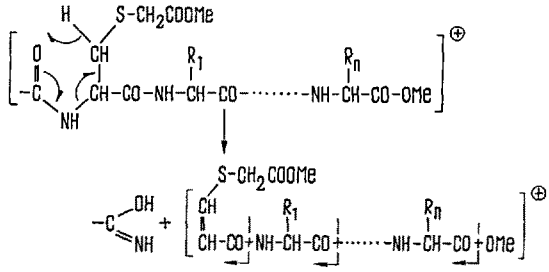
Peaks corresponding to the ions resulting from such fragmentation (see peaks at m/e 486, 458, 387, 330 and 302 in Figure 3) are usually quite strong and are accompanied by peaks lower by 73 and 106 m.u. (see peaks at m/e 444, 413, 411, 380, 352, 314, 281, 257, 224 and 196 in Figure 3). The latter are due to elimination, respectively, of a carbomethoxymethyl group or of methyl mercaptoacetate which occurs alongside the amino acid type of fragmentation. It is noteworthy that, in contrast to the cystine peptides, those with S-β-aminoethylcysteine and S-carboxymethylcysteine residues practically suffer no elimination of the side chain as a whole (i.e. no rupture of the C_α-C_β bond).

Still another process is characteristic of S-carboxymethylcysteine acylpeptide esters, namely, rupture of the



(7)

N-C_α-bond of the carboxymethylcysteine residue. This reaction, which we observed earlier with peptides containing aromatic and heterocyclic amino acids[1,2], is accompanied by migration of a hydrogen atom leading to ion (7) which undergoes further fragmentation by the amino acid mechanism. For instance in Figure 3 peak at m/e 346 corresponds to the ion of type (7), while its subsequent fragmentation gives the peaks at m/e 315, 287, 216, 188 and 159.

The findings described here show that the mass spectrometric method can be successfully employed for determining the amino acid sequence in cysteine-containing peptides formed in the partial hydrolysis of proteins.

Выводы. Показано, что масс-спектрометрический метод определения аминокислотной последовательности может быть с успехом применен к пептидам, содержащим остатки цистина, S-β-аминоэтилцистеина и S-карбоксиметилцистеина.

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Effect of Sodium Citrate on the Translocation of Bacterial DNA in *Solanum lycopersicum* esc.

It has been shown that foreign DNA can be taken up by plants[1-4]. After some depolymerization, though without modification of its primary and secondary structures, the DNA enters the cell nuclei where it appears to combine with the tomato DNA and replicate.

Since sodium citrate is known to precipitate or chelate bivalent cations, and because these ions, especially Ca^{++}, are known to be an important factor of cell permeability[5], plants were treated with sodium citrate in an attempt to increase the uptake of exogenous DNA.

Sodium citrate is known to inhibit DNAse I by its action on Mg^{++} ions[6] and so may also reduce the depolymerization of the foreign DNA translocated in the plants.

Plants of *Solanum lycopersicum* var. Tuckwood were used in the following experiments. Cut shoots (5–9 cm long) from adult plants with young leaves were placed in a solution of 0.15 M sodium citrate (tribasic) for 2 h. They were transferred to a solution of ^{3}H-DNA prepared from *Escherichia coli* (200 γ/ml) in dilute saline citrate solution for 6 h prior to placing in water for 48 h. As controls, cut shoots were placed in water instead of the citrate solution prior to similar feeding periods in the DNA solution and water.

A sample of internode (0.5 cm long) was removed from each plant, fixed for 3 h in Clark fixative and embedded in wax prior to sectioning for autoradiographic studies[7]. Some sections were used directly whilst others were (1) extracted with dilute acid to remove any labelled low-polymer polynucleotides, and (2) digested with DNAse I. Autoradiographs were exposed for 10 days.

After discarding the terminal portion of the shoot which had dipped in the feeding solutions, the DNA of the remainder of the material was extracted by a method already described[3]. This DNA was analyzed by centrifugation and chromatography on DEAE-cellulose columns[8].

A parallel study was made concerning the effects of citrate on the synthesis of endogenous DNA. Cut shoots were treated as described above except that the ^{3}H-DNA was replaced by a solution of ^{3}H-thymidine with a similar specific activity to that of the bacterial ^{3}H-DNA.

The bacterial DNA utilized in the experiments was extracted by the method of Marmur[9] from a thymine-less strain of *E. coli* (CR 34) which had been cultured on a medium containing ^{3}H-thymine.

