RESOLUTION OF A STAPHYLOCOCCAL PHOSPHOTRANSFERASE SYSTEM INTO FOUR PROTEIN COMPONENTS AND ITS RELATION TO SUGAR TRANSPORT*

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A phosphotransferase system (PTS) first isolated from gram-negative bacteria (Kundig, et al, 1964) was recently shown to mediate the transport of at least 9 carbohydrates in Salmonella typhimurium (Simoni, et al, 1967). The PTS involves two reactions:

(Enzyme I) Phosphoenolpyruvate (PEP) + HPr $\rightleftarrows$ P-HPr + Pyruvate

(Enzyme II) P-HPr + Sugar $\rightarrow$ Sugar-P + HPr

HPr, the phosphate carrier protein, is a low molecular weight (9700) histidine-containing protein, found in the soluble fraction of cell homogenates along with Enzyme I. Both components are required for all sugars phosphorylated by the PTS. The sugar specificity of the system is determined by the family of membrane-bound Enzymes II, many of which are inducible. Mutants lacking Enzyme I or HPr are pleiotropic carbohydrate negative (car^-); such mutants have been isolated from Escherichia coli, Aerobacter aerogenes (Tanaka, et al, 1967) and S. typhimurium (Simoni, et al, 1967).

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*Supported by USPHS Grant AM-9851. The proteins of the PTS are designated I, II, and HPr. The bacterial source is designated by subscript; e.g., I_{Ec} and I_{Sa} refer to Enzymes I from E. coli and S. aureus respectively. Fractions exhibiting specificity towards sugars such as II or the new protein component, III, are indicated by superscript; e.g., II^{gal}_{Sa} and II^{mtl}_{Sa} represent Enzymes II obtained by growth of S. aureus on galactose and mannitol respectively.

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A car$^-$ mutant of the gram-positive organism Staphyloccocus aureus has been reported by Egan and Morse (1965). The mutant is unable to utilize or transport a large number of carbohydrates. Preliminary evidence for the existence of a PTS in S. aureus has been presented (Kennedy and Scarborough, 1967; Hengstenberg, et al, 1968; Anderson, et al, 1968).

The present study demonstrates that S. aureus contains a PTS analogous, but not identical, to that of gram-negative organisms, and that the car$^-$ mutant is deficient in Enzyme I.* More important, however, fractionation of the S. aureus PTS showed that certain sugars were phosphorylated only in the presence of a new family of sugar-specific inducible components, hereafter designated III.

EXPERIMENTAL

Wild type S. aureus (BS5601, a derivative of NTCC 8511) and its car$^-$ mutant (5202F) were kindly provided by Dr. M. L. Morse; only the results obtained with these cells are reported, although they were identical in all respects to those obtained with wild type NTCC 8511 and car$^-$ mutant HS635 kindly provided by Dr. Ruth Korman (1963). Previously described methods were used for growth of cells in minimal and broth media, induction for lactose utilization and thiomethyl β - D galactopyranoside (TMG) transport, and examination of cell types for their ability to utilize and/or ferment various carbohydrates (Egan and Morse, 1965; Korman, 1963; Korman and Berman, 1958; McClatchy and Rosenblum, 1963; Laue and MacDonald, 1968).

Crude extracts prepared by sonication of galactose-induced wild type cells phosphorylated TMG when supplemented with PEP, but a more effective method for rupturing the cells involved the use of purified lysostaphin (Schindler and Schuhardt, 1965; kindly provided by Dr. Peter Tavormina, Mead, Johnson and Co.) in the presence of DNAase. The extract from galactose-induced wild type cells was resolved into four fractions as follows: II^{gal}_{Sa} was present in the membrane

*The Enzyme I defect in the car$^-$ mutant has also been observed by Hengstenberg and Morse, and by E. P. Kennedy (private communications).

Table 1

Requirements for Phosphorylation of TMG and Mannitol by the S. aureus Phosphotransferase System

Complete incubation mixtures contained the following components (μmoles) in 350 μl: K phosphate buffer, pH 7.05, 20; PEP, 2; dithiothreitol, 0.5; KF, 5; $MgCl_2$, 2; Enzyme I (0.04mg) and HPr (0.07mg) purified from wild type cells grown on 1% Bactopeptone medium containing 1% galactose. In addition, the following components were added to the incubation mixtures: Experiment A; ^{14}C- TMG (methyl labeled, 8×10^5 cpm/μmole), 1.0 μmole; Enzyme II (0.18 mg) and factor III (0.51 mg) purified from cells grown in Bactopeptone-galactose medium. Experiment B; D-mannitol-1-^{14}C (5.9×10^5 cpm/μmole), 1.0; Enzyme II (0.07mg) and factor III (0.69mg) obtained from cells grown in 1% Bactopeptone-1% mannitol medium. After 60 min at 37°, the reactions were stopped by heating at 100° for 3 min, and aliquots assayed by paper electrophoresis. The strips containing the sugar-P were counted by liquid scintillation techniques and have not been corrected for background, boiled controls, etc.

