Phosphoenolpyruvate-Dependent Phosphotransferase System of *Staphylococcus aureus*: Factor III^{lac}, a Trimeric Phospho-Carrier Protein That Also Acts as a Phase Transfer Catalyst†

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ABSTRACT: Factor III^{lac} (FIII) consists of three identical subunits. It could be shown that each of the subunits carries a phosphoryl group upon phosphorylation (P-FIII) with phosphoenolpyruvate (PEP), enzyme I, and histidine-containing phospho-carrier protein (HPr). The phosphoryl group is bound to a histidyl residue in P-FIII. Each subunit of FIII contains four histidyl residues. After tryptic cleavage a peptide was isolated that contained one other histidyl residue besides the active center histidine. By further cleavage of the peptide T-2 with V-8 *Staphylococcus aureus* protease it could be shown that His-19 in the sequence of the peptide T-2 is the active center histidine. Another peptide (1–38), caused by incomplete tryptic cleavage, could be isolated. It inhibited the phospho-transfer reaction from PEP to the sugar molecule at the step of factor III–enzyme II recognition. It competes with factor III for the binding site of enzyme II, the membrane component. It is a very hydrophobic peptide. This hydrophobic region is buried in factor III. But upon phosphorylation of factor III it is turned out. Thus P-FIII binds to Triton X-100 micelles whereas factor III does not. This conformational change caused by phosphorylation could be shown by proton nuclear magnetic resonance methods [Kalbitzer, H. R., Deutscher, J., Hengstenberg, W., & Rösch, P. (1981) *Biochemistry 20*, 6178–6185], by circular dichroism spectroscopy, and by the Ouchterlony double-diffusion method. Antibodies against FIII do not precipitate P-FIII.

The phosphoenolpyruvate-dependent phosphotransferase system (PTS)[1] was first discovered by Roseman and coworkers for *N*-acetyl-D-mannosamine transport in *Escherichia coli* in 1964 (Kundig et al., 1964). A few years later the PTS for lactose uptake in *Staphylococcus aureus* was described (Hengstenberg et al., 1967; Simoni et al., 1973a,b; Simoni & Roseman, 1973). It consists of four enzymes that catalyze the following reactions:

$$\text{PEP} + \text{enzyme I} \overset{Mg^{2+}}{\rightleftharpoons} \text{P-enzyme I} + \text{pyruvate} \quad (1)$$

$$\text{P-enzyme I} + \text{HPr} \rightleftharpoons \text{P-HPr} + \text{enzyme I} \quad (2)$$

$$\text{P-HPr} + \text{factor III} \rightleftharpoons \text{P-factor III} + \text{HPr} \quad (3)$$

$$\text{P-factor III} + \text{galactoside} \xrightarrow[\text{enzyme II}]{Mg^{2+}} \text{P-galactoside} + \text{factor III} \quad (4)$$

All four components have been purified or partially purified. Enzyme I has a molecular weight of 80000 (Stein et al., 1974). HPr has been intensively studied by NMR methods because of its low molecular weight of 7685 (Beyreuther et al., 1977; Gassner et al., 1977; Schmidt-Aderjan et al., 1979; Rösch et al., 1981). Enzyme II has been found to have a molecular weight of 50000 on $NaDodSO_4$–polyacrylamide gels (Schäfer et al., 1981) but may be more complex in its native membrane-bound form.

Factor III^{lac} consists of subunits, and each subunit can be phosphorylated with the phosphoryl group bound to the N-3 atom of a single histidyl residue (Hays et al., 1973). These findings have been confirmed by ^{1}H NMR investigations (Kalbitzer et al., 1981). NMR measurements also revealed strong conformational changes of factor III upon phosphorylation. It has previously been shown that phosphorylation leads to a destabilization of the trimeric structure of factor III and that phosphorylated factor III in contact with the cell membrane dissociates into its subunits (Hengstenberg, 1977).

In this paper, we demonstrate two domains of factor III. One includes the active center histidine. The other is a hydrophobic region that binds enzyme II. This hydrophobic region of the protein becomes exposed to the protein surface upon phosphorylation.

Materials and Methods

Enzyme I. Enzyme I was purified according to Deutscher (1979).

HPr Protein. HPr was purified according to Beyreuther et al. (1977).

Factor III. FIII was isolated from strain S 305 A, which is constitutive for lactose transport. Cells were grown in a 100-L Chemap fermenter to OD_{578} = 8–10. Cells (1000 g) were broken in a Dynomill (Fa. Bachofen). The crude extract was centrifuged 2 times for 2 h at 3000*g*. The supernatant was applied to a DEAE-cellulose column (DE-23, 12 × 30 cm), eluted with a gradient of 0–0.6 M NaCl (10 L) in standard buffer (0.05 M Tris-HCl, 10^{-4} M DTT, 10^{-4} M PMSF, and 10^{-4} M EDTA). The FIII-containing fractions were pooled, and FIII was precipitated with ammonium sulfate at 60% saturation. After centrifugation the pellet was dissolved in standard buffer and applied to a Sephadex G-100 column (9 × 110 cm). After a second precipitation with ammonium sulfate (55% saturation) and dissolution of the pellet in standard buffer, the FIII-containing solution was brought to pH 5.4 with acetic acid. FIII precipitated at this pH, and after centrifugation the pellet was dissolved again in standard buffer. FIII was chromatographed on a second DEAE-cellulose

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[1] Abbreviations: PTS, phosphoenolpyruvate-dependent phosphotransferase system; PEP, phosphoenolpyruvate; HPr, histidine-containing phospho-carrier protein; FIII, factor III^{lac}; P-FIII, phosphorylated factor III^{lac}; NMR, nuclear magnetic resonance; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; *o*-NPG, *o*-nitrophenyl β-D-galactopyranoside; $NaDodSO_4$, sodium dodecyl sulfate; DDTBB, dimethyl dithiobis(butyrimidate); DEAE, diethylaminoethyl.

