THE INFLUENCE OF ASPARTIC-SEMIALDEHYDE ON THE BIOSYNTHESIS OF DIAMINOPIMELIC ACID BY E. COLI*

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Studies with various organisms have shown that the carbon chain of aspartate is incorporated into lysine and diaminopimelic acid (Abelson et al., 1953 Gilvarg, 1958) and several other aminoacids.

Aspartic semialdehyde has been established as a branch point in the conversion of aspartate to diaminopimelic acid, homoserine, threonine and methionine. The formation of the aldehyde from aspartate takes place through β-aspartyl-phosphate by the action of aspartokinase (Black and Wright, 1955).

Detailed analysis of the further pathway of biosynthesis of diaminopimelic acid has been reported by Gilvarg (1957, 1958) and Rhuland (1959).

The present report deals with the direct influence of aspartic-semialdehyde on the biosynthesis of diaminopimelic acid accumulated by E. coli 26-26 in the culture medium.

MATERIAL AND METHODS

Cells of E. coli 26-26 were grown at 30 C on a glucose (3 %)-salt medium supplemented with 0.1 g/l of L-lysine 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 measurements at 675 mμ in a Beckmann spectrophotometer.

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Aspartic-semialdehyde-2-^{14}C (Municio and Vega, 1966) was added to the growth medium at different stages of the first logarithmic phase of growth and cells were centrifuged at the end of this growth. The supernatant solution was brought to pH 2 with hydrochloric acid and allowed to stand at 0 - 5 C. When precipitate is formed it is centrifuged, washed and lyophilized. This product has been shown to be a lipopolysaccharide-protein (Meadow, 1958. Municio et al., 1963, 1964). The supernatant was passed through a column of Dowex 50 (H form) from which aminoacids were recovered by elution with 0.2N NH_4OH; the effluent was passed through a column of Amberlite CG 50 to obtain the neutral compounds in the effluent.

Cell wall preparations were obtained by mechanical disruption shaking with small glass beads in Mickle's apparatus in accordance with a Salton and Horne's (1951) similar method or in a Omni Mixer highspeed homogenizer (Sharon and Jeanloz, 1964).

Cytoplasmic proteins were precipitated from the supernatant at 0 C with 10% thrichloroacetic acid and purified according to Siekevitz (1952).

Analytical studies of aminoacids obtained by acid hydrolysis (6 N HCl, 110 C, 8 hours) from cell walls, cytoplasmic proteins and lipopolysaccharide-protein, were comparatively carried out by paper chromatography. Diaminopimelic acid was determined as described by Gilvarg (1958).

The radioactivity of "infinitely thick" samples of different products was determined. The radioactivity in fractions from paper chromatrograms was determined in a Packard Tri-Carb liquid scintillation counter.

RESULTS

Figure 1 shows the effect of aspartic-semialdehyde concentration and addition time on the growth of E. coli 26-26. It is evident that increasing concentrations of aspartic-semialdehyde delay the growth of the microorganisms, depending on the time of its addition to the culture medium. At zero time, there is an increase of the lag phase but the growth pattern is the same as for the control; if the addition takes place during the logarithmic phase of growth, there is no change in it but the post-logarithmic phase starts later with increasing concentrations of aspartic-semialdehyde.

Extracellular diaminopimelic acid is simultaneously accumulated reaching a maximum level (Municio et al., 1960) coinciding with the lysis phase. Figure 2 shows the influence of addition time and concentration of aspartic-semialdehyde on the levels of extracellular diaminopimelic acid. The addition of aspartic-semialdehyde at time (a) virtually stops the increase of the level of

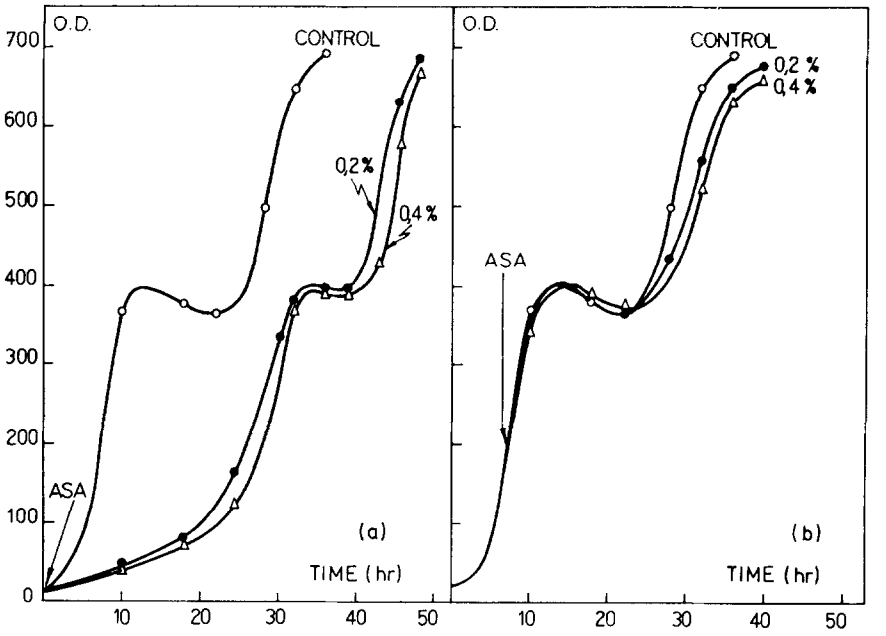

Fig. 1. Influence of different concentrations of aspartic-semialdehyde (ASA) on the growth of E. coli 26-26 at two addition times.

