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Phosphorylation of Streptozotocin During Uptake via the Phosphoenolpyruvate:Sugar Phosphotransferase System in *Escherichia coli*

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Mutants of *Escherichia coli* K-12, *Staphylococcus aureus*, and *Bacillus subtilis* defective in the general components (enzyme I, or HPr, or both) of the phosphoenolpyruvate:sugar phosphotransferase system are shown to be resistant to the antibiotic streptozotocin. It is shown here, employing ^{32}P-labeled phosphoenolpyruvate, that wild-type cells of *E. coli* phosphorylate streptozotocin, whereas with a phosphotransferase system-defective mutant of *E. coli* the drug is recovered in an unaltered, free form. The internal accumulation of streptozotocin at the steady-state level was about 70 times that of the concentration in the external medium. The antibacterial action of streptozotocin, as well as the uptake of the drug, was inhibited by *N*-acetyl-D-glucosamine. The uptake of the antibiotic was extremely sensitive to *p*-chloromercuribenzoate. It is concluded that streptozotocin is taken up by *E. coli* via the phosphoenolpyruvate:sugar phosphotransferase system and consequently accumulates in the cell at first as streptozotocin-phosphate.

Streptozotocin (Streptozocin) is a broad-spectrum antibiotic which also displays antitumor and diabetogenic activities (19). It is produced by *Streptomyces achromogenes* subsp. *streptozoticus* (21). Chemically it has been shown to be a 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose (see Fig. 1) (22) and, hence, an *N*-acetylglucosamine derivative. The antimicrobial activity is assumed to be due to an interaction of the antibiotic with deoxyribonucleic acid, inducing rapid degradation (18).

Since the target of streptozotocin is an intracellular one, this drug has to pass through the layers of the cell envelope of the bacterium to influence cell growth. It is well established that the envelope of bacteria is almost impermeable to hydrophilic compounds (23) unless the bacterium provides a specific uptake mechanism. Bacteria are therefore equipped with an abundant number of specific transport systems for nutrients, i.e., amino acids, sugars, vitamins, and ions (1). The close structural analogy of streptozotocin with *N*-acetyl-D-glucosamine prompted us to investigate the possibility that streptozotocin could utilize the bacterial transport systems for the uptake of *N*-acetyl-D-glucosamine.

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This report shows that in *Escherichia coli* streptozotocin is taken up via the phosphoenolpyruvate:sugar phosphotransferase system (PTS) and is thus initially accumulated in the cell as a phosphate ester derivative. Our definition of this mechanism for the accumulation of streptozotocin has led J. Lengeler to devise an elegant procedure for the selection of rare bacterial mutations (14). This procedure is based on the fact that killing by streptozotocin is restricted to cells which have transported the drug.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* K-12 L191 [F$^-$, *thi met his arg lac galT rpsL pts(I, H)*] was obtained from J. Lengeler. *Staphylococcus aureus* S305A (wild type) and the mutant strains S710A (*ptsI*) and S797A (*ptsH*) (7) were obtained from W. Hengstenberg. The *Bacillus subtilis* strains 60015 (wild type) and 61310 (phosphoenolpyruvate transferase mutant) (15) were provided by E. Freese. Cells were grown in a complex medium K1 (9) or on minimal medium M9 (16) with shaking on a rotary water bath shaker at 37°C.

Agar diffusion test. The susceptibility to streptozotocin and other antibiotics of various bacterial strains was tested by the disk method on complex medium agar plates. Standardized paper disks (6 mm in diameter) soaked with the antibiotic were used. After 12 h of incubation at 37°C, the diameter of the inhibitory zones was determined.