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Table I: Purification Scheme of Factor III[lac]

	mg of protein	sp act. [μmol min^{-1} (mg of protein)$^{-1}$]	purification factor	yield (%)
crude extract	116 400	0.021		
ion-exchange chromatography (DE-23)	13 700	0.155	7.4	86
ammonium sulfate precipitation (60%)	11 200	0.183	1.2	82
gel filtration (Sephadex G-100)	1 150	0.792	4.3	37
ammonium sulfate precipitation (55%)	490	1.33	1.7	26
acid precipitation (pH 5.4)	190	3.3	2.4	25
ion-exchange chromatography (DE-52)	140	3.7	1.1	21

column (DE-52, 1.6 × 27 cm) and was eluted with a gradient of 0–0.5 M NaCl (500 mL) in standard buffer. The FIII-containing fractions were concentrated by pressure dialysis (Amicon, UM-2 membrane) and then desalted on a Sephadex G-25 column eluted with 0.05 M NH_4HCO_3. After the fractions were freeze-dried, 100–140 mg of electrophoretically pure FIII was obtained (Table I).

In order to assay FIII, we used a mutant complementation assay with strain S 714 G, which lacks active FIII. The solution had the following composition: 2×10^{-2} M $MgCl_2$, 10^{-2} M PEP, 0.4×10^{-2} M ONPG, 0.4×10^{-2} M DTT, and 50 μL of crude extract S 714 G. The final volume was 0.5 mL. In some cases an artificial system with the purified PTS components was used. Then the assay solution, again 0.5 mL, contained the following components: 2×10^{-2} M $MgCl_2$, 10^{-2} M PEP, 0.4×10^{-2} M ONPG, 0.4×10^{-2} M DTT, 10 μg of 6-P-galactosidase, 10 μg of enzyme I (after ammonium sulfate precipitation), 5 mg of membrane fraction, and 10 μg of HPr. In both cases when FIII is added, ONPG is phosphorylated to ONPG-6-P, which is cleaved by 6-P-galactosidase to galactose 6-phosphate and *o*-nitrophenol, the production of which is measured at 405 nm.

Phosphorylated Factor III. To obtain P-FIII, we used the following reaction mixture: 10 mg of FIII, 5 mg of HPr, 2 mg of enzyme I (after ammonium sulfate precipitation), 5×10^{-2} M PEP, 10^{-2} M Mg^{2+}, and 2×10^{-2} M DTT. NH_4HCO_3 (0.05 M), pH 8.2, was added to give a final volume of 0.5 mL. When radioactively labeled ^{32}P-FIII was synthesized, 50–100 μCi of ^{32}PEP was also added. After incubation for 10 min at 37 °C P-FIII was separated from P-enzyme I and P-HPr on a Sephadex G-75 column (1.6 × 50 cm). P-FIII was then concentrated by pressure dialysis to a final volume of 0.5–1 mL (Amicon, UM-2 membrane) for further experiments. P-FIII in this concentrated form has a half-life of 2 days at 4 °C but is much more stable when frozen at −18 °C.

Enzyme II. Enzyme II was purified according to Schäfer et al. (1981).

Tryptic Fragments. Tryptic fragments of FIII or ^{32}P-FIII were prepared according to Kalbitzer et al. (1981).

^{32}P-Labeled Phosphoenolpyrwate [^{32}P]PEP. [^{32}P]PEP was synthesized and prepared according to Lauppe et al. (1972). As [^{32}P]PEP decomposes slowly, the specific activity related to [^{32}P]PEP had to be estimated before each quantitative experiment. We used (3-nitro-4-aminophenyl)ethyl β-D-thiogalactopyranoside (A_{420} = 5.4 × 10^6 mol^{-1}·cm^2), synthesized by P. Schaedel (unpublished data). This sugar was also phosphorylated by the *S. aureus* lactose PTS. In a parallel assay containing 1 mg of FIII, 0.5 mg of HPr, 0.2 mg of enzyme I, 50 μL of membrane fraction (0.1 g/mL), Mg^{2+}, PEP, DTT, and [^{32}P]PEP, the above sugar was phosphorylated to the 6-^{32}P compound. The phosphorylated sugar was separated from other radioactive compounds and from the unphosphorylated sugar by paper electrophoresis and was then eluted. Via its extinction at 420 nm the specific activity due to [^{32}P]PEP could be evaluated.

