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Phosphorylmuramic Acid in *Lactobacillus casei*. Its Presence in Protein and in a Nucleoside-pyrophosphate Fraction

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A method has been developed for the isolation of nucleotide bound muramic acid peptides from *Lactobacillus casei*. The amino acid composition of these peptides has been studied and compared with acid resistant phosphopeptides from hydrolyzed phosphoproteins. A closely related amino acid composition has been found. Evidence for the existence of nuleotide bound phosphorylmuramic acid is given and the specific activity for such a compound is consistent with the view that it could be a precursor in the synthesis of phosphoproteins in *Lactobacillus casei*.

In 1955 a new phosphorylated amino acid was isolated from a hydrolysate of the protein residue of *L. casei* [1,2]. Recently it was identified as a phosphorylated derivative of 3-O-α-carboxyethyl-D-glucosamine [3] which has also been named muramic acid *. In one of the earlier communications [2] we also reported the presence of several UDP-derivatives in a cold 10 % TCA extract of the microorganism.

Park and Johnson [4] in 1949 observed that uridine nucleotides accumulated in *Staphylococcus aureus* which was inhibited by penicillin. Later they were identified as UDP linked to Mur or Mur-peptides [5]. Uridine nucleotide accumulation was also found in a penicillin-sensitive *Lactobacillus helveticus* [6] where the effect of penicillin was far less conspicuous. Evidence has also been obtained which suggests that the UDP-Mur-peptides are precursors of the bacterial cell wall in a metabolic pathway inhibited by penicillin.

* The following abbreviations are used: Mur = muramic acid; MurP = O-phosphorylmuramic acid; P = inorganic phosphate; ^{32}P = radioactive inorganic phosphate; cpm = counts per minute; cps = counts per second; AMP = adenylic acid; ADP = adenosine diphosphate; ATP = adenosine triphosphate; AT^{32}P = radioactive adenosine triphosphate; CMP = cytidylic acid; GTP = guanosine triphosphate; UMP = uridylic acid; UDP = uridine diphosphate; UTP = uridine triphosphate; DPN = diphosphopyridine nucleotide; TCA = trichloroacetic acid; Ala = alanine; Glu = glutamic acid; Leu = leucine; UV = ultra violet; E_{260} = optical density at 260 mμ.

The previous observations [2,3] may also throw some light on the mechanism for the synthesis of protein bound MurP where the phosphoryl group most probably is bound to the sixth carbon atom of the glucosamine moiety. The UDP-linked MurP reported in this paper may be an intermediate in the biosynthesis of protein bound MurP.

MATERIALS AND METHODS

Lactobacillus casei (7469) was grown in the medium previously described [3]. Bacterial growth was estimated by measuring the turbidity of suspensions with filter No. 59 in the Klett-Summerson photometer. The growth of the microorganism was inhibited by penicillin as shown in Table 1.

Chromatographic separation of acid soluble nucleotides. The cultivations were made with the suspension of microorganisms of step 5 according to previous description [3] grown for 18 h and diluted with fresh medium 1:30. Most of the experiments were made with 90 liter cultures and with the addition of 10 μg of benzylpenicillin per ml when one-half the maximum of growth was reached. The cultivation technique and the extraction methods were the same as in the preceding paper [3]. In all radioactivity experiments the medium contained 0.33 mC ^{32}P per liter from the time of incubation. The cold TCA extract from each experiment was shaken with ether to remove the TCA and then neutralized to pH 6 to 7 by the addition of a few drops of 1 N NaOH. The solution was evaporated *in vacuo* to a volume of about 30 ml and mostly run on two 70 $\times$ 3 cm Dowex 1 formate (2 % DVB) columns and eluted mainly according to Hurlbert *et al.*[7] The eluate was collected in fractions of about 30 to 40 ml every 15 min.