Quantitative streptozotocin determination. The method employed to determine streptozotocin is

a modification of the method described by Forist (5), which makes use of the reactivity of the *N*-nitroso group in the molecule. In this reaction the acid hydrolysis of the *N*-nitroso residues forms nitrous acid, which in turn causes in the presence of *N*-(1-naphthyl)-ethylenediammonium dichloride and sulfanilic acid the formation of an azo compound. This formation can be measured optically at 546 nm. To a portion (0.1 ml) of the sample, 0.2 ml of 6 N HCl and 1 ml of reagent solution are added. [The reagent solution consists of 0.5% sulfanilic acid and 0.1% *N*-(1-naphthyl)-ethylenediammonium dichloride in 30% acetic acid.] The reaction mixture is incubated at 60°C for 45 min. After cooling the sample, the absorbance at 546 nm is recorded with a spectrophotometer (Eppendorf, Hamburg, West Germany), and the amount is calculated from a standard curve.

Uptake measurements. Cells were harvested at an optical density at 578 nm of about 0.5 and washed twice with saline at 10°C. After resuspension of the cells in glycerol-M9 minimal medium, the concentration of the bacteria was adjusted to about 1.25 mg of cells (dry weight) per ml. The cells were preincubated in an Erlenmeyer flask with 100 μg of chloramphenicol per ml in a rotary water bath shaker at 37 or 27°C (as indicated) for 20 min.

Uptake of *N*-acetylglucosamine was initiated by the addition of 1 μmol of *N*-acetyl-D-[1-^{14}C]glucosamine per ml with a specific radioactivity of 4 mCi/μmol. Samples of 200 μl were withdrawn at the indicated intervals and rapidly vacuum filtered through 0.45-μm pore-size cellulose acetate membrane filters (Sartorius-Membranfilter GmbH, Göttingen, West Germany), which had been presoaked with 0.1 M LiCl solution. The filters were dried in an oven at 60°C for 30 min, 5 ml of toluene-based scintillator fluid was added, and the radioactivity was determined in a scintillation counter.

Uptake of streptozotocin was similarly determined with a concentration of 100 μg of streptozotocin per ml. Cellulose acetate filters had to be used for this assay rather than cellulose nitrate, since the nitro groups of the latter interfere with the chemical determination of streptozotocin. After rinsing the filters with 0.1 M LiCl, they were placed into tubes, and the reagents for the streptozotocin assay were added as described above. At the end of the incubation the filters were removed, the cells were spun down, and the absorbance in the supernatant was determined as described.

Synthesis of ^{32}P-labeled phosphoenolpyruvate. ^{32}P-labeled phosphoenolpyruvate was synthesized by the method developed by Lauppe et al. (13). The reaction product was separated by high-voltage electrophoresis on paper, eluted from the paper with H_2O, concentrated by evaporation, and immediately neutralized with 0.05 M Tris-hydrochloride buffer (pH 7.5). The ^{32}P-labeled product was characterized by an enzymic assay system represented by the following reaction: adenosine 5′-diphosphate + phosphoenolpyruvate → adenosine 5′-triphosphate + pyruvate. ^{32}P-labeled phospoenolpyruvate was obtained in a yield of 40 to 50% with a specific radioactivity of about 1 mCi/μmol.

In vivo phosphorylation of streptozotocin. Cells of *E. coli* K-12 were grown in complex medium K1 and harvested at an optical density of about 0.5. The cells were washed twice with saline and finally resuspended in 0.05 M Tris-hydrochloride buffer (pH 7.5) containing 5 mM $MgCl_2$ and 0.1 mM dithiothreitol, yielding a concentration of about 0.5 g of cells per ml of buffer. The cells were cooled in an ice bath and were sonicated with four bursts at maximal power output for 15 s each with a Branson Sonifer. To a portion of 0.4 ml of these sonicated cells were added 50 μl of 0.1 M phosphoenolpyruvate, 25 μl of freshly synthesized ^{32}P-labeled phosphoenolpyruvate (about 50 μCi), and 10 μl of 500 mM streptozotocin. After 10 min of incubation at room temperature, the cell debris was pelleted by centrifugation. The components of the supernatant were separated by high-voltage electrophoresis on paper. The product was eluted from the paper with water for further characterization.