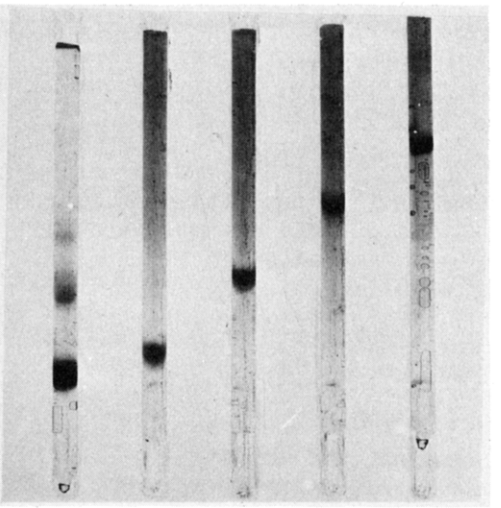

FIGURE 1: $NaDodSO_4$ gels of cross-linking experiments. Gels from left to right: 100 μg of FIII after cross-linking as described under Materials and Methods, 5 μg of cytochrome *c*, 5 μg of chymotrypsinogen, 5 μg of ovalbumin, and 5 μg of bovine serum albumin. The anode was at the bottom of the figure.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gels contained 7.5% acrylamide and 0.4 M Tris–glycine, pH 9.3. The gels were stained with Coomassie Brilliant Blue. Triton gels also contained 0.1% Triton X-100; urea gels, 8 M urea. $NaDodSO_4$ gels contained 7.5% acrylamide and 0.1% $NaDodSO_4$.

Charge-Shift Electrophoresis. Charge-shift electrophoresis was performed according to Helenius & Simons (1977).

Cross-Linking. The cross-linking reaction was carried out in 200 μL of triethanolamine, pH 8.1. The reaction mixture contained 100 μg of FIII and 1.5 mg of dimethyl dithiobis-(butyrimidate) (DDTBB). After a 15-min incubation at 37 °C an additional 0.75 mg of DDTBB was added, and the reaction mixture was incubated again for 15 min.

Fingerprint Technique. Electrophoresis and chromatography were performed as described by Beyreuther et al. (1977) with the difference that 0.05 M NH_4HCO_3, pH 8.6, was used as the electrophoresis buffer instead of pyridine/acetate, pH 6.5. Orange G was used as a tracking dye. The peptides were detected after spraying with 0.025% Fluram (Roche, Basel, Switzerland) in acetone. Peptides containing histidyl residues were visualized with Pauly reagent (Pauly, 1915).

Results

Subunit Structure of FIII. To determine the subunit structure of FIII, we carried out cross-linking experiments. After being cross-linked with dimethyl dithiobis(butyrimidate), two additional bands appeared on $NaDodSO_4$–polyacrylamide gels with a molecular weight of 24 000 and 35 000, respectively, beside the band of FIII protomers with a molecular weight of 12 000 (Figure 1). When mercaptoethanol was added to a final concentration of 10^{-3} M before applying to $NaDodSO_4$ gels, these two additional bands disappeared. Thus FIII

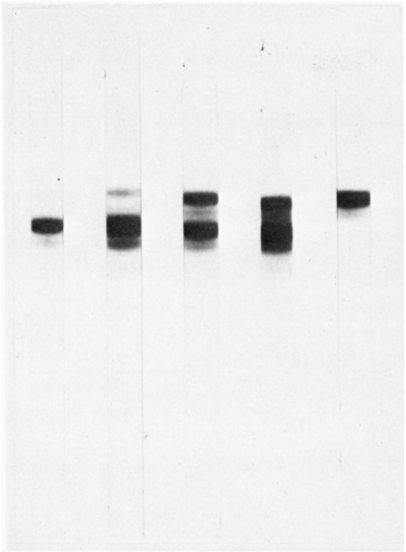

FIGURE 2: Urea gels. Gels from left to right: 10 μg of P-FIII, 20 μg of P-FIII, 10 μg of P-FIII + 5 μg of FIII, 20 μg of P-FIII + 10 μg of FIII, and 10 μg of FIII. The anode was at the bottom of the figure.

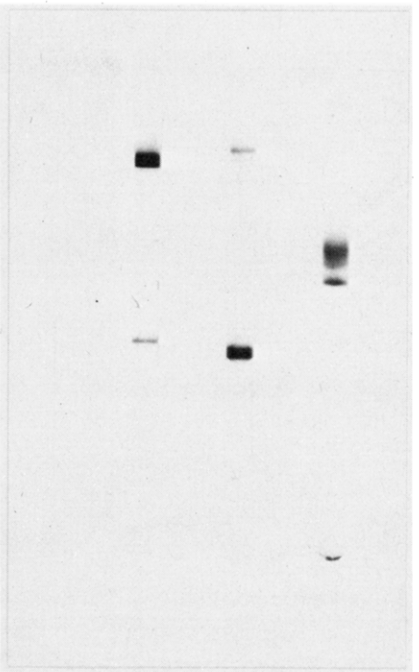

FIGURE 3: Triton gels. Gels from left to right: 20 μg of P-FIII and 20 μg of FIII (these gels were run for 1.5 h) and 20 μg of P-FIII (this gel was run for 4 h). The anode was at the bottom of the figure.

consists of three identical subunits with a molecular weight of 12000. According to Hays et al. (1973) two or three of the subunits are phosphorylated. For proof of this, FIII has been phosphorylated with enzyme I, HPr, and ^{32}PEP, whose specific activity had been determined as described under Materials and Methods. In three different experiments FIII was phosphorylated 2.6-, 2.9-, and 2.9-fold, suggesting a molecular weight of 35000 for FIII.

