THE PRESENCE OF A MUCOPEPTIDE IN THE MEDIA OF AN E. COLI MUTANT AND ITS RELATION TO THE CELL WALL

A.M.Municio, T.Diaz and A.Martinez

Institute of Chemistry. Madrid

Received March 26, 1963

Previous reports from this laboratory (Municio et al. 1960) have described the growth characteristics of a lysine-requiring mutant of E.coli and the way in which diaminopimelic acid (DAP) is accumulated in the culture media.

At the end of the logarithmic phase of growth of this mutant is observed a similar type of phenomenon to that observed by Toennies and Shockman (1953) and Toennies and Gallant (1949) in lysine requiring organisms growing in lysine-limited media, and by Meadow and Work (1956, 1957) in DAP-requiring mutants of E. coli. The observed phenomenon is an evident process of cell lysis which takes place under the experimental conditions previously described (Municio et al. 1960) and that corresponds to a sharp increase of DAP concentration in the medium.

This process of cell lysis is followed by a post-logarithmic phase of growth in which the bacteria are reorganized. The organisms harvested from the loga - rithmically and post-logarithmically growing cultures have a different biochemical behaviour. So, bacteria isolated from media in the last growth step grow well in DAP media, they cannot accumulate this amino acid in the culture media and the growth curve is different to the initial one.

This paper is to present evidence showing that during the lysis phase a mucopeptidic substance is present in the media and that it is related to the cell wall.

MATERIAL AND METHODS

Cells of E.coli (ATCC 12408) were grown at 30ºC in sucrose-glycerol-lactic acid (1.5-1.5-0.4%)-salt medium supplemented with 0.1 g/l of L-lysine. During assay periods, cell suspensions were incubated in special glass bottles (Municio et al.,1961)and shaken to achieve an oxygenation of 0.3-0.6 mmol O_2/l.min. The rate of growth was estimated by absorbance mea - surements at 675 mu in a Beckman Spectrophotometer. Lysis was confirmed by electron microscopy.

100 ml Samples of free-cell media at different stages of growth were allowed to stand at pH 2 and 5ºC. When precipitate is formed it is centrifugated, washed with distilled water and lyophilized.

Analytical studies (amino acids, sugars and amino-sugars) of the substance isolated from culture media and of the cell-wall preparations were comparatively carried out by the identification methods described by Salton and Pavlik (1960). Cell-wall preparations were obtained from cultures in a prelytic phase of growth by mechanical disruption shaking with small glass beads (Prismo Safety Corp.) in Mickle's (1948) apparatus in accordance with a Salton and Horne's (1951) similar method and lyophilized.

Agar gel electrophoresis and immunoelectrophoresis were essentially that of Uriel and Grabar (1956), using a potential gradient of 8.3 volt/cm.

Toxicity was examinated in lots of 20 g mice by intravenous injection of 0.5 ml volumes. Rabbits, 7 to 9 lb, were inoculated intraperitoneally at weekly intervals and exsanguinated from the heart. Serolo - gical tests were performed as described by Kabat and Mayer (1961).

RESULTS AND DISCUSSION

When cells from overnight stationary cultures were incubated in lysine limited shake cultures, it was found that the presence of the mucopeptide reached a peak in coincidence with the lysis following depletion of lysine. The bacterial reorganization maximum seems to be simultaneous (Fig. 1) with a disminution of the

mucopeptide level in the culture. In degenerated strains of this mutant, in which the lysis phase is not observed or it is greatly reduced, the mucopeptide amount expelled into the media is null or very poor.

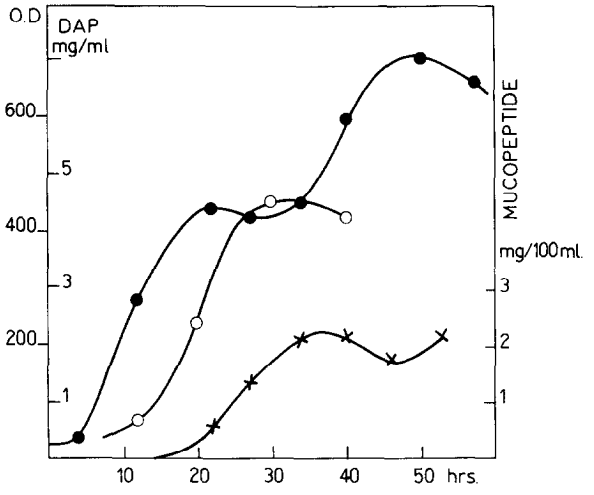

Figure 1. Comparison of bacterial growth -●-, DAP biosynthesis -o-, and mucopeptide level -+-

The agar gel electrophoresis shows the existence of three different proteins (components A, B and C) and small amounts of lipoprotein and polysaccharide material. Component B, which is in the highest proportion, does not migrate, while component A has a relative mobility (Grabar et al., 1960) U_R = 1.09 and component C, U_R = 0.44.