High-voltage paper electrophoresis. The sample was applied to electrophoresis paper (MN 827, Machery and Nagel, Düren, West Germany) that had been soaked in 0.05 M ammonium acetate buffer, pH 4.5, and blotted before use. The electrophoresis was carried out in 0.05 M ammonium acetate buffer, pH 4.5, for 1 h at 1,500 V and 70 to 80 mA. The reaction products were localized on the paper by autoradiography (X-ray film T4, Agfa Gevaert; exposure time, 4 to 14 h) and also by the streptozotocin specific chemical assay on the strips of the electrophoresis paper, as described above.

Acid phosphatase reaction. A sample (150 μl) of the presumed streptozotocin-phosphate in water was mixed with 30 μl of 0.15 M sodium acetate buffer, pH 5, and 20 μl of acid phosphatase (0.1 mg/ml; 2 U/mg). After incubation for 20 min at room temperature, the sample was concentrated by lyophilization and dissolved in 15 μl of H_2O. Portions (5 μl) of the treated samples were applied to cellulose thin-layer sheets (Merck, Darmstadt, West Germany). The chromatograms were developed in isopropanol-pyridine-glacial acetic acid-water (8:8:1:4, vol/vol) at room temperature for 4 h. The dried chromatogram was cut into strips for the detection of streptozotocin activity by the described chemical method or by counting the radioactivity.

Chemicals. *N*-acetyl-D-glucosamine, chloramphenicol, and phosphoenolpyruvate (disodium salt) were obtained from Serva (Heidelberg, West Germany). Adenosine di- and triphosphate, glucose-6-phosphate, acid phosphatase, and pyruvate kinase were from Boehringer (Mannheim, West Germany). Streptozotocin and nojirimycin were obtained from Upjohn Overseas Co. (Kalamazoo, Mich.). The source of the radiochemicals was Amersham Buchler (Buckinghamshire, United Kingdom). The specific activities were as follows: *N*-acetyl-D-[1-^{14}C]glucosamine, 45 mCi/mmol; D-[*U*-^{14}C]glucose-6-phosphate (sodium salt), 150 mCi/mmol; D-[1-^{14}C]glucose, 60 mCi/mmol; 3-*O*-methyl-D-[*U*-^{14}C]glucose, 50 mCi/mmol. The [^{32}P]orthophosphate in dilute HCl solution had a specific activity at reference date of 30 to 100 Ci/mg and was immediately used for synthesis. All other chemicals were obtained, in the purest grade available, from Merck (Darmstadt, West Germany).

RESULTS

Resistance of PTS-defective mutants of *S. aureus*, *B. subtilis*, and *E. coli* to the action of streptozotocin. A mechanism for the transport of carbohydrates, widespread in bacteria, is the group translocation process catalyzed by the phosphoenolpyruvate:sugar PTS involving the following reactions: (i) phosphoenolpyruvate + HPr $\xrightarrow{\text{enzyme I}}$ P-HPr + pyruvate; (ii) P-HPr + sugar $\xrightarrow{\text{enzyme II complex}}$ sugar-P + HPr. In the first step of the overall reaction sequence, only general proteins with no specificity regarding the sugar substrate participate, whereas the second step, resulting in the phosphorylation of the sugar, depends on sugar-specific proteins (8, 20). Thus, mutants altered in either of the two general factors, HPr or enzyme I, are defective in the uptake via the PTS of all sugars.

A mutant of *S. aureus* in the enzyme I (S710A), as well as a mutant in the HPr protein (S797A) (7), was tested for susceptibility to streptozotocin (Fig. 1); to nojirimycin (Fig. 1) (10), which is a 5-amino-5-deoxy-D-glucopyranose; and, for comparison, to a few other water-soluble antibiotics. A double mutant of *E. coli* K-12 (L191), which is defective in the function of both enzyme I and the HPr protein, was compared with the corresponding wild type. A mutant of *B. subtilis* which is deficient in enzyme I or in the heat-stable protein HPr, strain 61310 (15), was also included in these susceptibility determinations.