Separation of FIII and P-FIII. FIII and P-FIII cannot be separated either by ion-exchange chromatography or by polyacrylamide gel electrophoresis. But adding 8 M urea to the polyacrylamide gels allowed a good separation of FIII and P-FIII (Figure 2). Under these denaturing conditions the phosphorylated and unphosphorylated FIII protomers show a different migration behavior during electrophoresis. An even better separation of FIII and P-FIII could be obtained by adding 0.1% Triton X-100 to the polyacrylamide gels. FIII migrates as in normal polyacrylamide gels, but P-FIII is retarded, suggesting that P-FIII binds to Triton X-100 micelles whereas FIII does not (Figure 3). Running the gels for a longer period revealed that the slowly migrating P-FIII band is not homogeneous but consists of at least three bands. These multiple bands may be caused by Triton X-100 itself.

Figures 2 and 3 show the urea gels and Triton gels, respectively, of 2.9-fold phosphorylated FIII. It is obvious that under these conditions all subunits of FIII are phosphorylated. This means that in the phosphorylated state each subunit of FIII carries one phosphoryl group, and this provides additional evidence that FIII consists of three identical subunits.

"Active Center" Histidine. From ^{1}H NMR investigations (Kalbitzer et al., 1981) and from alkaline hydrolysis (Hays et al., 1973) FIII is known to bind the phosphoryl group to the N-3 position of a single histidyl residue. According to amino acid analysis FIII contains four histidyl residues. Tryptic cleavage of ^{32}P-FIII and separation of the tryptic fragments by gel filtration on Sephadex G-50 revealed two radioactivity-containing peaks. One belongs to inorganic phosphate that had been cleaved from ^{32}P-FIII during tryptic digest. The other belongs to the active center histidine. The peak shows also absorption at 280 nm, emphasizing that the active center peptide, T-2, contains at least one tyrosyl residue. This agrees well with the ^{1}H NMR investigations of this peptide (Kalbitzer et al., 1981) where we found the signals of one tyrosyl and two histidyl residues in the low-field part of the spectrum of T-2, and it agrees well with the amino acid composition of the peptide -Glu-Ala-Gln-Gly-Asp-Asp-Ile-Ala-Tyr-Ser-Val-Thr-Met-Met-His-Gly-Gln-Asp-His-Leu-Met-Thr-Thr-Ile-Leu-Leu-Lys-.

The phosphorylated active center peptide, P-T-2, has been further purified by ion-exchange chromatography on DEAE-cellulose. By this purification step it has also been separated from unphosphorylated T-2 produced during the cleavage and purification procedure. ^{1}H NMR investigations of the unphosphorylated and phosphorylated active center peptides revealed that one of the histidyl residues (designated His-II) carries the phosphoryl group attached to the N-3 position of the imidazole ring (Kalbitzer et al., 1981). But as this peptide still contains another histidyl residue, a further cleavage with V-8 *Staphylococcus aureus* proteinase was carried out. The digest was separated by fingerprint techniques and gave just one radioactive spot but two spots containing histidyl residue according to the Pauly assay (Figure 4A).

The fingerprint of the unphosphorylated peptide T-2 after cleavage with V-8 protease differed from that of the phosphorylated peptide in the migration behavior of just one spot (Figure 4B). This is the histidine- and radioactivity-containing spot. Both histidine-containing spots were isolated from the thin-layer plate and extracted from cellulose according to Chen (1976). The following amino acid analysis revealed significant differences of both peptides. The peptide carrying the phosphoryl group (V-2) contained one lysine and three leucines that were absent in the other histidine peptide (V-1). On the contrary, the peptide V-1 contained one valine, one serine, and one tyrosine that were absent in the V-2 peptide. Thus it is clear that the histidine in position 19 of the tryptic active center peptide carries the phosphoryl group.

Inhibitory Peptide. After tryptic cleavage another functional peptide T-X could be isolated. This peptide migrated faster than the active center peptide on Sephadex G-50. After further purification on DEAE-cellulose it could be shown that it contains the first 38 amino acids of the N terminus:

