SHORT COMMUNICATION

Specific Proline Accumulation in an *acrA* Mutant of *Escherichia coli* K12 Grown in Salt-hypertonic Medium

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Free proline is specifically accumulated in an *acrA* mutant of *Escherichia coli* K12 when cultured in a medium containing excess NaCl.

INTRODUCTION

It has been reported previously that an acriflavine sensitivity mutation, *acrA*, lying at min 10 on the *Escherichia coli* chromosome (Nakamura *et al.*, 1975), leads to loss of a protein (referred to as *acrA*⁺ protein) of the inner membrane (Nishimukai *et al.*, 1973) and to sensitivity to salt hypertonicity (Nakamura, 1977).

Recent studies of several organisms (Measures, 1975; Stewart & Lee, 1974; Tempest *et al.*, 1970) have shown that an intracellular accumulation of amino acids occurs to equilibrate the intracellular and the extracellular osmotic pressures as the salt concentration of the medium is increased. The aim of the present paper was to establish whether a specific amino acid accumulation occurs in the *acrA* mutant in the presence of a large amount of NaCl, and to examine what defect in its metabolic balance occurs as a result of stress, leading eventually to cell death in the case of salt hypertonicity.

METHODS

Escherichia coli K12 strains used were the *acrA* mutant N43 and the wild-type *acrA*⁺ strain N90 (Nakamura, 1965, 1977). Broth medium PGY, which contained 0.3 % (w/v) NaCl, and hypertonic media with NaCl were prepared as described previously (Nakamura, 1977). For determination of the amino acid pool size, bacteria grown in the normal and the hypertonic media (200 ml) were harvested by centrifugation and washed with saline. The pellets were extracted with a 5 % (w/v) trichloroacetic acid solution at 0 °C for 30 min. The amino acid extracts were applied to an automatic amino acid analyser (Beckman model 120B, equipped with the accelerated elution system). The general procedure from harvesting the bacteria to the addition of trichloroacetic acid was according to Tempest *et al.* (1970).

RESULTS AND DISCUSSION

The compositions of the amino acid pools of strains N43 and N90 grown at 37 °C for 24 h in the normal medium (PGY) and in the hypertonic media with NaCl added to 4, 5, 6 and 10 % (w/v) are shown in Table 1. The inoculum size was 1 % for the normal and the 4 % NaCl cultures of N43 and for the normal and 5 % NaCl cultures of N90, and 50 % for the 5, 6 and 10 % NaCl cultures of N43. During incubation, survival of the bacteria was 100, 50, 2.7 and < 0.01 % in 4, 5, 6 and 10 % NaCl cultures of N43, respectively, and 100 % in the 5 % NaCl culture of N90. These survival ratios are considerably higher than

Table 1. *Composition of amino acid pools in strains N43 and N90 grown in PGY media hypertonic with NaCl**

Amino acid	Amino acid composition (%)							
	Strain N43					Strain N90		
	NaCl concn (% w/v)					NaCl concn (% w/v)		
	0	4	5	6	10	0	5	
Lys	22.7	5.8	4.7	9.5	7.0	15.2	5.4	
His	7.1	5.1	3.8	5.4	1.9	6.0	3.5	
Arg	10.9	8.1	4.1	5.7	15.0	10.6	6.7	
Thr	NG	NG	NG	NG	8.7	NG	NG	
Ser	NG	NG	NG	NG	7.9	NG	NG	
Glu	25.6	31.3	24.0	21.3	5.0	29.1	57.8	
Pro	NG	23.1	42.0	26.6	NG	NG	NG	
Gly	1.4	1.4	1.7	2.8	10.4	3.0	5.4	
Ala	31.1	4.2	5.3	8.7	19.2	5.8	4.0	
Val	7.6	3.7	3.6	4.7	6.3	4.5	NG	
Met	NG	2.6	1.2	NG	NG	NG	NG	
Ile	4.2	5.1	1.9	3.2	6.7	3.6	3.2	
Leu	7.1	4.5	3.4	5.7	11.7	6.1	3.9	
Tyr	5.5	2.6	2.3	3.2	NG	8.5	6.1	
Phe	4.6	2.6	2.0	3.2	NG	7.6	4.0	
Total [μ mol (g dry wt) ⁻¹]	2.38	16.3	15.4	6.00	2.40	3.30	5.95	

* Survival of the bacteria during incubation for 24 h was 100, 50, 2.7 and < 0.01 % in 4, 5, 6 and 10 % NaCl cultures of N43, respectively, and 100 % in the 5 % NaCl culture of N90. Amino acids not indicated in this table were not detectable or negligible (NG) in content.

those reported previously (Nakamura, 1977), probably because larger inocula were used in the present experiments in order to obtain larger numbers of bacteria.

The results indicate that: (i) the amounts of all amino acids were increased by the addition of 4 and 5 % NaCl, and the increase was greater in N43 than in N90; (ii) higher concentrations of salt decreased the amino acid pool size; (iii) there was a considerable accumulation of proline in N43 cultured in the presence of excess NaCl (increasing from an undetectable amount in the normal culture to 42 % in the 5 % NaCl culture) but no such accumulation in N90; (iv) pool sizes of lysine and alanine were decreased by the addition of 4 % or more salt; (v) free glutamic acid in the pool was accumulated in both strains in the hypertonic media; and (vi) no significant changes occurred in the pool sizes of amino acids other than lysine, alanine, proline and glutamic acid. The percentage of glutamic acid in all the amino acid pools appeared to be roughly constant and unaffected by the NaCl concentration up to at least 6 %.

Measures (1975) has shown that the accumulation of glutamic acid and proline in the pool occurs in either minimal or complete medium with a hypertonic concentration of NaCl. However, the present results show that a marked accumulation of free proline occurs in the *acrA* mutant N43, but not in the wild-type strain N90, under hypertonic conditions. Proline is synthesized from glutamic acid via glutamic acid semialdehyde. The end-product proline is an allosteric inhibitor of glutamate kinase and dehydrogenase which catalyse the conversion of glutamic acid to glutamic acid semialdehyde (Umbarger, 1978). A possible explanation could be that the allosteric inhibition of the enzymes by proline is relaxed in the *acrA* mutant by the hypertonic stress with NaCl, but enzyme activities are resistant to it.

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