The results of a conventional agar diffusion test, as shown in Table 1, clearly reveal that the PTS-defective mutants of *S. aureus*, *E. coli*, and *B. subtilis* are much more resistant to streptozotocin than the wild-type strains. The less-pronounced increase in the resistance with the *S. aureus* mutant S797A can be explained by the leakiness of this mutation (7). The mutants of *S. aureus* and *E. coli* are also more resistant to nojirimycin.

The resistance of the PTS-defective mutant of *Escherichia coli* to the action of streptozotocin was also observed in liquid cultures. The growth of the tight *pts* double mutant (I^-, H^-) *E. coli* K-12 L191 was not affected by 50 μg of streptozotocin per ml, whereas complete inhibition of growth was caused under identical conditions with the wild-type strain of *E. coli* K-12.

Antagonism between streptozotocin and *N*-acetyl-D-glucosamine. Agar diffusion tests demonstrate the antagonistic effect of *N*-acetyl-D-glucosamine on the action of streptozotocin. Growth inhibition curves (not shown) more clearly show that *N*-acetyl-D-glucosamine protects the cells against the bactericidal action of

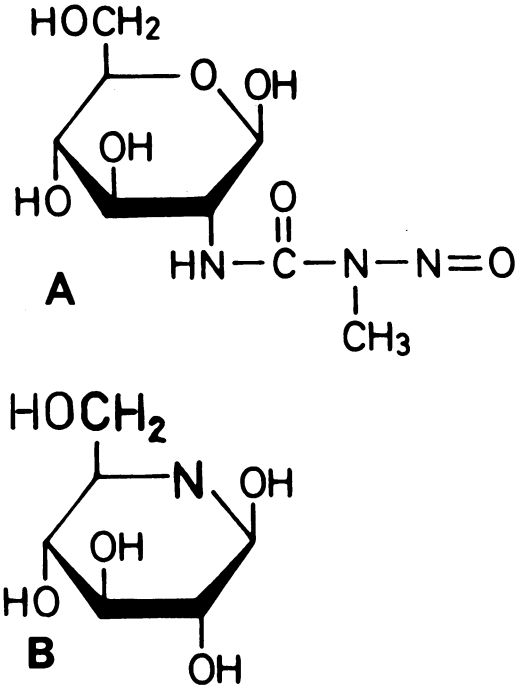

FIG. 1. *Structures of the antibiotics streptozotocin (A) and nojirimycin (B).*

TABLE 1. *Agar diffusion tests with PTS-defective mutants of E. coli K-12, S. aureus, and B. subtilis*[a]

Antibiotic	Disk potency (μg)	Diam of zone of inhibition (mm)						
		E. coli K-12		*S. aureus*			*B. subtilis*	
		Wild-type	L191 *pts* (*I*, *H*)	S305A wild type	S710A *ptsI*	S797A *ptsH*	60015 wild type	61310 *ptsI* or *H*
Streptozotocin	20	10.5	0	22	0	11.5	14	0
Nojirimycin	50	15	0	21	0	ND	ND	ND
Erythromycin	50	17	18	23	22	23	21	25
Neomycin	50	14	14	13	12	13	13	14.5
Fosfomycin	35	30	31	27	25	25	ND	ND

[a] All readings made after 12 h of incubation on complex agar medium at 37°C. ND, Not determined.

streptozotocin. An excess of 10 times the streptozotocin concentration was necessary, however, to neutralize the action of the antibiotic on *E. coli*.