The elution was started with water and about 25 fractions were collected. In the system, which followed, a 600 ml mixing volume was used. The four elution ranges which approximated 1 N formic acid, 4 N formic acid, 0.2 M ammonium formate in 4 N formic acid and 0.4 M ammonium formate in 4 N formic acid were obtained by changing the reservoir to contain these solutions at tubes 25, 100, 250 and 350. Altogether 500 to 600 fractions were collected in each series. In two experiments the TCA extract was run with the elution system of Strominger [6].

Partial hydrolysis of the Schneider protein residue was performed with 11 N HCl at 37°C during 3 days according to Flavin [8]. The hydrolysate was evaporated *in vacuo*

Table 1. Growth-inhibition of *Lactobacillus casei* by penicillin given as photometric readings of the growth culture. Penicillin was added after 3.5 h of growth.

Incubation time, h	Control culture	Cultures with penicillin		
		1 000 μg/ml	100 μg/ml	10 μg/ml
0	25			
0.5	27			
1	30			
1.5	42			
2	55			
2.5	85			
3	112			
3.5	152			
4	184			
4.5	230	210	210	206
5.5	290	230	240	230
24	420	235	245	250

several times to remove excess of HCl and applied to a 7 × 50 cm Dowex 50 (8 % DVB) column. Elution with 0.01 N HCl was used as for the elution of MurP [3]. Excess of HCl was then removed from the different fractions separated on the Dowex 50 column as described above. The residues were dissolved in small volumes of water and applied to Dowex 1 formate columns (dimensions 2 × 45 cm). Elution was carried out with a gradient 0 → 1 M formic acid and with a volume of 200 ml in the mixer.

Analytical methods. The nucleotides were assayed by optical density determinations of suitable dilutions from each tube using a 1 cm quartz cuvette in a Beckman model DU spectrophotometer at 250, 260 and 280 mμ. The ratios used for identifying the UDP-derivatives were the following [9]: 250/260 = 0.73 and 280/260 = 0.39.

The radioactivity of the different fractions from the column chromatography of the TCA soluble nucleotides and protein residue was measured in glass cups with a L.K.B. Robot Scaler. Scanning of the paper chromatograms of nucleotides and MurP-peptides was made according to Ågren *et al.*[10] Determinations of specific activity values were made on stainless steel planchettes with the Tracerlab Superscaler. At least 1 000 counts were measured. The specific activities of the different nucleotides and of MurP were calculated as counts per min per μmole of compound. All activity values were recalculated to the time of incubation.

The amino acid composition of the UDP-peptides and the peptides isolated by partial hydrolysis of phosphoproteins was determined by two dimensional paper chromatography [11]. The UDP-peptides were hydrolyzed with 1 N HCl for 3 h at 100°C and the peptides from phosphoproteins with 2 N HCl for 20 h at 120°C in sealed tubes. In some cases the solvents used for the identification of Mur were suitable, as listed in a preceding paper [1]. In chromatography Whatman No. 1 or Munktell OB papers were used, and for ionophoresis Whatman No. 3 where the buffer solutions were either a 0.1 M solution of pyridine-acetic acid at pH 5 or 1 N acetic acid.

RESULTS

Isolation of UDP-linked Mur-peptides. In Fig. 1 an elution diagram is presented for the TCA extract prepared from a 45 liter culture passing through a Dowex 1 column with the formic acid system. 15 mC radioactive phosphate had been added at the inoculation and penicillin after 3.5 h of growth. The culture was harvested after 4.5 h of growth in the middle of the log phase. In several points the diagram was similar to that obtained with TCA extracts of yeast [12,13]. The peaks corresponding to CMP, DPN and AMP appeared in the same position. UMP and ADP were found in the expected position after the inorganic phosphate. After ADP eight or sometimes nine E_{260}-absorbing peaks appeared, in Fig. 1, Nos. 1—8. The two first peaks after ADP generally coincided with the elution of two yellow coloured fractions from the columns. The first fraction appeared in the same position as a UDP-linked peptide containing Mur and up to four other amino acids. The 280/260 ratio of the eight different UV peaks were 0.40, 0.50, 0.40, 0.51, 0.37, 0.40, 0.38 and 0.39, respectively.

