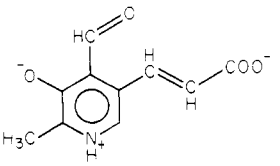
Covalently Modified Peptides Isolated from Aspartate Aminotransferase after Reaction with Pyridoxal 5′-Sulfate†

Jane Schmidt, Robert D. Scott, and David E. Metzler*

ABSTRACT: The apoenzyme form of cytosolic aspartate aminotransferase of pig hearts was allowed to react at room temperature with 1 equiv of pyridoxal 5′-sulfate. The resulting covalently modified enzyme was degraded with pepsin. Fluorescent tri-, tetra-, and hexapeptides were isolated and characterized as fragments of the active site sequence: Phe-Ser-Lys*-Asn-Phe-Gly-Leu. This sequence contains a modified form (Lys*) of lysine-258 that is known to form a Schiff base with pyridoxal phosphate in the active site. The peptides were further degraded by acid hydrolysis to give a fluorescent derivative of lysine with light absorption and chemical properties similar to those of the original modified enzyme. A related series of peptides were obtained from apoenzyme after reaction with the 5-carboxyethenyl analogue of pyridoxal 5′-phosphate.

The apo form of the cytoplasmic aspartate aminotransferase of pig heart reacts with pyridoxal 5′-sulfate at pH 8.3 in an unusual way (Yang et al., 1974). An initially broad absorption band is converted over a period of 1 h to a narrow band centered at 402 nm at pH 8 and at 386 nm below pH 5. The low-pH form has a brilliant blue-white fluorescence. A similar spectrum is generated by reaction of pyridoxal sulfate with cysteine, serine, and other β-substituted amino acids and amines. Inorganic sulfate is liberated during the reaction with cysteine. An NMR study suggested a mechanism whose first step is addition of the nucleophilic group in the β position of the amine to a Schiff base formed initially. For the reaction with enzyme, it was proposed that an unknown nucleophile from the protein adds to the Schiff base (Scheme I). The adduct would then be converted to an *o*-quinonoid structure by elimination of sulfate (Yang et al., 1974). This quinonoid intermediate would be converted on to the final product. Recently, as described in the preceding paper, Scott et al. (1982) have characterized this final product as the bicyclic pyrrolopyridine shown in Scheme I.

A very similar reaction has been observed for a 5-*trans*-carboxyethenyl analogue of pyridoxal-P (Miura & Metzler, 1976). The product has a greenish yellow fluorescence. In this case, the double bond of the side chain appears to become saturated in a reaction sequence quite similar to that of Scheme I.



5-*trans*-carboxyethenyl analogue of pyridoxal-P

In the present work we report the isolation and partial characterization of several peptides from the active site of the apo form of aspartate aminotransferase after incubation of the latter with pyridoxal sulfate or with the carboxyethenyl analogue. A preliminary report has been published (Schmidt et al., 1980).

Experimental Procedures

The α subform of cytoplasmic aspartate aminotransferase of pig hearts was prepared by the method of Jenkins et al. (1959) as modified by Martinez-Carrion et al. (1967, method A). The apoenzyme was obtained by the procedure of Scardi et al. (1963) as modified by Furbish et al. (1969). Information on enzyme activity and molar absorptivities is given in preceding papers (Yang et al., 1974).

Pepsin, trypsin, and chymotrypsin were purchased from the Worthington Biochemical Corp. Aminopeptidase M was purchased from the Pierce Chemical Co. Pronase P (protease from *Streptomyces griseus*) as well as carboxypeptidase Y from baker's yeast was obtained from Sigma Chemical Co. Pyridoxal 5′-sulfate and the 5-*trans*-carboxyethenyl analogue of pyridoxal phosphate were prepared in this laboratory according to published procedures (Yang et al., 1974; Miura & Metzler, 1976).

N-Ethylmaleimide, 5,5′-dithiobis(2-nitrobenzoic acid), 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride), pyridoxal 5′-phosphate, and pyridoxamine 5′-phosphate were purchased from Sigma Chemical Co. *N*-Dansyl amino acids were either purchased from Sigma or prepared from dansyl chloride and the free amino acids (Gray, 1972). All other chemicals were from commercial sources and were of reagent grade or better.

The modification reaction was carried out by mixing the apoenzyme (10^{-4} M based on the monomer) in 0.02 M triethanolamine buffer at pH 8.3 with 1 equiv of pyridoxal 5′-sulfate or of the 5-carboxyethenyl analogue from 10 mM stock solutions. The reaction with pyridoxal sulfate was allowed to proceed for at least 2 h and that with the carboxyethenyl analogue for 20 h at room temperature or at about 37 °C. The pH was lowered to about 2.5 by the dropwise addition of 40% formic acid. The amount of the characteristic chromophore produced from pyridoxal sulfate was estimated by using a molar absorptivity of 5.3×10^3 M^{-1} cm^{-1} at 385 nm [Yang et al. (1974), Figure 3A].