Uptake of streptozotocin in whole cells of *E. coli*. The uptake of streptozotocin was determined with *E. coli* K-12 pregrown on various carbon sources. Independent of the composition of the growth medium, the steady-state level of streptozotocin at 37°C was achieved after only about 6 min of incubation. At 27°C the uptake did not reach saturation in 10 min (Fig. 2). The steady-state level of streptozotocin present in cells pregrown in minimal medium supplemented with glucose was only half the value obtained with cells cultured in glucose-free media. An unpredicted uptake pattern was found with cells grown in the presence of *N*-acetyl-D-glucosamine as sole carbon source. As shown in Fig. 2, the uptake curve is sigmoidal in character, remaining low for the first 2 min. Thereafter, the uptake increases to the level obtained with cells grown in glycerol containing minimal medium or complex medium. Maximal transport is obtained with cells from the mid-logarithmic to stationary growth phase (data not shown).

The accumulation of streptozotocin at the steady-state level can be calculated by using the fact that 1 mg (dry weight) of cells is equivalent to 2.7 μl of cell water (24). A steady-state level of about 72 nmol of streptozotocin per mg of cells was reached in 6 min at 37°C at an external concentration of 0.37 mM. The cell accumulated streptozotocin to an internal concentration of about 70 times that of the external medium, assuming that the antibiotic was free and not bound. For comparison, the intracellular concentration of *N*-acetyl-D-glucosamine at the steady-state level (Fig. 2) was about 72 mM, given an external concentration of 1 mM. Thus, the cells generate a similar concentration gradient for both streptozotocin and *N*-acetyl-D-glucosamine.

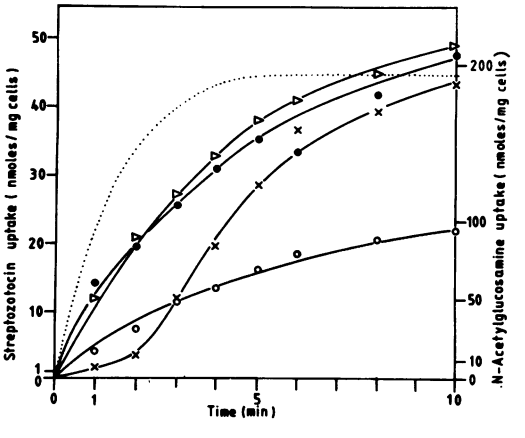

FIG. 2. *Time course of streptozotocin uptake by E. coli cells grown on various carbon sources. E. coli K-12 was grown in different media, washed twice with saline, and resuspended in standard uptake medium (glycerol-M9 minimal medium). Uptake of streptozotocin* (———) *and N-acetyl-D-[1-^{14}C]glucosamine* (·····) *at 27°C was measured as described in the text. The cells for N-acetylglucosamine uptake measurements were grown in complex medium K1. Symbols: △, complex medium K1; ●, glycerol-M9 minimal medium; ○, glucose-M9 minimal medium; ×, N-acetylglucosamine-M9 minimal medium.*

Inhibition of streptozotocin accumulation by sulfhydryl reagents. The effect of various poisons of energy metabolism and of the sulfhydryl reagent *p*-chloromercuribenzoate on the uptake of streptozotocin was investigated. Whereas sodium cyanide and 2,4-dinitrophenol at a concentration of 1 mM lowered the rate of uptake of *N*-acetyl-D-glucosamine by about 50% (55 and 43%, respectively), no inhibition of uptake of streptozotocin was observed under identical conditions. The most effective inhibitor of streptozotocin uptake was *p*-chloromercuribenzoate, which at a concentration of 1 mM completely inhibits the accumulation of both *N*-acetyl-D-glucosamine and streptozotocin. These results indicate that the overall accumulation of *N*-acetyl-D-glucosamine differs from that of streptozotocin, although both processes are extremely sensitive to *p*-chloromercuribenzoate, which has been shown to denature the enzyme I of the PTS (20).