Only peak 1 and 7 seemed to contain a muramic acid peptide linked to UDP. Paper chromatograms of the hydrolyzed peptides showed the presence mainly of muramic acid, alanine, glycine, glutamic acid and another ninhydrin positive substance which so far has not been identified. The total yield of UDP-linked Mur-peptides was about 10 μmoles which is much less than Strominger [6] obtained from cultures of *L. helveticus*. This yield was not increased to any greater extent when penicillin was present in the log phase cultures. Of interest is the observation that ATP, GTP and UTP were not present in

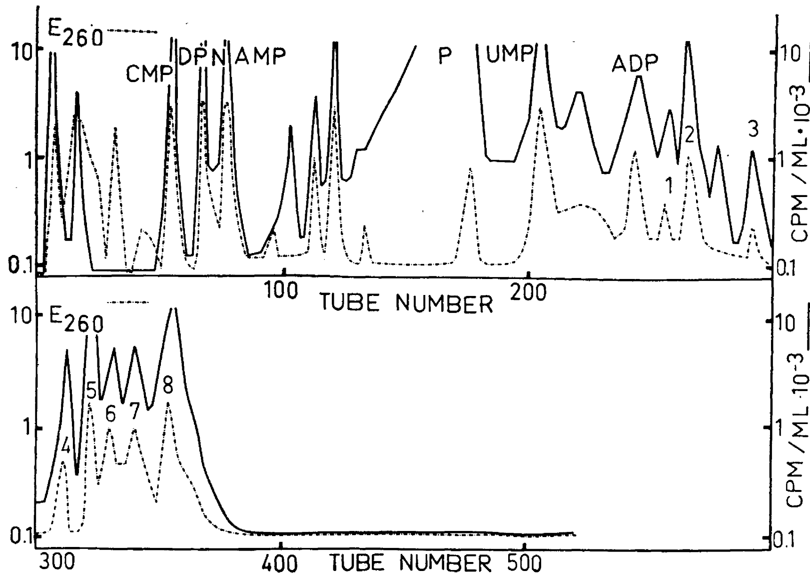

Fig. 1. Acid-soluble nucleotides from 80 g of wet cells after 4 h of incubation with 15 mC of radioactive inorganic phosphate. The nucleotides were separated by gradient elution with the reservoir content changed at the tubes numbered as follows: *25*, 1 N formic acid; *100*, 4 N formic acid; *250*, 0.2 M ammonium formate + 4 N formic acid; *350*, 0.4 M ammonium formate + 4 N formic acid; column dimensions 3 × 70 cm; Dowex 1 formate form. The continuous line represents radioactivity values, the broken line represents E_{260} values.

our TCA extracts of *L. casei*. The elution system used by Strominger [6] has also been used but according to our experience has no advantage over that used by us. It is worth mentioning that in the region between peaks C and D in the Strominger system there was no Mur-containing material in the TCA extracts of *L. casei* while Strominger found considerable amounts in extracts from *S. aureus*. The stepwise elution system used by Strominger we also found more laborious.

Isolation of UDP-linked MurP. It has been previously mentioned in this paper that UDP-linked MurP could be an intermediate in the biosynthesis of protein bound MurP. Several experiments were carried out to isolate such a compound in the TCA extracts of *L. casei*. As it could be supposed that the yield of such a compound should be very low no attempts were made to isolate a separate fraction, containing this material. Instead, all substances emerging from the columns after the ADP peak had appeared were pooled. Ammonium ions were removed by rapidly passing the eluate through a short cooled Dowex 50 filter which was afterwards washed with an equal volume of cold water. The formic acid eluate was lyophilized and extracted with a small volume of water. Hydrolysis was carried out with 0.1 N HCl at 100°C for 7 min which is far in excess of that used by Caputto *et al.*[14] for the liberation of glucose