Experiment A		Experiment B	
Omissions	^{14}C-TMG-P cpm	Omissions	^{14}C-Mannitol-P cpm
None	16,200	None	17,600
PEP	660	PEP	1,100
$MgCl_2$	650	$MgCl_2$	1,300
Enzyme I	1,300	Enzyme I	4,900*
HPr	960	HPr	1,480
Enzyme II^{gal}_{Sa}	800	Enzyme II^{mtl}_{Sa}	1,300
Factor III^{gal}_{Sa}	1,400	Factor III^{mtl}_{Sa}	2,350

*Factor III^{mtl}_{Sa} contained significant Enzyme I activity.

fraction isolated by centrifugation at 100,000g (the pellet was washed exhaustively with buffer before use); I_{Sa} and III^{gal}_{Sa} were separated from HPr_{Sa} by precipitation of the former at pH 3.7; HPr_{Sa} was further purified by DEAE-cellulose chromatography; I_{Sa} and III^{gal}_{Sa} were separated by DEAE-cellulose chromatography, using a linear gradient of KCl ranging from 0.17 to 0.40 M in a solution containing 0.01 M phosphate buffer, pH 7.5, 0.001 M dithiothreitol, and 0.001 M EDTA. Fraction III was eluted at 0.26 M KCl, while I was eluted at 0.32 M. The details

Table 2

Sugar Specifity of Enzymes II and Factors III

Incubation conditions and assays were performed as described in Table 1. All mixtures contained Enzyme I and HPr as well as the other components described in Table 1 with the following exceptions. One set of tubes contained labeled TMG while the other contained labeled mannitol. Enzymes II and factors III, isolated from galactose or mannitol induced cells, were added as indicated. The rate of the reaction in complete incubation mixtures was limited by the quantity of fraction III.

Protein Fractions Added		Product Formed	
II_{Sa}	III_{Sa}	^{14}C-Mannitol-P cpm	^{14}C-TMG-P cpm
II^{mtl}	III^{mtl}	20,100	0
II^{mtl}	---	1,100	0
II^{mtl}	III^{gal}	1,250	100
II^{gal}	III^{gal}	2,600	22,300
II^{gal}	---	2,150	2,800
II^{gal}	III^{mtl}	4,700	3,200

of the purification procedure will be reported elsewhere.

Except for component III, the following criteria indicated that the S. aureus and E. coli PTS were analogous: (a)Nucleotide triphosphates did not replace PEP. (b)TMG was phosphorylated at the C-6 position* as shown by isolation of the product in about 80% yield as the crystalline dipotassium salt which was characterized by its typical X-ray diffraction pattern (kindly performed by Dr. Peter Laue). (c)A number of experiments indicated that I_{Sa} transferred ^{32}P from ^{32}P-PEP to HPr_{Sa}. The ^{32}P-HPr_{Sa} exhibited the same pH dependent sensitivity to hydrolysis as ^{32}P-HPr_{Ec}, and was also hydrolyzed by pyridine at pH 7.5. These preliminary results suggest that ^{32}P-HPr_{Sa} contained ^{32}P linked to a histidine residue, as does ^{32}P-HPr_{Ec} (Anderson, et al, 1968).

That the E. coli and S. aureus PTS were not identical was suggested by the fact that substitution of purified E. coli HPr for HPr_{Sa} in the S. aureus PTS

*The crystalline TMG-6-P was kindly isolated by Dr. Werner Kundig.

Table 3

Activity of Phosphotransferase Components in Crude Extracts of Induced and Noninduced S. aureus Cells

Incubation mixtures contained ^{14}C-TMG as the substrate and the other components described in Table 1 except that an excess of 3 purified components of the PTS system was added to assay the fourth component in the crude extracts. Product formation was proportional to time of incubation and protein concentration of the crude extract. Specific activity of each component in the crude extract is expressed as μmoles TMG-P formed per mg protein in 30 min at 37°.