Effect of various carbohydrates on the uptake of streptozotocin. The in vivo experiments on the antagonistic effect of *N*-acetyl-D-glucosamine on the action of streptozotocin very likely represent a competition for the same uptake system. To demonstrate this possibility, inhibition experiments were performed between the uptake of streptozotocin and increasing concentrations of *N*-acetyl-D-glucosamine and other sugars. As shown in Fig. 3, *N*-acetyl-D-glucosamine is a potent inhibitor for uptake. An equal concentration of *N*-acetyl-D-glucosamine is sufficient to achieve maximum inhibition of uptake of streptozotocin, that is, almost complete inhibition. Glucose also decreases the uptake of streptozotocin, but a residual uptake of about 20% remains unaffected. No reduction in the accumulation of streptozotocin was found in the presence of free D-glucosamine, which is also transported in *E. coli* via the PTS. In the presence of lactose, which in *E. coli* does not utilize the PTS, there was no decrease in the uptake of streptozotocin observed, as was expected.

Phosphorylation of streptozotocin dur-

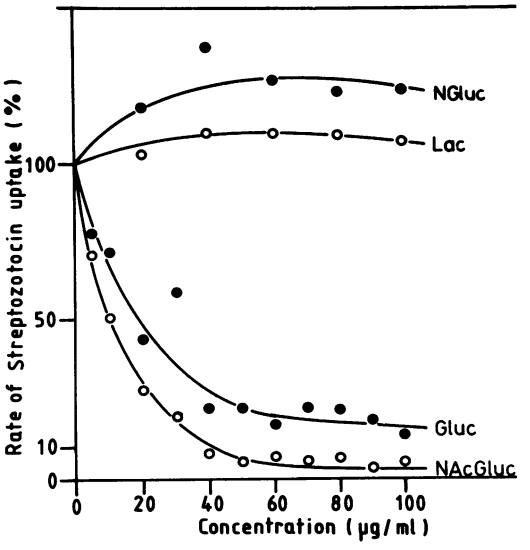

FIG. 3. *Effect of various carbohydrates on the uptake of streptozotocin in E. coli. Uptake of streptozotocin by E. coli K-12 was determined at a concentration of 50 µg/ml of the antibiotic for 1 min at 37°C in the presence of the indicated amounts of sugars. The sugars were added 15 s before streptozotocin. The results are given as percentages of a control without addition of carbohydrate.*

ing uptake in *E. coli*. Evidence has been presented that galactose can be taken up by certain mutant strains of *E. coli* by a facilitated diffusion process, mediated by a component of the PTS but without subsequent phosphorylation (12). A similar mechanism could be envisaged for the transport of the unusual substrate, the antibiotic streptozotocin. The results presented so far clearly demonstrate that streptozotocin uptake depends on a functional PTS and that *N*-acetyl-D-glucosamine competes for uptake. The question remains, however: is streptozotocin phosphorylated at the expense of phosphoenolpyruvate during uptake, as would be expected for a normal substrate of the PTS?

This was investigated by the incubation of permeabilized cells of *E. coli* with streptozotocin in the presence of ^{32}P-labeled phosphoenolpyruvate. This in vivo system has the capacity to transfer phosphate residues from the added phosphoenolpyruvate to PTS sugars like α-methyl-glucoside (13). We succeeded in phosphorylating streptozotocin with this system. The phosphorylation of the antibiotic was demonstrated by high-voltage electrophoresis to separate the reaction products from the reaction mixture. As shown in Fig. 4, one ^{32}P-labeled spot gave a positive colorimetric test for streptozotocin. A second spot, also containing the ^{32}P label but showing no reaction in the streptozotocin-specific test, had an R_f value similar to the presumed streptozotocin-phosphate. It was not attempted to further characterize this latter material, which may represent the phosphorylated form of a degradation product of streptozotocin. No ^{32}P-containing material other than phosphate and phosphoenolpyruvate was detected in an identical assay employing the PTS-defective mutant of *E. coli* K-12, L191 (Fig. 4).