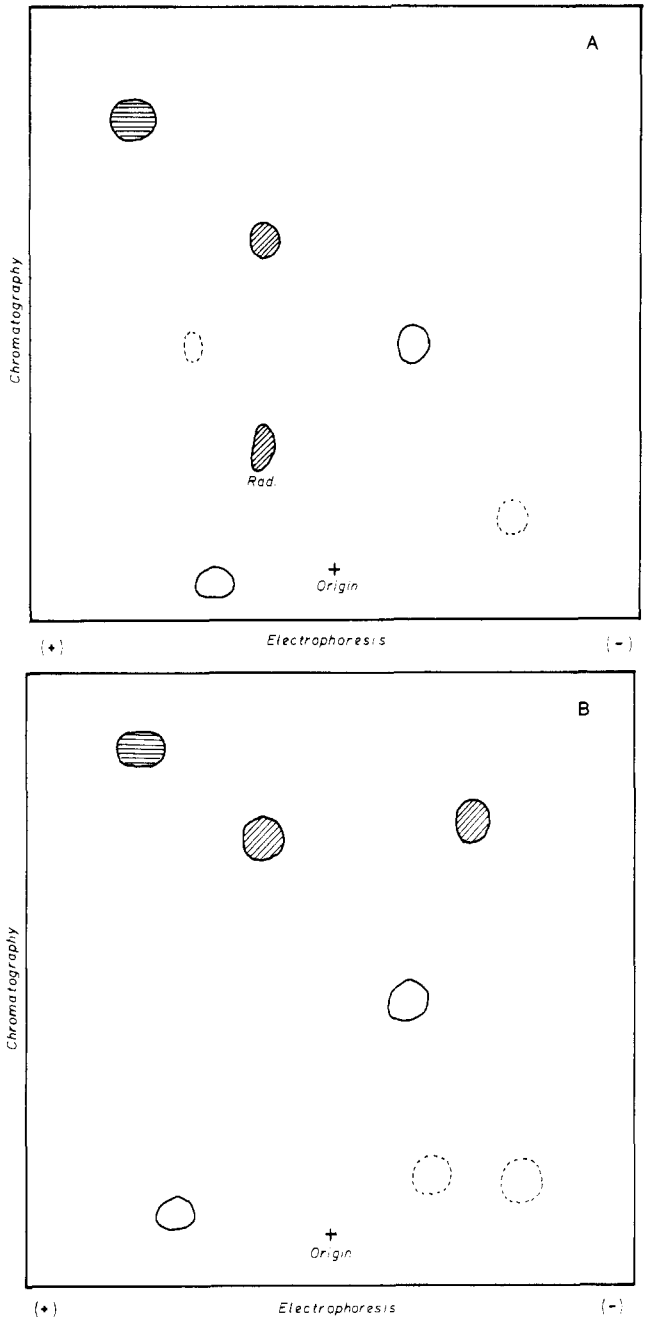

FIGURE 4: Fingerprints of (A) phosphorylated peptide T-1 (^{32}P-T-1) and (B) unphosphorylated peptide T-1 after cleavage with V-8 *Staphylococcus aureus* protease: (spots with horizontal hatching) orange G; (spots with diagonal hatching) histidine-containing spots.

Met-Asn-Arg-Glu-Glu-Val-Gln-Leu-Leu-Gly-Phe-Glu-Ile-Val-Ala-Phe-Ala-Gly-Asp-Ala-Arg-Ser-Lys-Phe-Leu-Glu-Ala-Leu-Thr-Ala-Ala-Gln-Ala-Gly-Asp-Phe-Ala-Lys-. This peptide was produced in small amounts (0.5 mg/20 mg of FIII) and was caused by incomplete tryptic cleavage. It may represent a domain structure. The sequencing of this peptide revealed that it is a mixture of two tryptic fragments. The larger part contains the above sequence; from the smaller part the first three amino acids are cleaved off, and it begins with Glu-Glu-Val.

An interesting finding was that this peptide, T-X, was able to inhibit the assay for FIII, which means PTS-dependent phosphorylation of ONPG. If to the artificial assay system, as described under Materials and Methods, were added 10 μg of FIII and 10 μg of this peptide, no phosphorylation of ONPG could be observed. This inhibition could be overcome by

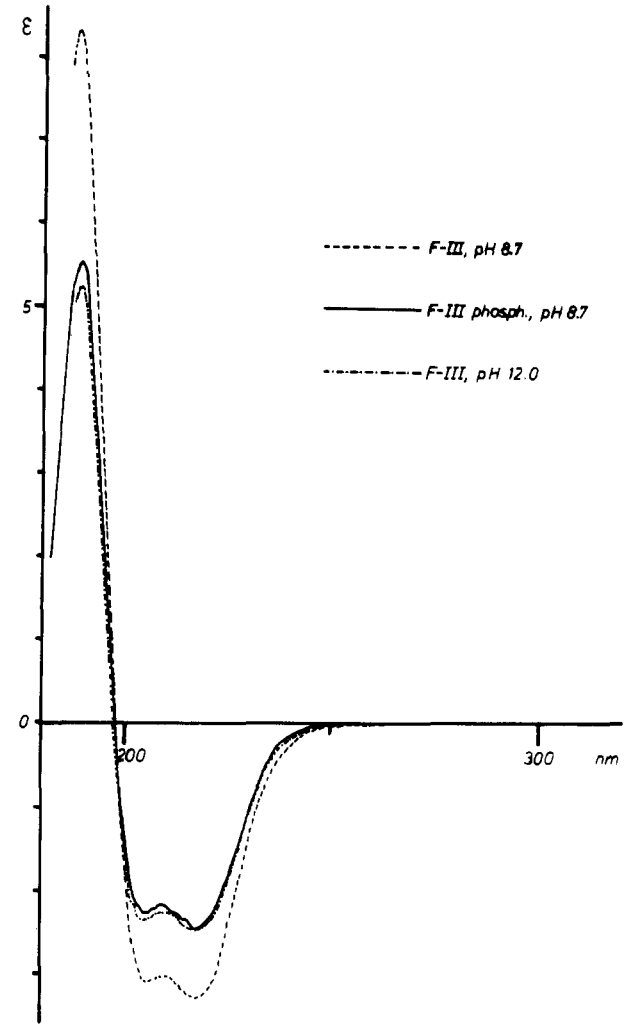

FIGURE 5: CD spectrum of FIII, pH 8.7 (broken line), of P-FIII, pH 8.7 (solid line), and of FIII, pH 12.0 (dotted line). All curves are evaluated for a protein concentration of 1 mg/mL.

adding an excess of FIII or enzyme II (membrane fragments) but not by an excess of enzyme I or HPr (Table II). These results can be interpreted that the tryptic peptide T-X represents the binding region of FIII to enzyme II and that it competes with FIII for enzyme II in the reaction mixture. Then an excess of either FIII or enzyme II could abolish the inhibition whereas enzyme I and HPr have no effect.