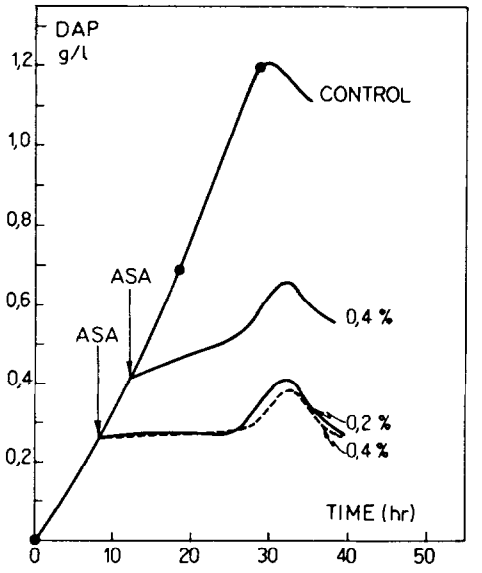

Fig. 2. Influence of addition of aspartic-semialdehyde (ASA) on the biosynthesis of extracellular diaminopimelic acid by E. coli 26-26.

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the aminoacid in the medium but the rate of biosynthesis is restored in coinci dence with the post-logarithmic phase of growth; addition at time (b) produces

a substantial decrease in the rate of accumulation of diaminopimelic acid.

Since the addition of aspartic-semialdehyde (0.2 and 0.4 per cent) to the culture during the logarithmic phase of growth does not exert any substantial modification of the growth patter, it will be necessary to establish its influence on the biosynthetic pathway of diaminopimelic acid.

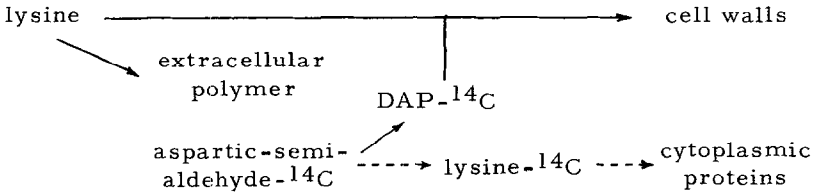
Furthermore, the presence of aspartic-semialdehyde induces the presence in the culture medium of higher amounts of valine and isoleucine (Municio and Vega, 1966).

Isotopic studies with aspartic-semialdehyde-2-^{14}C added to the medium during the logarithmic phase of growth and analysis of diaminopimelic acid and lysine from cell walls, cytoplasmic proteins and extracellular lipopolysaccharide protein (Municio et al., 1963), have led to the results in Table I.

Table 1. Precursor behaviour of aspartic-semialdehyde-2-^{14}C

Material	Diaminopimelic acid		Lysine	
	Intensity	Radioactivity	Intensity	Radioactivity
Cell walls	+	2+	2+	-
Cytoplasmic proteins	-	-	3+	2+
Extracellular lipopolysaccharide protein	-	-	3+	-

Bearing in mind the absence of diaminopimelic decarboxylase during the logarithmic phase of growth of E. coli 26-26 and the presence of lysine-^{14}C in the cytoplasmic proteins, the existence of two ways of lysine biosynthesis can be confirmed:



The levels of diaminopimelic acid-^{14}C accumulated into the culture medium are shown in Table 2.

Table 2. Evolution of diaminopimelic acid (g/l) in the medium of E. coli 26-26 using glucose (3%) as carbon source

Time (hr)	8	10	15	20	25	28
Control	0.01	0.02	0.11	0.55	1.00	1.10
Aspartic-semi-aldehyde (0.4%)	0.01	0.01	0.04	0.06	0.10	0.14

Control of the radioactivity incorporated into the aminoacids isolated from the culture medium shows a certain higher radioactivity in isoleucine than in diaminopimelic acid.

Hence, the participation of aspartic semialdehyde in the biosynthesis of diaminopimelic acid and isoleucine is apparent, utilization of the former being preferentially pointed in the direction of the formation of isoleucine.

The lower utilization of aspartic semialdehyde in the biosynthesis of diaminopimelic acid should be considered in relation to its inhibitory effect on the normal biosynthesis of the aminoacid. The existence of a feedback control of aspartokinases (EC 2.7.2.4) has been shown in E. coli (Stadman et al., 1961) and one of the described aspartokinases is inhibited in its action and repressed in its formation by lysine. But this fact alone can not satisfactorily explain the mentioned behaviour of aspartic semialdehyde in E. coli 26-26 in spite of its being a lysine-requiring mutant.

Patte et al., (1964, 1965) have shown the co-operative inhibitory effect of several aminoacids and related substances on the lysine-sensitive aspartokinase from E. coli mutants.

The results described herein would suggest the role of aspartic-semialdehyde as a co-operative substance to the inhibitory effect of lysine. When

depletion of lysine takes place in the culture medium (Municio et al., 1961, 1963) the rate of accumulation of diaminopimelic acid is again restored but the level of the aminoacid present in the control is not reached because of the presence of diaminopimelic acid carboxy-lyase in the post-logarithmic phase of growth.

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