Pardee, J. D., & Spudich, J. A. (1982) *J. Cell Biol. 93*, 648–654.
Rich, S. A., & Estes, J. E. (1976) *J. Mol. Biol. 104*, 777–792.
Rouayrenc, J.-F., & Travers, F. (1981) *Eur. J. Biochem. 116*, 73–77.
Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Tobacman, L. S., & Korn, E. D. (1982) *J. Biol. Chem. 257*, 4166–4170.
Tseng, P. C.-H., & Pollard, T. D. (1982) *J. Cell Biol. 94*, 213–218.
Waechter, F., & Engel, J. (1975) *Eur. J. Biochem. 57*, 453–459.
Weeds, A. G. (1982) *Nature (London) 296*, 811–816.

Amino Acid Sequence of the Amphiphilic Phosphocarrier Protein Factor III^{Lac} of the Lactose-Specific Phosphotransferase System of *Staphylococcus aureus*†

K. Stüber,‡ J. Deutscher,*,§ H. M. Sobek,§ W. Hengstenberg,§ and K. Beyreuther‡

Institute of Genetics, University of Köln, D-5000 Köln, Federal Republic of Germany, and Department of Microbiology, Ruhr-Universität Bochum, D-4630 Bochum, Federal Republic of Germany
Received July 13, 1984

ABSTRACT: The lactose-specific factor III of the phosphotransferase system of *Staphylococcus aureus* is an amphiphilic trimeric protein composed of identical subunits. It is hydrophilic in its unphosphorylated state and can be isolated from the cytoplasmic protein fraction. It becomes a constituent of the membrane-bound phosphotransferase complex upon phosphorylation of a single histidyl residue. The sequence of *S. aureus* factor III^{Lac} was determined and revealed that the subunits consist of 103 residues corresponding to a M_r of 11 367 and of 34 101 for the native trimer: Met-Asn-Arg-Glu-Glu-Val-Gln-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-Ala-Asp-Ala-Leu-Ile-Glu-Glu-Gly-Asn-Asn-Cys-Ile-Ala-Gln-Ala-His-Arg-Ala-Gln-Thr-Ser-Leu-Leu-Ala-Lys-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-Asp-Leu-His-Lys-Lys-Leu-Leu-Glu-Phe-Tyr-Lys-Arg-Gly. According to this sequence and previous work histidine residue 82 located in the C-terminal part of the polypeptide chain is phosphorylated at the N-3 position by phosphoenolpyruvate, enzyme I, and histidine-containing phosphocarrier protein. The N-terminal part of the protein comprising approximately one-third of the chain exhibits in vitro affinity toward membrane-bound enzyme II^{Lac}.

The phosphotransferase system (PTS)[1] is the major active transport system for carbohydrates and is extensively studied at the biochemical level (Roseman et al., 1982). Recent genetic and biochemical studies show that this multienzyme system is also involved in regulation (Dills et al., 1980).

The lactose-specific PTS of *Staphylococcus aureus* consists of the inducible proteins factor III^{Lac} and enzyme II^{Lac} and the constitutively expressed proteins enzyme I and HPr. These four proteins interact as shown by the following reaction scheme:

$$\text{PEP} + \text{enzyme I} \xrightleftharpoons{Mg^{2+}} \text{P–enzyme I} + \text{pyruvate} \quad (1)$$

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

$$3\text{P–HPr} + \text{factor III}^{Lac} \rightleftharpoons \text{P}_3\text{–factor III}^{Lac} + \text{HPr} \quad (3)$$

$$\text{P}_3\text{–factor III}^{Lac} + 3\text{lactose} \xrightarrow[\text{enzyme II}^{Lac}]{Mg^{2+}} 3\text{lactose-6-P} + \text{factor III}^{Lac} \quad (4)$$

As already reported earlier, the structure of the factor III^{Lac} protein is of great interest since it catalyzes the phosphotransfer between the water phase of the cytoplasm (eq 1–3) and the lipid phase of the membrane (eq 4). We therefore designated the protein a phase-transfer catalyst (Deutscher et al., 1982). One of the unusual properties of the protein that supports this feature is to occur in the phosphorylated form in a conformation with increased hydrophobicity. The hydrophobic properties of this protein are conferred by the first 40 residues of the amino-terminal sequence. This region also carries the binding site for the factor III–enzyme II interaction. The active center peptide isolated earlier in the phosphorylated and nonphosphorylated forms is located on a separate functional domain. The elucidation of the primary structure of factor

† This research was supported by the Deutsche Forschungsgemeinschaft (SFB 74 and He 896/9-4).
‡ Institute of Genetics, University of Köln.
§ Department of Microbiology, Ruhr-Universität Bochum.