Another interesting result was that the peptide T-X is very hydrophobic. Besides three phenylalanyl residues, it contains many alanyl, leucyl, and valyl residues. So we assume that this is also the region that is buried in FIII but is turned to the surface upon phosphorylation and causes the hydrophobic behavior of P-FIII.

Structural Changes upon Phosphorylation of FIII. Structural changes upon phosphorylation have been intensively studied by ^{1}H NMR methods (Kalbitzer et al., 1981). Another evidence was that the α helix content of FIII is reduced from 42% to 25% in P-FIII as measured by CD spectroscopy (Figure 5). This is a drastic change of secondary structure, which could also be induced by bringing the FIII solution to pH 12. This is in agreement with the NMR data, where we also found similar changes of the FIII spectrum at pH 12 as caused by phosphorylation.

Further evidence came from Ouchterlony double-diffusion tests. Antibodies against FIII do not precipitate P-FIII (Figure 6). This means that the surface of FIII is largely altered upon phosphorylation and supports the results with Triton gels where

Table II

(A) Inhibition of PEP-Dependent ONPG Phosphorylation by Peptide T-X and Its Abolition by an Excess of FIII or Enzyme II[a]

addition	10 μg of FIII	10 μg of FIII, 10 μg of T-X	10 μg of FIII, 10 μg of T-X, 20 μg of EI	10 μg of FIII, 10 μg of T-X, 50 μg of HPr	60 μg of FIII, 10 μg of T-X	10 μg of FIII, 10 μg of T-X, 5 mg of EII fraction
OD_{405}	1.05	0.06	0.05	0.06	0.85	0.71

(B) Dependence of Inhibition by Peptide T-X from the Amounts of FIII and Enzyme II, Respectively[b]

addition	10 μg of FIII	15 μg of FIII	20 μg of FIII	35 μg of FIII	60 μg of FIII
OD_{405}	0.05	0.17	0.32	0.58	0.83
addition	0.5 mg of EII fraction	1 mg of EII fraction	2.5 mg of EII fraction	5 mg of EII fraction	
OD_{405}	0.12	0.35	0.56	0.68	

[a] For all experiments the artificial reaction mixture as described under Materials and Methods was used. The incubation time was 10 min at 37 °C. [b] The reaction mixture had the same composition as above, with the exception that in all cases 10 μg of peptide T-X was added.

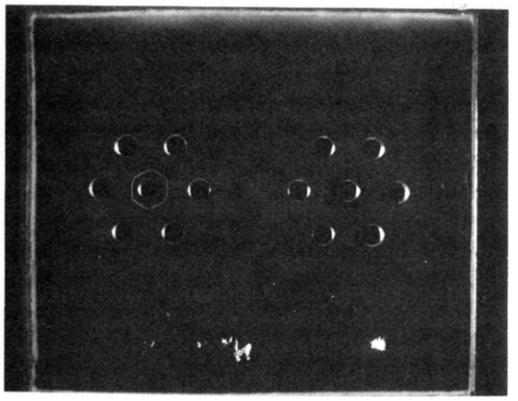

FIGURE 6: Ouchterlony double-diffusion tests on FIII and P-FIII. In both cases antiserum against FIII was placed in the central hole. On the left 10 μL of a solution containing 15 μg of FIII, 10 μg of HPr, 5 μg of enzyme I, and 10^{-4} M $MgCl_2$ was applied in the other holes. On the right the solution also contained 10^{-2} M PEP. Under these conditions P-FIII is built.

FIII changed from hydrophilic to hydrophobic behavior. This last change was further studied by gel filtration on Sephadex G-100 equilibrated with 0.1% Triton X-100 in 0.05 M NH_4HCO_3, pH 8.6. Whereas FIII did not alter its migration behavior in the presence of 0.1% Triton, ^{32}P-FIII migrated markedly faster (Figure 7). This shows again that P-FIII binds to Triton micelles whereas FIII does not. The different behavior of FIII and P-FIII against detergents was also studied by charge-shift electrophoresis. FIII and P-FIII migrate the same distance in the direction of the anode in the presence of Triton X-100. If 2% cetyltrimethylammonium bromide was added, FIII still migrates the same distance whereas P-FIII remains at the origin or migrates a small distance in the direction of the cathode (Figure 8). This shows that P-FIII is also able to bind to detergents other than Triton X-100. The middle slot of charge-shift electrophoresis shows a considerable amount of slow-moving factor III material that is not observed if Triton alone is used. We assume that cetyltrimethylammonium bromide led to slight denaturation of the protein, accompanied by detergent binding, which should produce a slow-moving component.

