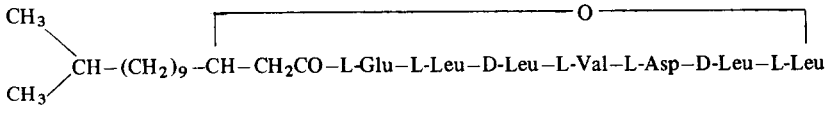
Protoplast-bursting factor was isolated from 30 hr culture supernatants using essentially the ether extraction procedure of Arima et al. [2] and assayed using the method of May and Elliott [1].

Acid and alkali treatments were performed in ether for one hour at room temperature, the final concentrations of both HCl and NaOH being 0.1 N. The sodium salt of the hydrolysed antibiotic was insoluble in ether and thus precipitated on its formation. The precipitate was collected by centrifugation and washed six times with equal volumes of ether to remove any unreacted starting material.

3. Results and discussion

The molecule possesses a hydrophilic side (fig. 1), a hydrophobic reverse side (fig. 2) and a hole bounded by hydrophilic groups. The ability of the factor to disrupt membranes is probably due to a detergent-like hydrophobic and hydrophilic orientation of the molecule, permitting it to assimilate into the membrane and cause structural disorganisation of the latter.

When the two D-leucine residues in the space filling model were changed to the laevo configuration (fig. 3) it was not possible to obtain a conformation of the molecule which did not have at least one peptide bond grossly distorted together with associated non-bonded interactions involving side chains. Moreover, it was observed that under such conditions the



Scheme 1.

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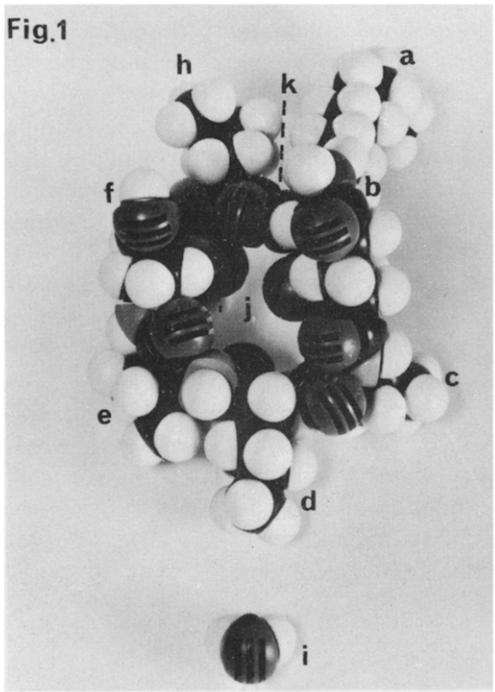

Fig. 1. Hydrophilic side.

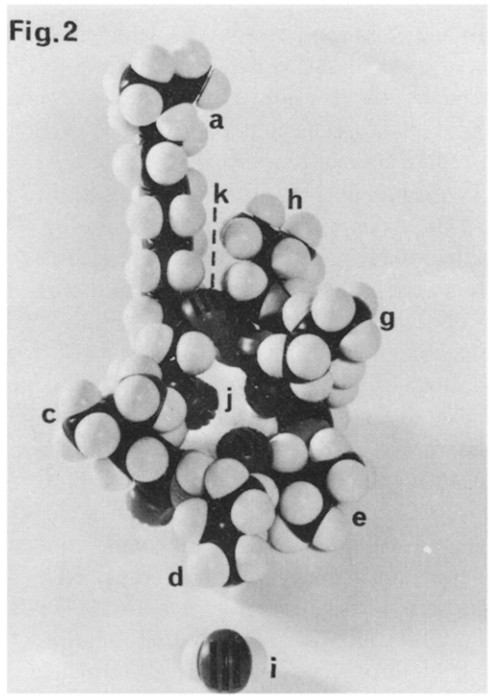

Fig. 2. Hydrophobic side.

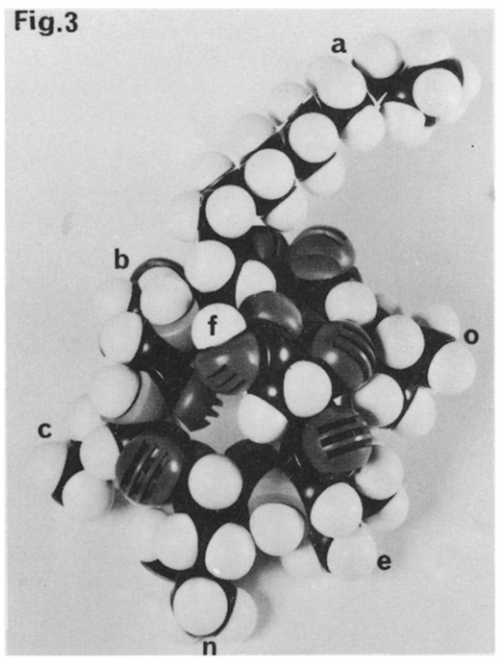

Fig. 3. All amino acids in the laevo form.