[1] Abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; DABITC, 4-(*N*,*N*-dimethylamino)-4′-isothiocyanatoazobenzene; PTS, phosphotransferase system; FIII^{Lac}, factor III specific for lactose; P-FIII^{Lac}, FIII^{Lac} phosphorylated at His-82; HPr, histidine-containing phosphocarrier protein; P-HPr, HPr phosphorylated at His-15; PTH, phenylthiohydantoin; HPLC, high-pressure/performance liquid chromatography; EDTA, ethylenediaminetetraacetic acid; PEP, phosphoenolpyruvate; Tris, tris(hydroxymethyl)-aminomethane.

0006-2960/85/0424-1164$01.50/0

III^{Lac}, presented in this paper, allows now the precise localization of these functional domains.

Materials and Methods

Purification of FIII^{Lac}. A large-scale procedure previously described by Deutscher et al. (1982) was used with slight alterations to remove minor impurities. The FIII^{Lac} protein solution from the DE-52 ion exchange column was concentrated by pressure dialysis to about 5 mL and subsequently chromatographed in 0.5-mL portions on a LKB TSK G-2000 SW column (7.5 × 600 mm) at a flow rate of 0.7 mL/min with Jasco HPLC equipment. The elution buffer contained 0.1 M sodium phosphate, pH 6.8, 0.1 M NaCl, and 0.5% NaN_3. Fractions containing FIII^{Lac} activity were pooled, desalted, and freeze-dried.

Amino Acid Analysis. For amino acid analysis, the samples (0.1–1 nmol) were hydrolyzed in vacuo in 6 N HCl (containing 1% phenol, v/v) at 110 °C for 24–96 h and analyzed on a Beckman Model 121 M or Durrum D 500 amino acid analyzer.

Fingerprinting Technique. Fingerprints were performed with 1–5 nmol of digested factor III^{Lac} on selected batches of cellulose thin-layer plates (Schleicher & Schüll, Dassel, FRG) showing minimal electroosmosis of neutral peptides at pH 6.5. First dimension was electrophoresis at pH 6.5 in the buffer system of Michel (1951). The plates were thoroughly dried after electrophoresis and then developed perpendicular to the first dimension for 8–16 h in the systems of Baglioni (1961) (pyridine/1-butanol/water, 35:35:30 v/v) or Waley & Watson (1953) (pyridine/1-butanol/acetic acid/water, 10:15:3:12 v/v/v/v; PBAW-system). Plates were stained with fluorescamine (0.01% fluorescamine and 1% pyridine in ethanol).

Carboxamidomethylation. Factor III^{Lac} (10 mg) dissolved in 1-mL reaction buffer (6 M guanidine hydrochloride, 2 mM EDTA, 0.5 M Tris-HCl, pH 8.2) was preincubated for 30 min at 50 °C. Dithiothreitol (50 μmol) was added, and the mixture was kept for 4 h at 50 °C. The solution was brought to 37 °C, 12.5 μCi (462.5 kBq) of iodo[1-^{14}C]acetamide was added, and the mixture was reacted for 15 min at 37 °C in the dark. After addition of cold iodoacetamide (100 μmol), the reaction was continued for another 20 min at 37 °C in the dark. The mixture was passed through a column of Sephadex G-50 superfine (2 × 83 cm), equilibrated, and developed with 0.1% ammonium hydrogen carbonate buffer, pH 8.2. The radioactive fractions containing carboxamidomethylated factor III^{Lac} were pooled and lyophilized.

Manual Sequencing. Peptides (1–10 nmol) isolated from fluorescamine-stained fingerprints according to Beyreuther (1977) were sequenced manually with the DABITC/phenyl isothiocyanate double-coupling method of Chang (1980). The thiohydantion derivatives were identified on thin-layer polyamide sheets (2.5 × 2.5 cm) as described by Yang (1979).