Enzymatic cleavage of the presumed streptozotocin-phosphate. The presumed streptozotocin-phosphate formed during incubation with wild-type *E. coli* was eluted from the electrophoresis paper and incubated in the presence of phosphatases. The reaction products were separated by thin-layer chromatography. Enzymatic digestion with alkaline phosphatase resulted in a complete disappearance of any detectable streptozotocin activity, probably due to the known lability of the antibiotic at high pH. Acid phosphatase, which is active at pH 5, cleaved the phosphorylation product into free streptozotocin and phosphate (Fig. 5). Streptozotocin has been shown to be most stable at pH 4. It can be seen that all streptozotocin-specific activity is detected at a position where free

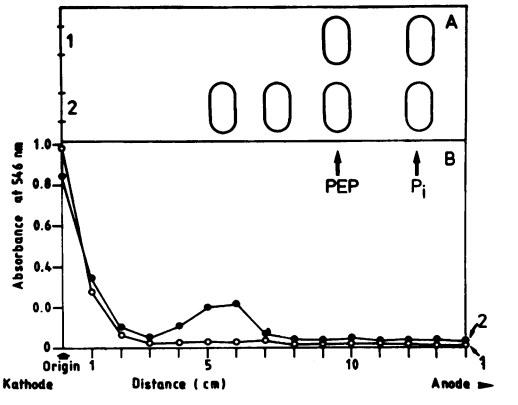

FIG. 4. *Separation of the reaction products of a streptozotocin phosphorylation experiment with sonicated cells of E. coli K-12. Cells of E. coli K-12 wild type (●) (2) and of the PTS-defective mutant L191 (○) (1) were sonicated as described in the text. To 0.8 ml of cell suspension 100 µl of unlabeled 0.1 M phosphoenolpyruvate (PEP), 50 µl of ^{32}P-labeled phosphoenolpyruvate (about 50 µCi), and 20 µl of 0.5 M streptozotocin were added and incubated for 10 min at room temperature. After centrifugation of the samples, the supernatant was subjected to high-voltage electrophoresis on paper in 0.05 M ammonium acetate buffer, pH 4.5, for 1 h at 1,500 V and 70 to 80 mA. Radioactivity distribution on the paper was localized by autoradiography (A); for the detection of streptozotocin activity, employing the described chemical method, the paper was cut into strips (B).*

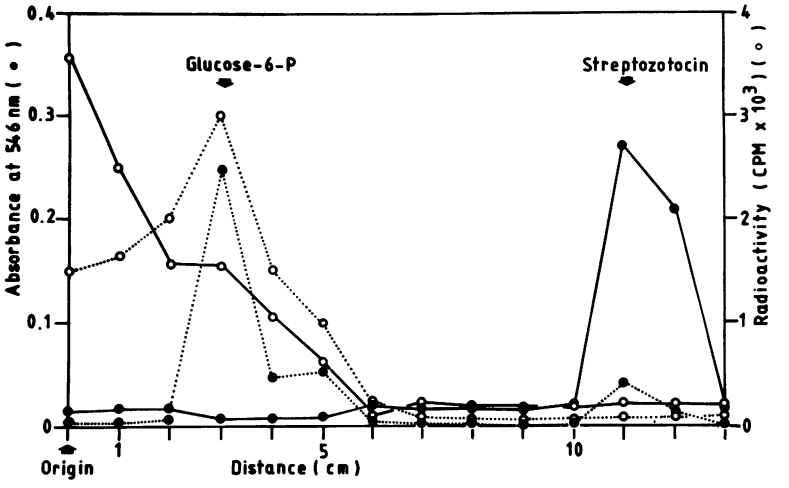

FIG. 5. *Separation by thin-layer chromatography of the reaction products of a phosphatase digestion of the presumed streptozotocin-phosphate. The product of the phosphorylation reaction containing both ^{32}P-label and streptozotocin activity (peak I in Fig. 4) was eluted from the electrophoresis paper and treated with acid phosphatase* (———). *As a control, an identical sample was treated in the same way except that the enzyme was omitted from the reaction mixture* (·····). *The concentrated samples were applied to cellulose thin-layer sheets. The chromatograms were developed in isopropanol-pyridine-glacial acetic acid-water (8:8:1:4, vol/vol) at room temperature for 4 h. Radioactivity* (○) *and streptozotocin activity* (●) *were detected as described in the legend to Fig. 4.*