Discussion

Factor III represents one of the few proteins that consist of three identical subunits. Thus the catalytical part of aspartate transcarbamylase is built of three protomers (Warren

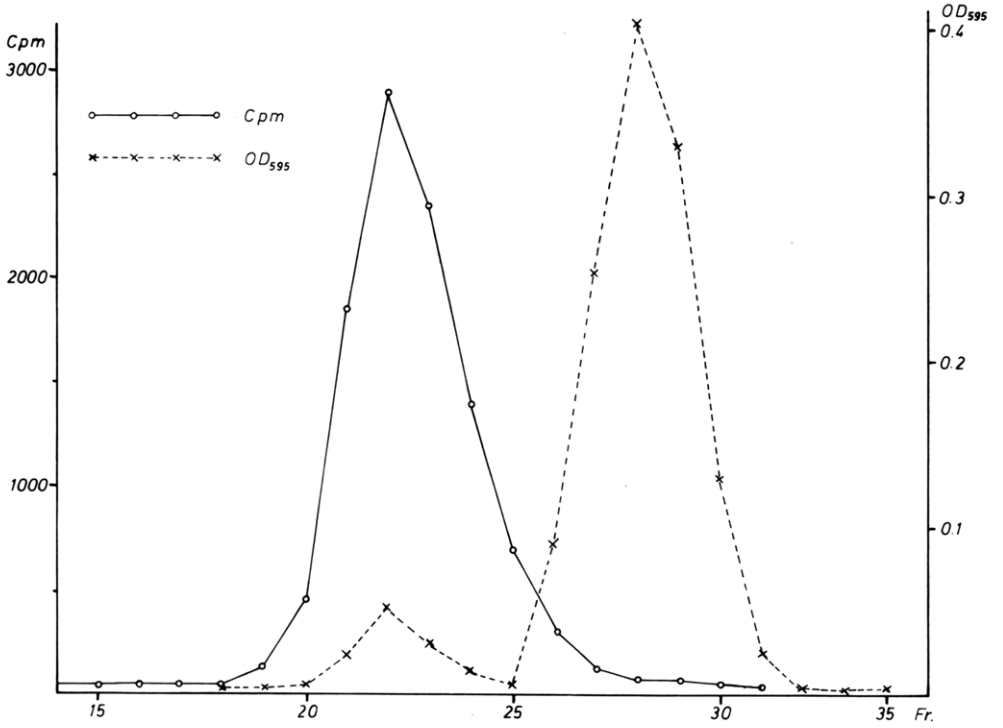

FIGURE 7: Chromatographic behavior of FIII and ^{32}P-FIII on a Sephadex G-100 column, equilibrated with 0.05 M NH_4HCO_3, pH 6.8, and 0.1% Triton X-100. Protein concentration (broken line) was determined according to Bradford (1976). ^{32}P-FIII was prepared as described under Materials and Methods.

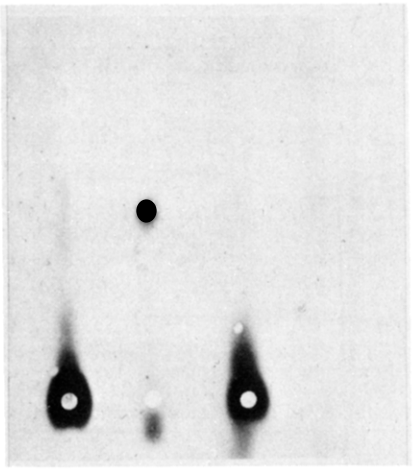

FIGURE 8: Charge-shift electrophoresis. Samples from left to right: 0.3 mg of P-FIII, 0.1 mg of FIII, and 0.3 mg of P-FIII in the presence of 2% cetyltrimethylammonium bromide. The anode was at the top of the figure.

et al., 1973). The separation of phosphorylated and unphosphorylated subunits on urea gels allowed also the observation that each of the subunits can carry one phosphoryl group upon phosphorylation with PEP, enzyme I, and HPr.

The phosphoryl group is bound to the N-3 position of His-19 of the active center peptide, T-2, which is produced by tryptic cleavage. Histidyl residues play a vital role during phosphotransfer reactions (Schneider, 1978). In P-enzyme I the phosphoryl group is also bound to the N-3 position of a histidyl residue (Stein et al., 1974), whereas in P-HPr the phosphoryl group is bound to the N-1 position of His-15 (Hengstenberg et al., 1976). The change of histidyl binding position during each transfer step from N-3 to N-1 and again to N-3 may be unimportant as 1- and 3-phosphohistidines have the same phosphorylation potential. But 3-phosphohistidine is kinetically more stable than 1-phosphohistidine (Schneider, 1978). For the phosphoryl group transfer to and from HPr an involvement of an arginyl residue has been demonstrated (Kalbitzer et al., 1982). This arginyl residue is two amino acids away from His-15 in the HPr protein from the different microorganisms thus far investigated. This is not the same for FIII, but the possibility cannot be excluded that through three-dimensional folding an arginyl or lysyl residue is brought to the neighborhood of the active center. X-ray crystallography would be necessary to prove this. Crystals of FIII were obtained from ammonium sulfate solutions, but they lacked the quality necessary for X-ray crystallography.