Automated Edman Degradation. Automated Edman degradation was carried out in an updated Beckman 890 B sequencer equipped with a Sequemat P-6 autoconverter (Beyreuther, 1977). The degradation was performed in the presence of 15 mg of polybrene. Polybrene (100 mg) was subjected to 10 cycles of Edman degradation in the presence of glycylglycine (2 μmol) and stored in 10% acetic acid (v/v) at 5 °C prior to use. The degradation program was achieved according to Hunkapiller & Hood (1980) and adapted to the updated Beckman 890 B sequencer. A hydrophilization step was added preceding thiazolinone extraction as described previously (Zaiss & Beyreuther, 1983).

PTH Identification. The identification of phenylthiohydantoin amino acids resulting from the Edman degradation was achieved by high-pressure liquid chromatography (HPLC). The PTH-amino acids were chromatographed on Zorbax CN columns (4.6 × 250 mm, Du Pont, Bad Nauheim, West Germany). The flow rate was 1 mL/min at ambient temperature or at 37 °C. The effluent was monitored at a wavelength of 269 nm. The amounts of PTH-amino acids injected varied from 250 to 10 pmol in a total volume of 5–10 μL of methanol. Buffer B was methanol/acetonitrile (17:3 v/v), and buffer A was sodium acetate (20 mM, pH 6.2) containing 1% acetonitrile (Hunkapiller & Hood, 1980). The program used linear gradients from 0 to 40% B in 6 min, from 40 to 60% B in 12 min, from 60 to 80% B in 4 min, and from 80 to 0% B in 4 min followed by reequilibration at 0% B for 10 min. Thin-layer chromatography was performed (Beyreuther, 1977) if more than 50 nmol of peptide was available for sequencing.

Peptide Separation. Peptide mapping and preparative separation of peptides was performed on Zorbax C8 reversed-phase columns (4.6 × 250 mm, Du Pont). The sample was dissolved in 10–50% acetic acid (v/v), and 0.05–0.15 mL was injected containing 1–100 nmol of digest. We employed linear gradients from 0 to 53% B in 80 min at 60 °C and at a flow rate of 1 mL/min. Solvent A was 25 mM ammonium acetate adjusted to pH 6.0 with acetic acid, and solvent B was 22 mM ammonium acetate buffer, pH 6.0, containing 60% acetonitile (v/v). The effluent was monitored at 210 nm, and peak fractions were collected as described by Stüber & Beyreuther (1981).

Proteolytic Cleavages. Proteolytic cleavage reactions were performed in 50 mM ammonium hydrogen carbonate, pH 8.2, at a protein concentration of 1 mg/mL. Prior to cleavage, the carboxamidomethylated protein was heat denatured at 100 °C for 10 min. After being cooled to 37 °C, the protease was added. Trypsin (TPCK-trypsin, Merck, Darmstadt, FRG) was used at a ratio of 1:50 (w/w, enzyme/substrate) at 37 °C for 16 h and stopped by adding formic acid to give pH 2.8. Digestions with *S. aureus* protease V8 (Miles, Frankfurt, Germany) were performed at ratios of 1:40 (w/w, enzyme/substrate) at 37 °C in the presence of 2 mM EDTA to inactivate the thermolytic activity present in the enzyme preparation. The reaction was terminated after 16 h by lyophilization. Thermolysin cleavage was performed at 22 °C in 50 mM NH_4HCO_3, pH 8.0, and 10 mM $CaCl_2$ and terminated after 16 h by lyophilization.

Secondary Structure Prediction. Computerized secondary structure prediction of factor III^{Lac} was performed according to Chou & Fasman (1974, 1977). The updated parameters of Argos et al. (1978) were used to write a program for secondary structure prediction. This program was written in PASCAL and run on a Zilog microcomputer system (MCZ 1/20) (Stüber, 1982).