streptozotocin migrates, and no streptozotocin activity remains at the position of the streptozotocin-phosphate. Although the employed thin-layer system has the disadvantage in that it separates free phosphate from sugar-phosphates only very poorly, a change in the distribution of ^{32}P-label can be seen. More label remains at the origin after the phosphatase digestion.

These results demonstrate that streptozotocin had been phosphorylated, concomitant with its accumulation into the cell.

DISCUSSION

A number of antibiotics, mostly antimetabolites, have been shown to be taken up by bacteria via the nutrient transport systems of the cell (6, 11). The vast majority of antibiotics, however, enter the cell by mechanisms still to be defined. This report demonstrates that the antibiotic streptozotocin is taken up by *E. coli* and very likely also by *S. aureus* and *B. subtilis* via the phosphoenolpyruvate:sugar PTS. Preliminary results suggest that also the antibiotic nojirimycin may be transported via the PTS. To our knowledge, it is the first example of an antibiotic transported via this uptake system.

In the case of *E. coli*, the experimental evidence is threefold. Firstly, a mutant deficient in the general proteins of the PTS, the enzyme I and the HPr protein, exhibits a greatly reduced susceptibility to streptozotocin compared with the wild type. Secondly, certain sugars which are transported in *E. coli* via the PTS, namely, D-glucose and *N*-acetyl-D-glucosamine, inhibit the uptake of streptozotocin and reverse the antibacterial action of the drug. Thirdly, streptozotocin is accumulated in wild-type cells of *E. coli* as the phosphate ester derivative. It is clear from these results that the PTS mediates the uptake of streptozotocin, but no attempt was made to clarify which specific protein of the enzyme II complex is involved. Detailed studies with the relevant mutants in these factors would have to be carried out to answer this question.

Whether or not an antibiotic is accepted by a particular nutrient transport system depends on the specificity of the uptake system. In general, transport systems are highly specific; however, a certain scope of variability in the structure of the substrates is tolerated by some uptake systems. A relatively broad variation tolerance has been found in the case of the dipeptide and oligopeptide transport systems in *E. coli* (17). Consequently, these systems were shown to transport non-physiological compounds like various peptide antibiotics (2, 3).

The PTS also seems to transport a large variety of substrates. For the specific enzyme II-B$^{'lac}$ of the system in *S. aureus*, it has been shown that substrates only had to possess a β-D-galactopyranoside ring but that the aglycon moiety could be modified a great deal. Even a non-physiological and bulky derivative such as 5′-(*N*-

dansyl)aminopentyl-1-thio-β-D-galactopyrano-side is phosphorylated and transported in *S. aureus* (8). Therefore, this moderate degree of specificity makes the PTS a suitable device for the uptake of non-physiological sugar derivatives including antibiotics. In two ways a controlled chemical modification of particular antibiotics could extend the utilization of the PTS for the uptake of antibiotics. Proper antibiotics could be chemically adapted to the structural requirements of the PTS. Also, it might be possible to hook impermeable inhibitors to sugar moieties, which then might function as a kind of vehicle to effect the passage of the inhibitory residue through the bacterial membrane. These approaches have been proven useful in the case of the peptide transport systems of bacteria (2, 4). In this respect the PTS might turn out to be an extremely qualified system because it is absent from eucaryotic cells, thus conferring on the drug a high specificity for the bacterial cell.

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