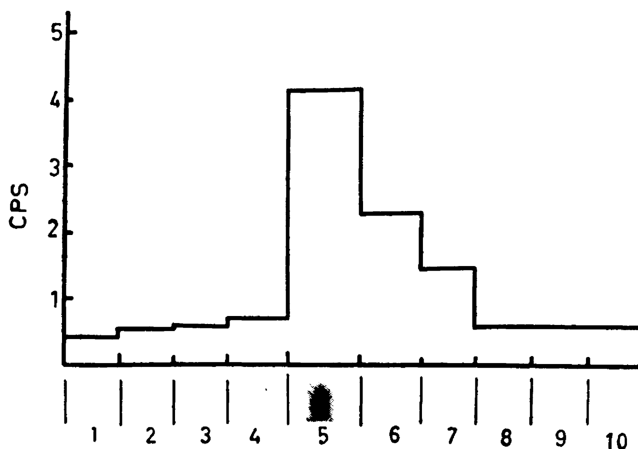

Fig. 2. Scanning curve (upper part of the figure) from a paper chromatographic strip of a hydrolysate of radioactive nucleotide bound MurP run parallelly with unlabeled MurP giving the ninhydrin spot on the lower strip. The paper strip with the chromatographically purified radioactive hydrolysate was cut in 10 equal pieces and the time for 1 000 counts was measured for each piece of paper.

from UDP-glucose. HCl was removed from the hydrolysate which was afterwards concentrated to a small volume and put on a Dowex 50 column and eluted with 0.01 N HCl. In the expected position for MurP a radioactive peak appeared. The identity of the material in this peak was checked by ionophoresis in a pyridine-acetate buffer at pH 5 and in paper chromatography (Fig. 2) and with a *iso*butyric acid-ammonia system [15]. In both cases radioactivity run parallel with added MurP. From Fig. 2 it is also obvious that the presence in this material of MurP-peptides could not be excluded. As described below such peptides leave the Dowex 50 column in about the same column volume as MurP.

It could not be excluded that the isolated MurP had been formed by splitting of UDP-linked Mur by a migration of the phosphoryl group from 1- to

Table 2. 25 mg sodium salt of UDP-acetyl glucosamine corresponding to 39.8 μmoles was hydrolyzed. The following products were obtained:

	μmoles	yield, %
Glucosamine-6-phosphate	0.01	0.025
Uridine-monophosphate	15	38 *
Inorganic phosphate	39	98
Glucosamine	23	58

* Calculated per uridine residue.

6-position. However, a more likely explanation seemed to be the existence of a UDP-linked MurP. As an example of a closely connected substance may be mentioned the recently isolated UDP-acetylglucosamine phosphate from the oviduct of laying hen [16]. The possibility of the postulated migration was tested in a model experiment with UDP-glucosamine. 25 mg of a commercially available substance (Sigma, 95—100 % pure, from yeast) was hydrolysed under the same conditions as the rest protein fraction (2 N HCl, 20 h and at 100°C).[15] The different components of the hydrolysate were separated on a Dowex 1 (2 % DVB) column by gradient elution with formic acid and the results are given in Table 2.

Glucosamine was determined in the fraction eluted with water according to Blix' modification of the Elson-Morgan reaction [17]. To detect the phosphate a small amount of carrier free ^{32}P-labeled orthophosphate was added before the separation. Phophorus was determined according to an earlier described method [10]. Glucosamine-6-phosphate was essayed by comparison on the paper chromatogram strips with known amounts of the pure substance prepared mainly according to Brown [18]. For development of the chromatograms *iso*-butyric acid-0.5 N ammonia (10:6) and propionic acid-benzene-water (58:25:25) were used. UMP was determined from UV absorption data at 260 and 280 mμ of a aliquot of the collected UMP peak.

The extremely low yield of glucosamine phosphate obtained in this experiment seemed to be insignificant when considering a possible migration of the phosphoryl group from 1- to 6-position as a source of error. The small amount found may be due to an impurity in the UDP-glucosamine preparation obtained from yeast.