Results

Sequence 1–55. Automated Edman degradation of [^{14}C]-carboxamidomethylated factor III^{Lac} gave unambiguous information of the sequence spanning residues 1–58 (Figure 1). Cysteine residue 49 was identified on the basis of radioactivity introduced by radioalkylation. The same result was obtained for four independent Edman degradations, which, however, did not all proceed beyond residue 50. Only two extended runs gave information on the sequence following residue 50. Sequence 1–55 includes five tryptic peptides, four of which were isolated from analytical fingerprints and sequenced manually (Figure 1). The tryptic peptide spanning residues 4–21 was not isolated in amounts sufficient for identification from the fingerprint. Out of the nine expected thermolytic peptides

Table I: Amino Acid Compositions of All V8 Peptides Covering the Sequence of Factor III^{Lac}

amino acid	V8.1	V8.2	V8.3	V8.4	V8.5	V8.6	V8.6a	V8.7
Asx	0.86 (1)	0.28 (0)	1.18 (1)	1.74 (2)	1.70 (2)	3.95 (4)	0.00 (0)	0.29 (0)
Thr	0.01 (0)	0.13 (0)	0.51 (0)	1.05 (1)	1.28 (1)	2.93 (3)	0.17 (0)	0.21 (0)
Ser	0.12 (0)	0.20 (0)	0.96 (1)	0.19 (0)	1.25 (1)	1.18 (1)	0.15 (0)	0.28 (0)
Glx	1.80 (2)	2.00 (2)	1.26 (1)	3.15 (3)	2.75 (3)	1.88 (2)	0.79 (1)	0.44 (0)
Pro	0.11 (0)	0.00 (0)	0.04 (0)	0.08 (0)	0.09 (0)	0.00 (0)	0.00 (0)	0.13 (0)
Cys	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	+ (1)	0.00 (0)	0.00 (0)	0.00 (0)
Gly	0.39 (0)	1.19 (1)	1.14 (1)	1.22 (1)	1.18 (1)	2.33 (2)	0.19 (0)	1.12 (1)
Ala	0.19 (0)	0.46 (0)	2.53 (3)	5.90 (6)	3.66 (4)	2.31 (2)	0.15 (0)	0.41 (0)
Val	0.00 (0)	0.92 (1)	0.59 (1)	0.06 (0)	0.05 (0)	0.99 (1)	0.00 (0)	0.11 (0)
Met	0.66 (1)	0.16 (0)	0.23 (0)	0.11 (0)	0.16 (0)	2.46 (3)	0.58 (0)	0.17 (0)
Ile	0.11 (0)	0.25 (0)	0.58 (1)	0.92 (1)	1.00 (1)	1.83 (2)	0.15 (0)	0.20 (0)
Leu	0.00 (0)	1.92 (2)	1.55 (2)	1.95 (2)	2.20 (2)	3.39 (3)	2.75 (3)	0.39 (0)
Tyr	0.03 (0)	0.08 (0)	0.12 (0)	0.11 (0)	0.18 (0)	1.05 (1)	0.00 (0)	1.00 (1)
Phe	0.04 (0)	1.00 (1)	1.38 (2)	0.97 (1)	0.25 (0)	0.00 (0)	0.00 (0)	0.69 (1)
His	0.02 (0)	0.06 (0)	0.33 (0)	0.04 (0)	0.84 (1)	2.31 (2)	0.81 (1)	0.10 (0)
Lys	0.11 (0)	0.11 (0)	1.04 (1)	1.09 (1)	1.02 (1)	1.33 (1)	0.91 (1)	0.87 (1)
Arg	0.54 (1)	0.00 (0)	0.57 (1)	0.00 (0)	0.91 (1)	0.07 (0)	0.13 (0)	0.80 (1)
no. of residues	5	7	15	18	19	27	6	5