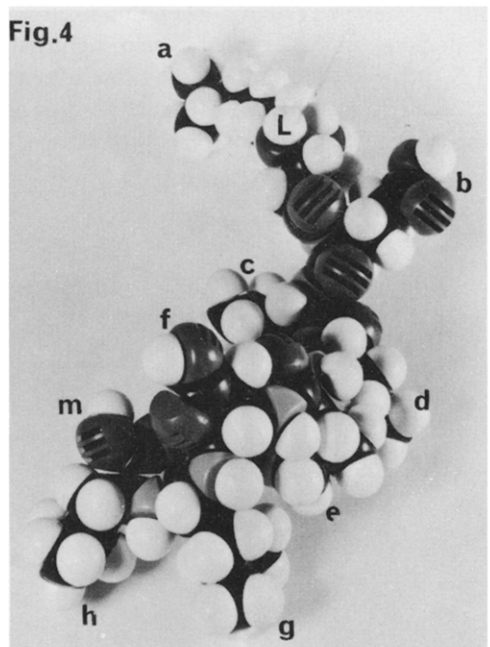

Fig. 4. Hydrolysed lactone ring.

Labelling: (a) C_{15}-β OH-isoacid; (b) L-Glu; (c) L-Leu; (d) D-Leu; (e) L-Val; (f) L-Asp; (g) D-Leu; (h) L-Leu; (i) water molecule; (j) hydrophilic hole; (k) lactone bond; (l) fatty acid hydroxyl group; (m) *C*-terminal leucine carboxyl group; (n) L-Leu replacing D-Leu (d); (o) L-Leu replacing D-Leu (g).

molecule no longer possessed its detergent-like orientation of hydrophobic and hydrophilic groups. Thus the leucine residues are needed in the dextro configuration to allow the formation of an unstrained cyclic peptide with surface active properties.

Disruption of the lactone bond (k), formed between the β-OH group of the fatty acid (1) and the *C*-terminal leucine residue, enabled the model to assume many random conformations (fig. 4). It was thought that this loss of molecular organisation could result in a loss of biological activity. This possibility was tested by alkaline hydrolysis of the lactone bond, followed by ether extraction to remove any unchanged starting material. The infra-red spectrum of the antibiotic shows a prominent absorbance in the region 1740–1710 cm^{-1}, which is characteristic for large lactones. On alkaline hydrolysis (0.1 N NaOH, 1 hr at room temperature) this peak diminished and was accompanied by an increase in absorbance in the regions 3650–3590 cm^{-1}, and 1550–1500 cm^{-1}, due to the formation of a bonded hydroxyl group and the *C*-terminal leucine carboxylate anion respectively. This opening of the lactone ring was accompanied by a loss of 97% of the protoplast-bursting activity. When this alkali-hydrolysed preparation was exposed to acid conditions (0.1 N HCl, 1 hr at room temp.), followed by ether extraction, 92% of the original activity was regained in the ether-soluble fraction. The infra-red spectrum of the restored antibiotic was identical to that of the starting material. A sample of the antibiotic stored in phosphate buffer at pH 8.0 gradually lost activity, the infra-red spectrum becoming identical to that of the alkali-treated preparation. Similarly, an alkali-treated sample of the protoplast-bursting factor was seen to gradually regain its ability to lyse protoplasts on standing in phosphate buffer at pH 7.0.

Thus it seems that the biological activity of the antibiotic is dependent on the integrity of the lactone bond, which can easily open and closed depending on the pH of the environment. The problem of how a cell can secrete a substance which is capable of rapidly destroying protoplasts made from the same cells is an intriguing one. Possibly the active ring is assembled only after the molecule has passed through the cell wall, the latter presumably protecting the underlying membrane from its action.

References

[1] B.K.May and W.H.Elliott, Biochem. Biophys. Res. Commun. 41 (1970) 199.
[2] K.Arima, A.Kakinuma and G.Tamura, Biochem. Biophys. Res. Commun. 31 (1968) 488.
[3] A.Kakinuma, A.Ouchida, T.Shima, H.Sugino, M.Isono, G.Tamura and K.Arima, Agr. Biol. Chem. 33 (1969) 1669.