Strain	Phenotype	Induced by*	Specific Activity in Extracts (x 10^2)			
			I	HPr	II	III
5601	Car+	---	52	6.8	0.1	0.1
5601	Car+	galactose	58	6.7	38	2.5
5601	Car+	gal-6-P	58	5.5	38	2.5
5202F	Car$^-$	---	0.32	3.0	0.1	0.1
5202F	Car$^-$	galactose	0.32	2.7	0.1	0.1
5202F	Car$^-$	gal-6-P	0.27	1.6	7.5	1.8

*All cells were grown in 1% Bactopeptone medium; galactose was added to a final concentration of 1%, and galactose-6-P to 0.02 M where indicated.

gave 5-10% of the TMG-P formed by the homologous system.

However, further investigation of the S. aureus system revealed another more important feature. Assay of S. aureus PTS with TMG or mannitol (or with several other sugars) showed that an additional factor was required for significant phosphorylation (Table 1). This factor, designated III, has not yet been detected in the E. coli system. Factor III^{gal}_{Sa} exhibited the following properties: (a)it was stable to heating at 100° for 3 min; (b)it was non-dialyzable and excluded by Bio-gel P-60 (exclusion limit approximately 65,000); (c)the factor was water soluble and retained full activity after extraction of a lyophylized preparation with chloroform-methanol (2:1); (d)it was inactivated upon treatment with crystalline papain or pepsin; (e)it exhibited sugar-specificity and was induced by growth of the cells on the specific sugar. Two factors III have been isolated, one from galactose-induced and the other from mannitol-induced cells, and they

showed negligible cross-reactivity (Table 2). These results therefore suggest that factors III are inducible proteins which are required for phosphorylation of the inducing sugars or their analogues.

Three types of studies were performed to investigate the function of III_{Sa}^{gal} : (1)Conceivably, the crude S. aureus PTS contained a TMG-6-P phosphatase, III was a phosphatase inhibitor, and TMG phosphorylation would only be detected in the presence of III. However, TMG-6-P was not hydrolzyed by the intact PTS or its components in the presence or absence of III or of PEP. (2)In studies on the rate and extent of transfer of ^{32}P from ^{32}P-PEP to HPr as catalyzed by Enzyme I, factor III did not influence the reaction. (3)In recent studies by Hanson and Anderson (1968), a fourth component was detected in Aerobacter aerogenes PTS.* This component was termed K_m component for fructose phosphorylation since it caused a large reduction in the K_m for fructose. In the present studies, the S. aureus PTS was studied with TMG over the concentration range 2.9×10^{-3} to 2.1×10^{-1} M. TMG phosphorylation was not detected in the absence of III_{Sa}^{gal}. The role of III is therefore not clear, and further studies will be required to determine whether the factors from S. aureus and A. aerogenes function in the same, or in a different manner.

As shown in Table 3, the defect in the car$^-$ mutant is the lack of Enzyme I. Induced wild type cells contained the four components of the PTS, while uninduced wild type cells lacked II_{Sa}^{gal} and III_{Sa}^{gal}. Morse, et al (1968) have recently reported that the inducer of the lac system in S. aureus is galactose-6-P, and not galactose. This report is confirmed by the present experiments. That is, the car$^-$ mutant is unable to utilize or phosphorylate galactose, and this sugar did not induce II_{Sa}^{gal} or III_{Sa}^{gal} in mutant. Galactose-6-P, on the other hand, did induce these proteins in the mutant, although, as expected, it did not affect the level of Enzyme I. These results suggest that III_{Sa}^{gal}, as well as II_{Sa}^{gal} and β-galactosidase, is under the control of the Staphylococcal lac operon.

*Private communication.

DISCUSSION

Egan and Morse (1965) showed that a car⁻ mutant of S. aureus was unable to utilize 8 carbohydrates as the result of a single mutation, and that the defect was in sugar transport. Furthermore, the wild type cells accumulated sugars either solely or primarily as derivatives which have recently been characterized as the corresponding phosphate esters (Hengstenberg, et al, 1967, 1968; Laue and MacDonald, 1968).

The present studies demonstrate a PTS in S. aureus analogous to that in gram-negative bacteria. The defect in the car⁻ mutant is the lack of Enzyme I, and this mutant is therefore analogous to the car⁻ mutants recently obtained from gram-negative organisms. These findings lend further support to the conclusion that sugar transport in bacteria is mediated by the phosphotransferase system.

In addition, two new protein factors (III) were isolated from induced S. aureus cells, each of which was specific and required for the phosphorylation of TMG and mannitol respectively. The precise role of this factor is not known, although it does not appear to affect the first reaction in the sequence. Further studies will be required to determine whether III is an intermediate in phosphate transfer, affects the binding of sugar or P-HPr to Enzyme II, or serves in another capacity.

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