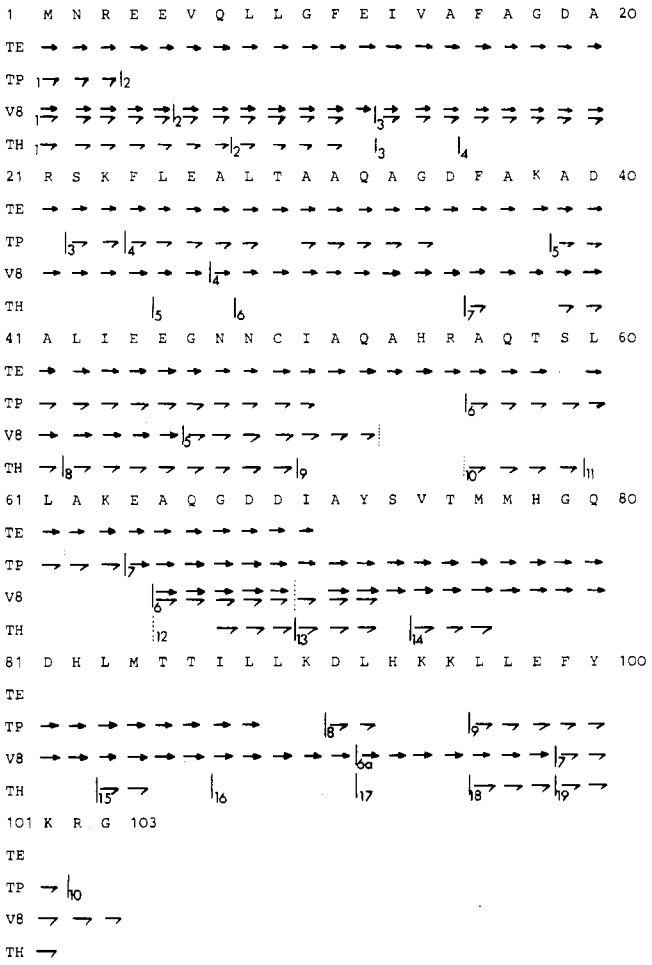

FIGURE 1: Amino acid sequence (one-letter code) of FIII^{Lac}. Amino acid residues were determined by automated sequence analysis (→) or by manual sequencing (DABITC method, half-arrow). TE means N-terminal sequence, TP means tryptic peptides, V8 means V8 proteolytic peptides, and TH means thermolytic peptides.

from sequence 1–55, only four were isolated from a fingerprint. They provide overlaps for TP.1 and TP.2 and V8 peptides V8.1, V8.2, V8.4, and V8.5. No further attempts were made to isolate the remaining tryptic and thermolytic peptides since the corresponding V8 protease peptides were isolated by reversed-phase HPLC and from fingerprints (Figure 2, Table I). The V8 peptide spanning residues 46–52 (Figure 1) results from cleavage at the partially deaminated glutamine residue 52. The corresponding V8 peptide going beyond residue 52

Table II: Amino Acid Composition of FIII^{Lac}

amino acid	quant	sequence data[a]	amino acid	quant	sequence data[a]
Asx	10.44	10	Met	3.67	4
Thr	5.27	5	Ile	4.40	5
Ser	3.67	3	Leu	11.20	13
Glx	15.34	14	Tyr	2.06	2
Pro	0.93	0	Phe	4.83	5
Cys	nd[b]	1	His	3.26	4
Gly	7.21	7	Lys	6.72	7
Ala	13.97	16	Arg	4.00	4
Val	3.40	3	Trp	0.00	0

[a] Total is 103. [b] nd, not determined.

was not isolated by reversed-phase HPLC but from fingerprints. The sequences determined for the V8 peptide are in full agreement with the sequence obtained by N-terminal automated Edman degradation of the protein (Figure 1).

Sequence 56–103. The C-terminal half of the factor III^{Lac} chain could contain a total of seven tryptic peptides (six lysyl and arginyl residues) on the basis of amino acid composition (Table II) and the knowledge of the sequence 1–55. We isolated four of these expected seven tryptic peptides (Figure 1). We found no free glycine from the C-terminus. Instead, a C-terminal dipeptide Arg-Gly was found having the same position in the fingerprint at pH 6.5 as found for Ser-Lys. A tryptic product corresponding to free lysine derived from position 95 was not found and is released if at all only in minute amounts. The four isolated tryptic peptides are due to the three residual lysyl residues of the C-terminal half of FIII^{Lac} (residues 56–103). The overlaps for the tryptic peptides were provided by the sequence information obtained from prolonged automated Edman degradations and by V8 peptides (Figure 1). The order of the tryptic peptides TP.5 and TP.6 is based on automated Edman degradations of the whole chain (Figure 1) and the amino acid composition of the intact V8 peptide spanning residues 46–64. The remaining overlaps are provided by the sequences of V8.6 for TP.7 and TP.8 and of V8.6a for TP.8, TP.9, and TP.10. The V8 peptides were isolated by reversed-phase HPLC with the exception of the V8 peptide spanning residues 46–64 (Table I). Peptides V8.6 and V8.6a were sequenced by automated Edman degradation and V8.7 was sequenced by manual Edman degradation (Figure 1). The sequence presented in Figure 1 does not include proline and tryptophan. The amino acid composition determined of several preparations of factor III^{Lac}, however, suggested the presence of approximately 1 molar equiv of proline (Table II). We have not been able to locate a proline