The analytical study of the mucopeptide and the cell wall preparations shows a close similitude between both products. On the other hand, this similarity is rein - forced by the fact that both products are toxic, and that the degree of toxicity for mice is similar and obviously superior to the toxicity of intact organims. (Table 1)

Table 1

MLD of mucopeptide, cell wall preparations and intact organims for mice (mean values)

Substances	mg/Kg weight
Mucopeptide	2.27
Cell wall	2.05
Organisms	66.00

The serological behaviour of the soluble mucopep - tide, purified cell walls and intact organisms has been tested by means of cross-reactions and results are given in Table 2(precipitin test). Mucopeptide solutions react with antisera obtained by rabbits immunization with purified cell wall of microorganisms harvested from cultures in prelytic phase of growth.

Table 2

Precipitin test of mucopeptide solution (24 mg/0.5 ml) with different antisera.

Antisera	Mucopeptide dilutions					
	1:10	$1:10^2$	$1:10^3$	$1:10^4$	$1:10^5$	$1:10^6$
Mucopeptide	3+	2+	1+	1+	1+	-
Cell wall	3+	3+	1+	-	-	-
Organisms	2+	1+	$\pm$	-	-	-

Results of agglutination tests are shown in Table 3.

Table 3

Agglutination test of intact organisms and cell wall by different antisera.

Antisera	Antisera dilutions						
	1:25	1:100	1:400	1:1600	1:6400	$1:10^4$	
Mucopeptide	4+	3+	3+	±	-	-	organism
Cell wall	4+	4+	4+	2+	±	-	organism
Organisms	4+	4+	3+	2+	1+	±	organism
Mucopeptide	2+	1+	±	-	-	-	cell wall
Cell wall	4+	4+	3+	1+	-	-	cell wall
Organisms	3+	3+	1+	-	-	-	cell wall

The mucopeptide antisera is able to agglutinate suspensions of intact organisms and cell walls, as well it is done by cell wall antisera.

The results of three series of immunoelectrophoresis are shown in Table 4.

Table 4.

Precipitation arcs of mucopeptide-antisera immunoelectrophoresis.

Antisera	Mucopeptide fractions		
	A	B	C
Mucopeptide	1	-	6
Cell wall	1	-	1
Organisms	1	-	-

The precipitation arcs corresponding to the same mucopeptide fractions exhibit in the three systems patterns with identical characteristic mobilities, shapes and relative intensities.

The mucopeptide isolated from culture media of the used E.coli mutant is, therefore, chemically and immunologically related to the cell wall.

REFERENCES

Grabar,P., Uriel,J., Courcon., Ann.Inst.Pasteur, 99, 13 (1960)
Kabat,E.A., Mayer,M.M., "Experimental Immunology", 2ª ed. 1961 (Ch.C.Thomas)
Meadow,P., Work,E., Biochem.J., 64, 11 (1956)
Meadow,P., Hoare,D.S., Work,E., Biochem.J.,66, 270 (1957)
Mickle,H., J.Roy.Microscop.Soc., 68, 10 (1948)
Municio,A.M., Angulo,J., Diaz,T., Herrera,J., Rivero, W., An.Real Soc.Españ.Fis.Quim.,56,311 (1960)
Municio,A.M., Angulo,J., Diaz,T., An.Real Soc.Españ. Fis.Quim., 57, 211 (1961)
Salton,M.R.J., Horne,R.W., Biochim.Biophys.Acta, 7, 177 (1951)
Salton,M.R.J., Pavlik,J.G., Biochim.Biophys.Acta, 39, 398 (1960)
Toennies,G., Shockman,G.D., Arch.Biochem.Biophys., 45, 447 (1953)
Toennies,G., Gallant,D.L., J.Biol.Chem., 177, 831 (1949)
Uriel,J., Grabar,P., Ann.Inst.Pasteur, 90, 427 (1956)