Isolation of phosphorylated peptides from the cell protein residue. If UDP-linked Mur or rather, similarly bound MurP or MurP-peptides, were intermediates in the biosynthesis of protein bound MurP this hypothesis would be supported by the isolation of phosphopeptides with the same amino acid content as in the UDP-linked peptides. Attempts were accordingly made to isolate such peptides from the partially hydrolyzed protein residues (11 N HCl at 37° during 3 days). In Fig. 3 a typical elution curve from a Dowex 50 column is presented for the protein hydrolysate from a 90 l culture of *L. casei* cultivated in the presence of penicillin. The peak after inorganic phosphate eluted between

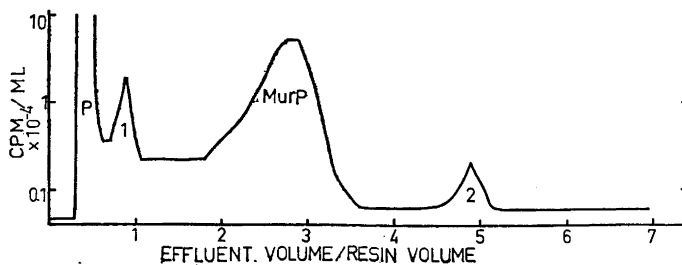

Fig. 3. Radioactivity curve from a hydrolysate of the protein residue from *L. casei* incubated for 4 h in a ^{32}P-containing medium. The curve is obtained by plotting the number of impulses per min (cpm) in 1 ml aliquots of each tube.

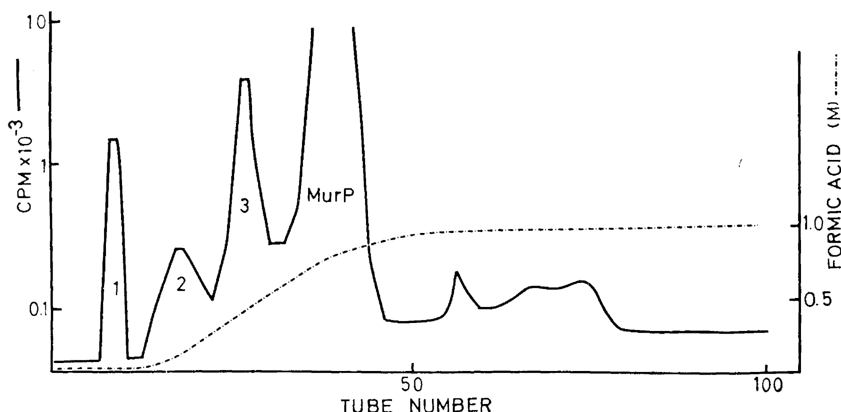

Fig. 4. Radioactivity curve from a mixture of MurP and MurP-peptides separated on a Dowex 1 column. The radioactivity curve (the continuous line) is obtained by plotting the number of impulses per min (cpm) in 1 ml aliquots of each tube. The broken line represents formic acid concentration.