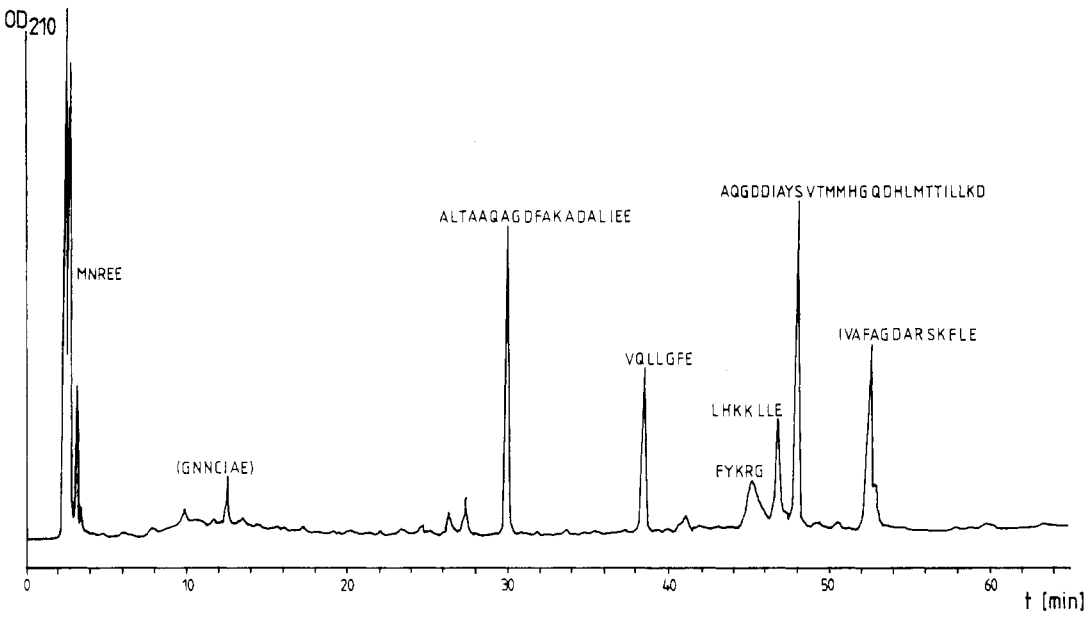

FIGURE 2: HPLC of V8 proteolytic digest of FIII^{Lac}. A total of 2.5 nmol of the digested protein was loaded on a Zorbax C8 reversed-phase column (4.6 × 250 nm) that was eluted with a linear gradient of 0–70% buffer B. Buffer A was 25 mM ammonium acetate, pH 6.0; buffer B was 60% acetonitrile in 25 mM ammonium acetate, pH 6.0. The flow rate was 1 mL/min at 60 °C. The effluent was monitored at 210 nm.

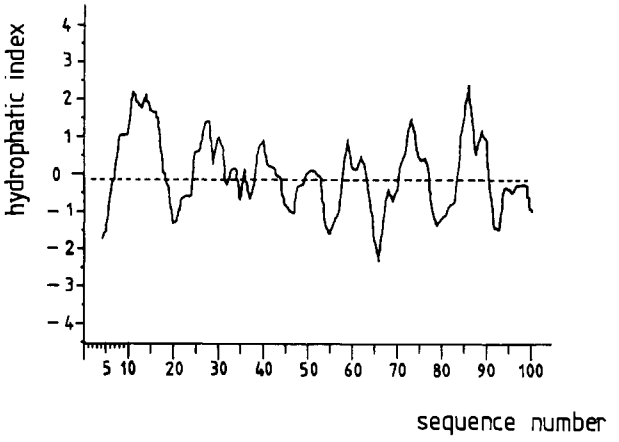

FIGURE 3: Hydropathy profile of FIII^{Lac} according to Kyte & Doolittle (1982).

residue in a molar equivalent in any of the isolated peptides. We have also not found proline in sequence 1–58 covered by automated Edman degradation of the whole chain. The remainder of the sequence, established by overlapping peptides and their sequences (residue 59–103), does also not contain a proline residue. Therefore, the proline found in the amino acid analysis of factor III^{Lac} is presumably not a constituent of the polypeptide.