As shown in Table I, shoots pretreated with citrate take up 3–4 times more exogenous DNA than the control plants. This result is confirmed by the autoradiographic study (Table II) where more labelled nuclei are found in each tissue from the citrate-treated shoots than the water-

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Table I. Radioactivity of tritiated insoluble DNA found in tomatoes (desintegrations/min)

	A		B	
	Control plant	Citrate treated plant	Control plant	Citrate treated plant
DNA ^3H molecules of 500,000 to $1 \times 10^6 M$	845,000 dpm	2,156,000 dpm	133,500 dpm	938,750 dpm
DNA ^3H molecules over $1 \times 10^6 M$	720,000 dpm	1,796,000 dpm	114,000 dpm	801,500 dpm

A and B are independent experiments performed with separately isolated extracts of *E. coli* DNA, A having a higher specific activity than B. DNA has been extracted from 3 plants each time.

Table II. Percentage of labelled nuclei of different tissues, found by autoradiography after 15 days exposure

	A		B	
	Control plant	Citrate treated plant	Control plant	Citrate treated plant
Collenchyma	1.3%	14%	0.5%	5%
Medullar parenchyma	8.5%	17%	4%	8.5%
Phloem and cambium	10.5%	24.5%	4%	11%

A and B correspond to plants of Table I. 1000 cells of each tissue on 10 sections were counted.

treated ones, though the increase is not the same for all tissues. Collenchyma, for instance, has 10 times as many labelled nuclei after citrate treatment as after water treatment, whereas medullar parenchyma has merely doubled the number of labelled nuclei after citrate treatment. Phloem, including the cambium, shows an increase of 2–3 times in the number of labelled nuclei after citrate treatment.

On the other hand, plants pretreated with citrate do not synthesize more DNA than the controls, nor do they take more ^3H-thymidine in their pool.

Furthermore, the quantity of newly synthesized DNA is smaller in all cases than the quantity of foreign DNA taken up by the plant[2].

Citrate pretreatment, however, does not appear to affect the depolymerization of the bacterial DNA translocated in the tomatoes, the proportion of the different fractions being similar in both cases (Table I).

The larger uptake of exogenous DNA after a sodium citrate treatment is probably due to an increase of cell permeability. The fact that more cells are labelled on autoradiographs seems to support this view. This could be due to an increase of pinocytosis. It is known that a surface absorption of charged particles (Na^+, K^+) is necessary to stimulate the plasmalemma to invaginate. Solutions of poorly dissociated molecules such as nucleic acids are not readily absorbed unless the necessary inducer ions are present[10]. Sodium citrate by acting on bivalent cations could activate pinocytosis by depressing ion competition.

The pecto-cellulosic wall seems also to play an important part in DNA uptake. This is emphasized by the fact that the increase of labelled nuclei after citrate treatment is much higher in collenchyma than in the other tissues counted. Collenchyma is a specialized support tissue with very thick walls.

Sodium citrate, by its action on calcium, an important constituent of non-cellulosic polysaccharides, changes the integrity of the cell wall, increasing thus water exchange, making the cell wall act like a sponge, and finally modifying its ionic layers. (Preliminary results show that plants pretreated with EDTA, 0.2 *M* also take up more foreign DNA.)

Cut shoots grown in citrate tend to have drooping stems. In our experiments, however, the plants recovered quickly, once put back into water. Moreover, their walls, cytoplasm and nuclei seem as well preserved as those of the control plants.

The citrate pre-treatment has failed to prevent depolymerization of the translocated, exogenous DNA. This may be explained in that, although sodium citrate inhibits neutral DNAse I, it does not inhibit and may even stimulate the acid DNAse II which occurs more commonly in plants[6]. It is possible, therefore, that the depolymerization observed is due to the action of DNAse II in the cytoplasm before the exogenous DNA reaches the nuclei.

The fact that citrate affects the translocation of foreign DNA without influencing in the least the synthesis of endogenous DNA proves that the 2 phenomena are distinct.

Résumé. L'effet du citrate de sodium sur la translation d'ADN d'origine bactérienne chez la tomate a été étudié biochimiquement et par autoradiographie. Les plantes prétraitées au citrate prennent plus d'ADN étranger que les plantes témoins. En revanche le citrate n'a pas d'effet sur la dépolymérisation du DNA pris par la plante. Les relations entre ces phénomènes et les différents modes possibles de pénétration du DNA étranger sont examinés.

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[12] Grant from 'Euratom'.