0.72 to 0.96 column volumes (effluent volume/resin volume) and the MurP peak appearing between 1.9 to 3.3 column volumes were separately rechromatographed on Dowex 1 formate columns after removal of excess HCl (column dimensions 0.9 × 50 cm and 2 × 40 cm, respectively). A few times the peak emerging from the Dowex 50 column between 4.9 and 5.1 column volumes was also rechromatographed on the smaller type of Dowex 1 column. Fig. 4 gives a typical elution curve for the rechromatography of the MurP peak from the Dowex 50 column on a Dowex 1 formate column. Elution was carried out with the gradient 0 → 1 M formic acid with a volume of 200 ml in the mixer. The large peak appearing when the acidity of the eluate reached a value which corresponded to about 0.60 M formic acid was practically pure MurP. The peak 3 eluted somewhat earlier at about 0.40 M formic acid was homogeneous by analysis with inophoresis and chromatographic procedures. After hydrolysis of a small part of the material paper chromatographic analysis showed three strong spots corresponding to Mur, alanine and glutamic acid and five weaker spots. After rechromatography of the phosphopeptide on a small Dowex 50 (4 % DVB) column and hydrolysis the peptide contained only alanine and Mur. The first peak in Fig. 4 appearing with water was also homogeneous when analyzed with ionophoretic and chromatographic methods. After hydrolysis paper chromatographic analysis showed spots for Mur and alanine and possibly for glutamic acid. Compared with peak 3 there was less alanine in relation to Mur in the material from peak 1. The small amounts of material available did not permit rechromatography on a second Dowex 50 column. A peak in the same position as 1 could also be eluted from the MurP fractions obtained by hydrolysis in the usual way [10] and then separated on a Dowex 50 column. This material was not homogeneous when analyzed by paper chromatography or ionophoresis in 1 M acetic acid. Three different

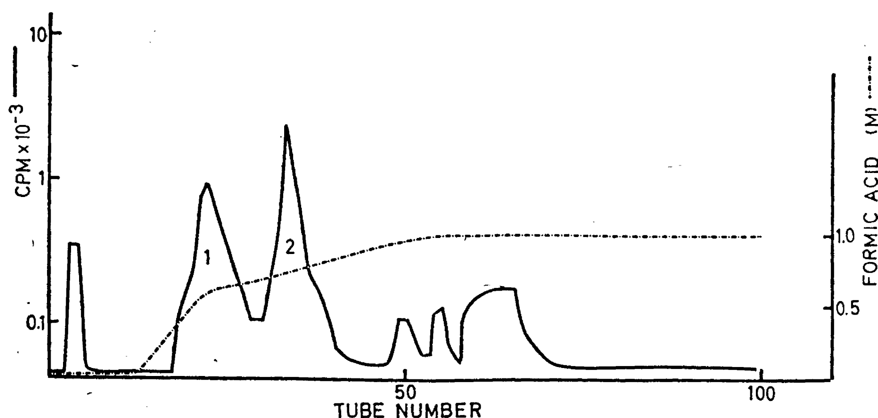

Fig. 5. Radioactivity curve from a mixture of MurP-peptides separated on a Dowex 1 column. The radioactivity curve is obtained by plotting the number of impulses per min (cpm) in 1 ml aliquots of each tube. The broken line represents formic acid concentration.

fractions were obtained. They all contained Mur, alanine and glutamic acid with about the same relation of Mur to alanine as in peak 1 (Fig. 4).

Fig. 5 demonstrates a typical Dowex 1 elution curve at the rechromatography of the material from peak No. 1 of the Dowex 50 curve (Fig. 3). The material corresponding to the two larger peaks 1 and 2 were homogeneous when analyzed with ionophoretic and paper chromatographic methods. After hydrolysis fraction 1 showed paper chromatographic spots corresponding to glutamic acid, alanine, Mur, valine, leucine and a spot in the position of γ-amino butyric acid. The amount of material corresponding to peak 1 allowed a rechromatography on a second Dowex 50 (4 % DVB) column. Only one radioactive fraction was obtained by elution with 0.01 N HCl. After hydrolysis of this material paper chromatographic analysis showed the presence of glutamic acid in reduced amounts, alanine, Mur and leucine. Fraction 2 from the Dowex 1 column (Fig. 5) was also hydrolyzed and analyzed by paper chromatography. Spots were obtained corresponding to the position of Mur, alanine and glutamic acid. Available amounts did not allow a rechromatography on a second Dowex 50 column.

The material from peak 2 on the Dowex 50 column (Fig. 3) was also rechromatographed on Dowex 1 columns. At least eight small radioactive fractions were obtained. The amounts of material in each fraction were extremely small and not further analyzed. A summary of the phosphopeptide compositions is given in Table 3. While a detailed sequence so far has not been established a certain agreement in amino acid composition between the different phosphopeptides can be noted.