Discussion

The sequence of factor III^{Lac} includes several clusters of hydrophobic residues (Figure 3). There is one pronounced hydrophobic cluster spanning residues 6–18, which, however, includes a single glutamic acid residue at position 12. Other less pronounced hydrophobic regions comprise residues 27–34, 70–77, and 83–89. All four histidine residues are outside these hydrophobic regions in a hydrophilic environment. The secondary structure prediction according to the rules of Chou & Fasman (Figure 4) shows seven regions with high probabilities for the formation of α-helices. The two proposed N-terminal helices correspond fairly to the two hydrophobic clusters (Figure 3). The single cysteine residue 49 and histidines-78 and -82, and presumably also histidine-93, are located on a

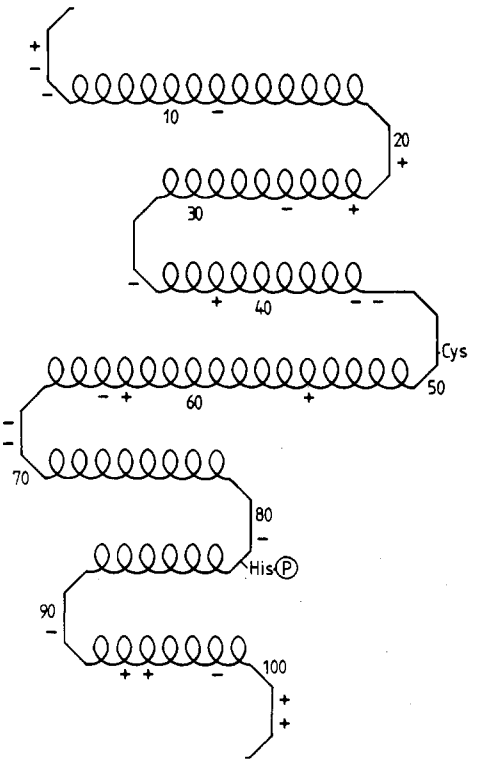

FIGURE 4: Secondary structure prediction for factor III^{Lac} according to the rules of Chou & Fasman (1974, 1977).

β-bend. The helical content determined by circular dichroism spectroscopy of the unphosphorylated trimer (about 50–53%) (Simoni et al., 1972; Deutscher et al., 1982) is in good agreement with the predicted value (about 50–60%). Circular dichroism measurements of phosphorylated factor III^{Lac} revealed a 20% reduction of the α-helical content.

This may apply to the N-terminal helix (residues 6–18) (Stüber, 1982). This supports our earlier suggestion that the amino-terminal part of the protein undergoes a drastic change upon phosphorylation (Deutscher et al., 1982). We assumed that in factor III^{Lac} the N-terminal region is buried in the protein and becomes exposed to the surface upon phosphory-

lation. Phosphorylation of factor III^{Lac} causes an increase in hydrophobicity as shown by detergent binding (Deutscher et al., 1982). The same could be expected if a segment of the protein composed of hydrophobic amino acids is turned to the surface. It is interesting that the N-terminal peptide, residues 4–38, interferes with factor III^{Lac} for binding to membrane-bound enzyme II^{Lac}. Defective factor III^{Lac} isolated from strain S 714G could still be phosphorylated by PEP, enzyme I, and HPr at about normal rate but was no longer able to transfer the phosphoryl group via enzyme II^{Lac} to the sugar (Sobek et al., 1984). The mutation was found to affect position 18 of the sequence: glycine was changed to glutamic acid. Thus, the binding site for enzyme II^{Lac} as well as the change in hydrophobicity and α-helical content upon phosphorylation could be related to the same part of the protein, the N-terminal region. The other functional domain of factor III^{Lac} carrying the histidine residue responsible for phosphoryl group transfer could be identified on the basis of earlier work and the sequence presented in Figure 1 (Deutscher et al., 1982). The phosphorylated nonapeptide, which was isolated and sequenced earlier, corresponds to residues 82–90 in Figure 1 and includes histidine-82 as 3-phosphohistidine according to Kalbitzer et al. (1981). Thus, the active site histidine residue 82 is identical with histidine B of the above-mentioned paper.

ACKNOWLEDGMENTS

We are thankful to N. Neifer and R. Engelmann for technical assistance.