The peptide T-X represents a hydrophobic domain of FIII and it is responsible for the amphiphilic behavior of this protein. In FIII this domain is buried, and FIII is hydrophilic. Upon phosphorylation the hydrophobic domain is turned outward to the protein surface, and P-FIII is hydrophobic. It binds to Triton X-100 micelles and to membrane vesicles. Thus, FIII functions as a phase transfer catalyst between the phosphoryl group transfer reactions in the cytoplasm (enzyme I, HPr) and the membrane-located sugar uptake.

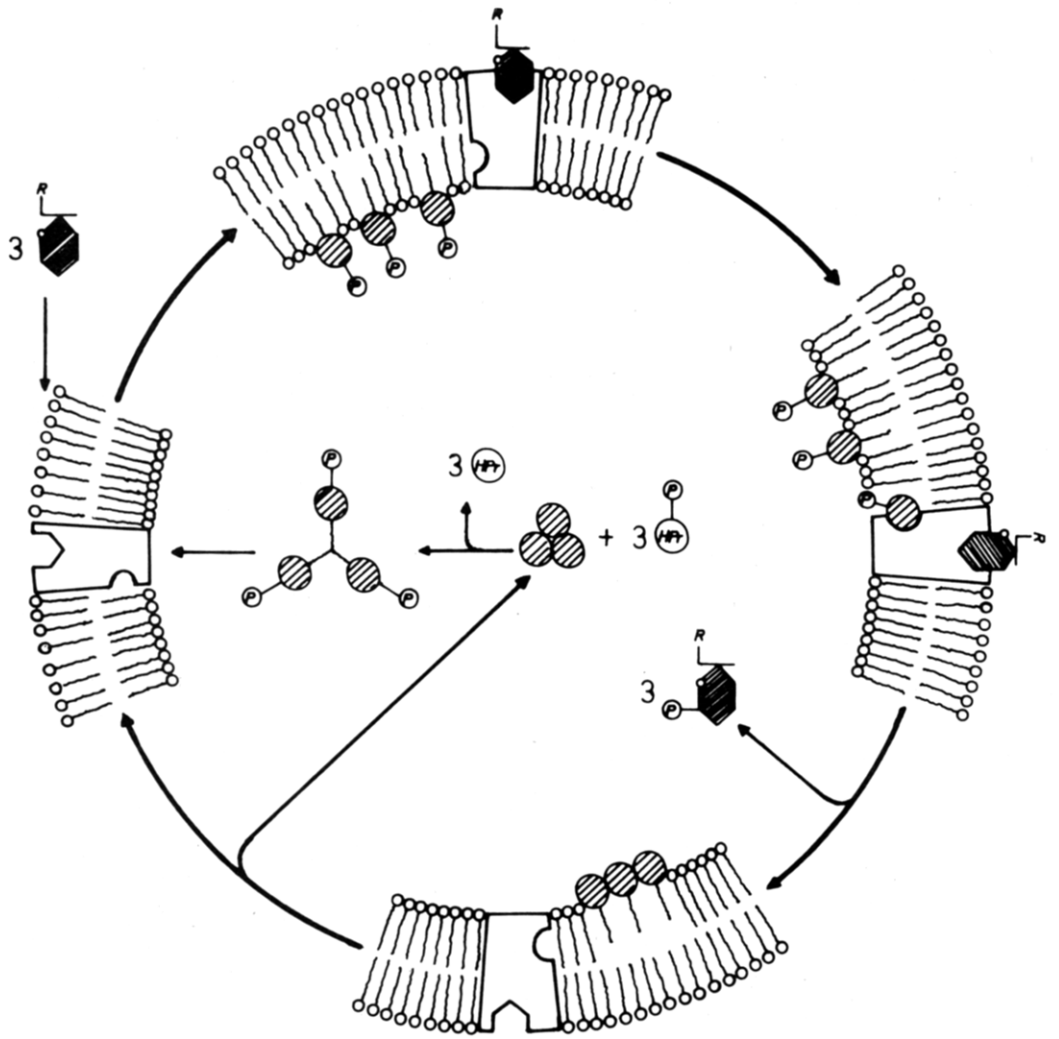

FIGURE 9: Tentative picture of function of FIII as a phase transfer catalyst.

The peptide T-X is also the binding site of FIII to enzyme II. Therefore, this peptide is able to inhibit ONPG phosphorylation in a competitive manner with respect to FIII or enzyme II. As it is known from earlier results that P-FIII in contact with the membrane dissociates to its subunits, we can give the following tentative picture of the function of FIII (Figure 9): Each of the three subunits of FIII is phosphorylated by P-HPr in the cytoplasm. This phosphorylation induces a structural change of FIII. A hydrophobic region represented by the peptide T-X is turned outward to the protein surface. Thus, P-FIII gains high affinity for the membrane. In contact with the membrane, P-FIII dissociates to its subunits. Subsequently, each of the subunits binds via the binding site T-X to an enzyme II molecule, which can then transport a sugar molecule from the cell exterior. The reactive ternary complex consists of P-FIII subunits, enzyme II, and sugar. The P-FIII subunit can then transfer its phosphoryl group to the sugar molecule, and an excess of energy is used for the uptake of the phosphorylated sugar. Three dephosphorylated FIII subunits may now aggregate in the membrane, become hydrophilic, and return to the cytoplasm. The cycle can then start again.

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