Specific activity values of protein bound MurP. In Table 4 a summary is given of the MurP values obtained from different types of experiments. 30 mC ^{32}P per 90 liter of culture were used in experiments Nos. 1 to 6, otherwise 40 mC except in experiment Nos. 13 and 14 where 60 and 100 mC, respecti-

Table 3. ^{32}P-Phosphopeptides isolated from the protein residue of *Lactobacillus casei.*

Phosphopeptide obtained by separation on a Dowex 50 column (Fig. 3)	Further separation on Dowex 1 columns (Figs. 4 and 5)	Amino acid composition
Peak 1	Peak 1	Glu, Mur, Ala, Leu
	Peak 2	Glu, Mur, Ala
MurP	Peak 1	Mur, Ala
	Peak 3	Mur, Ala

vely, were used. In the series marked a and b each represents comparable 45 liter cultures. Bactopeptone and liver furnished sufficient total phosphorus (0.057 %) for normal growth. The corresponding value for inorganic phosphate was 0.024 % and the specific activity of inorganic phosphate in the medium

Table 4. Specific activities of protein bound MurP.

Expt. No.*	^{32}P added after **	Penicillin added after **	Time for harvesting **	mC ^{32}P added per litre of culture	Specific activity ***	Dry cell weight in g
1 a	0	3	4	0.33	4 700	20.5
1 b	2.5	3	4	0.33	2 600	25.0
2 a	0	3	4	0.33	2 320	21.7
2 b	3	3	4	0.35	1 700	26.0
3 a	0	3	4	0.33	2 320	13.7
3 b	0	—	4	0.33	1 700	13.0
4	0	2	3	0.33	2 120	40.5
5 a	0	3	4	0.33	2 030	21.5
5 b	0	—	4	0.33	2 900	23.5
6 a	0	0	0.25	0.33	15	7.1
6 b	0	—	0.25	0.33	32	7.2
6 c	0	0	1	0.33	32	7.2
6 d	0	—	1	0.33	2 290	7.3
6 e	0	0	2	0.33	98	7.2
6 f	0	—	2	0.33	1 820	10.5
7	0	—	18	0.44	3 640	78
8	0	—	18	0.44	3 930	72
9	0	—	18	0.44	4 080	75
10	0	—	18	0.44	2 900	70
11	0	—	18	0.44	4 620	73
12	0	—	18	0.44	4 400	98
13	0	—	18	0.66	6 730	114
14	0	—	4	1.1	14 400	42

* Experiments with the same figures are run in parallels and from the same batch of cells.
** All times are given in hours.
*** Counts per min and μg of phosphorus.

at the time of inoculations was 5 010 in one experiment where 1.1 mC ^{32}P per liter of medium was used.

In order to increase the utilization of isotopic phosphate added to the medium inorganic phosphate was excluded in all experiments. Experiments Nos. 3 and 5 are comparable pairs and demonstrate that penicillin in these series had no significant influence on specific activity values of MurP when added in the log phase of growth. A comparison with the corresponding TCA extracts also seem to show rather similar amounts of UDP-linked Mur in the presence or absence of penicillin. Likewise experiments 1 and 2 are comparable pairs. Since penicillin had no influence on specific activity values in experiments Nos. 3 and 5 these series seem to show a dilution of radioactive MurP with already synthesized unlabeled or slightly labeled material. Whether MurP-phosphoproteins take an active part in exchange and transfer reactions or MurP represents structurally bound MurP cannot be decided from these figures only [19].

The experiments in series No. 6 were carried out in the same manner as by Strominger [6] with material from 6 comparable 15 liter cultures. The effect of penicillin on the specific activity is rather striking. The weight figures in Table 4 seem to indicate that when penicillin is present there is no growth and only a wery small incorporation of radioactivity in MurP. In the controls the weight figures and the photometer readings in Table 1 seem to indicate that as usual with the amount of inocula used the limit between lag phase and log phase is found after 1 h of incubation. Of special interest is the high specific activity in MurP at this time when it is questionable whether any new cell walls have been formed. The results are not in agreement with those obtained by Trucco and Pardee [20]. They studied the incorporation of uniformly labeled ^{14}C glucose in different cell fractions of *E. coli* up to 180 min of incubation and did not find any differences between specific activity values in controls and penicillin cultures for cell walls, cytoplasm or unbroken cells.