REFERENCES

Argos, P., Hanei, M., & Garavito, M. (1978) *FEBS Lett. 93*, 19.
Baglioni, C. (1961) *Biochim. Biophys. Acta 48*, 392–396.
Beyreuther, K. (1977) *Solid Phase Methods in Protein Sequence Analyses* (Previero, A., & Coletti-Previero, M. A., Eds.) p 107, Elsevier/North-Holland, Amsterdam.
Chang, J. Y. (1980) *Anal. Biochem. 102*, 384–392.
Chou, P. Y., & Fasman, G. D. (1974) *Biochemistry 13*, 222–245.
Chou, P. Y., & Fasman, G. D. (1977) *J. Mol. Biol. 115*, 135–175.
Deutscher, J., Beyreuther, K., Sobek, H. M., Stüber, K., & Hengstenberg, W. (1982) *Biochemistry 21*, 4867–4873.
Dills, S. S., Apperson, A., Schmidt, M., & Saier, M. H., Jr. (1980) *Microbiol. Rev. 44*, 385–418.
Hunkapiller, M. W., & Hood, L. E. (1980) *Biochemistry 19*, 2124–2133.
Kalbitzer, H. R., Deutscher, J., Hengstenberg, W., & Rösch, P. (1981) *Biochemistry 20*, 6178–6185.
Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105–132.
Michel, H. (1951) *Monatsh. Chemie 82*, 489.
Roseman, S., Meadow, N. D., & Kukuruzinska, M. A. (1982) *Methods Enzymol. 89*, 417–431.
Simoni, R. D., Hays, J. B., Nakazawa, T., & Roseman, S. (1973) *J. Biol. Chem. 248*, 957–965.
Sobek, H. M., Stüber, K., Beyreuther, K., Hengstenberg, W., & Deutscher, J. (1984) *Biochemistry 23*, 4460–4464.
Stüber, K. (1982) Doctoral Thesis, University of Cologne.
Stüber, K., & Beyreuther, K. (1981) in *HPLC in Protein and Peptide Chemistry*, pp 205–221, de Gruyter, Berlin and New York.
Waley, S. G., & Watson, J. (1953) *Biochem. J. 55*, 328.
Yang, C. Y. (1978) *Hoppe-Seyler's Z. Physiol. Chem. 360*, 1673–1675.
Zaiss, S., & Beyreuther, K. (1983) *Eur. J. Immunol. 13*, 508–513.

Purification and Characterization of a Biliverdin-Associated Protein from the Hemolymph of *Manduca sexta*†

Walter G. Goodman,*,‡ Bruce Adams,§ and Jeffrey T. Trost‡

Departments of Entomology and Chemistry, University of Wisconsin, Madison, Wisconsin 53706
Received May 16, 1984

ABSTRACT: A biliverdin binding protein, insecticyanin, has been isolated from the hemolymph of the fourth instar tobacco hornworm *Manduca sexta*. The protein has been purified to apparent homogeneity by conventional chromatography with a cumulative yield of 40–50%. The protein (M_w 71 600) is composed of three subunits (M_r 23 000). Each subunit binds one biliverdin molecule. Proton magnetic resonance spectroscopy and absorption spectroscopy demonstrate that the bilin is the biliverdin IX γ isomer.

The hemolymph of many invertebrates is blue due either to copper-containing proteins such as the hemocyanins or to biliverdin-associated proteins. A survey of the invertebrate hemolymph chromoproteins indicates that the majority of biliproteins are limited to the class Insecta, a diverse group that has evolved an oxygen transport system independent of circulating respiratory pigments. Although the functions of the biliproteins are not well understood, the tissue distribution of the proteins suggests that the blue chromophore, in conjunction with yellow pigments such as the carotenoids, gives rise to the cryptic green color observed in many phytophagous insects. A particularly rich source of biliproteins is found in the hemolymph and epidermis of the hornworm *Manduca sexta* (Dahlman, 1969; Cherbas, 1973; Goodman et al., 1982).

† This study was supported by the College of Agricultural and Life Sciences of the University of Wisconsin, the Johnson Wax Fund, and NIH (GM34257).
‡ Department of Entomology, University of Wisconsin.
§ Department of Chemistry, University of Wisconsin.

0006-2960/85/0424-1168$01.50/0