The specific activity of MurP from UDP-linked MurP was determined in experiment No. 5. To obtain sufficient amount of material all UDP peaks after ADP from 5a and 5b (Fig. 1) were pooled and MurP was isolated as previously described. The specific activity of this MurP phosphorus was 5 070 as compared with the mean value of 2 470 of the protein bound MurP phosphorus. This means that UDP-linked MurP could be a precursor to protein bound MurP.

DISCUSSION

With the method described in this paper it has been possible to isolate also from penicillin inhibited *L. casei* UDP-linked Mur-peptides of similar composition as previously found by Park and Strominger [5] in *S. aureus* and *L. helveticus*. Nucleotides of this type isolated from normal *L. casei* seemed to be identical with or closely related to the nucleotides from penicillin inhibited cultures. These observations suggest that the nucleotide bound peptides are normal metabolites and that they are themselves not toxic to the cell. The occurrence of these compounds in several species also suggests that they may have some general significance in bacterial metabolism.

The amino acids present in the UDP-linked Mur-peptides were of considerable interest. So far only glutamic acid, alanine, and Mur have been identified while a fourth amino acid also present is still of unknown character. According to Cummins and Harris [21] a hydrolysate of the bacterial cell wall of *L. casei* contained the following amino acids: aspartic acid, glutamic acid, alanine, Mur, and lysine. The unknown amino acid in our UDP-linked Mur-peptide was not identical with diaminopimelic acid or lysine which was found by Park [22] in his UDP-linked peptide by side of alanine, muramic acid and glutamic acid, also present in our UDP-linked peptide.

Under our experimental conditions there was usually no accumulation of UDP-linked peptides as a consequence of penicillin inhibition. That may be due to the fact that penicillin was added rather late in the log phase of growth when the effect of penicillin could be expected to act only for a comparatively short time. The conditions of experiment 6 might have been more favourable for studying an accumulation of UDP-linked peptides.

Park and Strominger [5] have suggested that the UDP-linked Mur-peptides are biosynthetic precursors of the bacterial cell wall and accumulate in penicillin treated *S. aureus* as a conseuqence of the interference by penicillin with the biosynthesis of the cell wall. From our point of view the biosynthesis of phosphoproteins is of general interest and with regard to the cell wall composition it has previously been demonstrated that about 80 % of the phosphoproteins in *E. coli* are located in the cell wall protein fraction [23]. It has previously been pointed out that UDP-linked MurP could be an intermediate in the biosynthesis of protein bound MurP. Of considerable interest was therefore the isolation of UDP-linked MurP with higher specific activity than protein bound MurP.

If it is assumed that UDP-linked Mur-peptides are in equilibrium with protein bound MurP-peptides it could be expected that phosphopeptides with a similar composition should be isolated from the hydrolyzed cell protein residue, containing the phosphoproteins. This actually was the case. One phosphopeptide containing Mur and alanine was found and a second one contained Mur, alanine and glutamic acid.

Some preliminary experiments have been carried out in an attempt to elucidate how a phosphorylation of Mur could be effected. Lyophilized cells of *L. casei* were disintegrated with a Raytheon magnetostriction oscillator and different fragment fractions were separated by differential centrifugation at 2 C°. Media containing the different fragment fractions, Mur, $AT^{32}P$, and magnesium ions were incubated for different times, but so far $Mur^{32}P$ could not be isolated. There may be two explanations of the failure to find a muramic acid kinase. Either the phosphorylation may only take place when Mur is activated by a nucleotide linkage or the phosphorylation may occur before the muramic acid moities are fused together.

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