The modified apoenzymes were digested with pepsin in a 1:100 weight ratio to the aspartate aminotransferase, at a pH of about 2.5. The pepsin was added from a freshly prepared solution (10 mg/mL) in 0.2 M acetate buffer, pH 4.7, or in 0.01 M formic acid. Digestion was allowed to proceed for 1 h at room temperature. Then a second portion of pepsin (equal in amount to the first) was added, and digestion was allowed

† From the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50011. *Received May 18, 1982.* This work was supported by Grant AM-01549 from the National Institutes of Health.

0006-2960/82/0421-5220$01.25/0

to proceed for 4 h longer. The solution was lyophilized and stored in a freezer.

Partial purification of the fluorescent peptides was accomplished by passage through a column of Sephadex G-15 in 0.01 M formic acid. The fluorescent fractions were combined, lyophilized, and subjected to thin-layer electrophoresis. A Desaga/Brinkman double-chamber electrophoresis apparatus was used with 40 × 20 cm thin layers of Sephadex G-25 superfine in a formic acid–acetic acid–water mixture (1:4:45 v/v) prepared according to the manufacturer's instructions. Lyophilized peptides containing about 0.4 μmol of the 386-nm chromophore were suspended in 20 μL of 0.1 M acetic acid and applied to the gel layer in a narrow line about 2 cm from the anode end of each plate. Electrophoresis was run at 800 V, 35–50 mA, for about 5 h until the fastest migrating fluorescent band approached the cathode end of the plate (Figure 1). The separated fluorescent bands were scraped from the plates, and the peptides were eluted with 0.01 M formic acid. They were sometimes subjected to a second thin-layer electrophoresis in 0.10 M ammonium acetate, pH 4.7, at 600 V, 25–40 mA, for 5 h.

In later experiments a lyophilized digest from 5 μmol of aspartate aminotransferase was suspended in 1 mL of 0.01 M formic acid, centrifuged, applied to a 2.7 × 100 cm column of Bio-Gel P-2 polyacrylamide beads, and eluted with 0.01 M formic acid at a rate of about 25 mL/h. Two fluorescent bands containing nearly equal amounts of chromophore and with a combined yield of about 65% were separated from most of the other peptides (Figure 2). The peptides were lyophilized and applied to 20 × 20 cm thin-layer chromatography plates (E. Merck-Brinkman, silica gel 60). About 1.9 μmol of chromophore was applied per plate in a narrow band with the aid of five consecutive 2-μL portions of 0.01 M formic acid. The chromatogram was developed with acetic acid–1-butanol–water (1:4:1 v/v). Four compounds with the characteristic fluorescence of the 386-nm chromophore separated. Each of these was scraped from the plates and eluted from the silica gel, with about 10 mL of 0.01 M formic acid. The four bands, of R_f values 0.03 (tripeptide), 0.07 (tetrapeptide), 0.20 (hexapeptide), and 0.26, contained about 37, 16, 21, and 0.3%, respectively, of the total chromophore applied to the plate. The first fluorescent band from the Bio-Gel column contained most of the three spots of higher R_f while the second band contained most of the peptide of $R_f = 0.03$. This peptide was obtained in pure form from the thin-layer chromatogram while the others were purified further by electrophoresis at pH 1.9 as described above.

The purified peptides were hydrolyzed in 6 M HCl at 110 °C for 22 h and analyzed by William Harris on a Durrum D400 amino acid analyzer (Table I). Amino-terminal residues were identified by dansylation and thin-layer chromatography (Gray, 1972). The third solvent was ethyl acetate–acetic acid–ethanol–water (500:34:34:20 v/v), and Gray's fourth solvent was not used.

So that the modified lysine (Lys*) could be obtained, about 2 μmol of the fluorescent peptides was dissolved in 2 mL of constant-boiling HCl and was heated at 110 °C in sealed, evacuated tubes for 16–22 h. The resulting hydrolysate was evaporated under vacuum to near dryness, diluted with 0.5 mL of 0.01 M formic acid, and fractionated on a 0.9 × 22.5 cm column of the sulfopropyl group containing ion exchanger, SP-Sephadex (40–120-μm beads). The column was eluted with a linear gradient of 0.007–0.4 M ammonium formate buffer, pH 2.9 (Figure 3). The fluorescent band that absorbed maximally at 382 nm was concentrated under reduced pressure

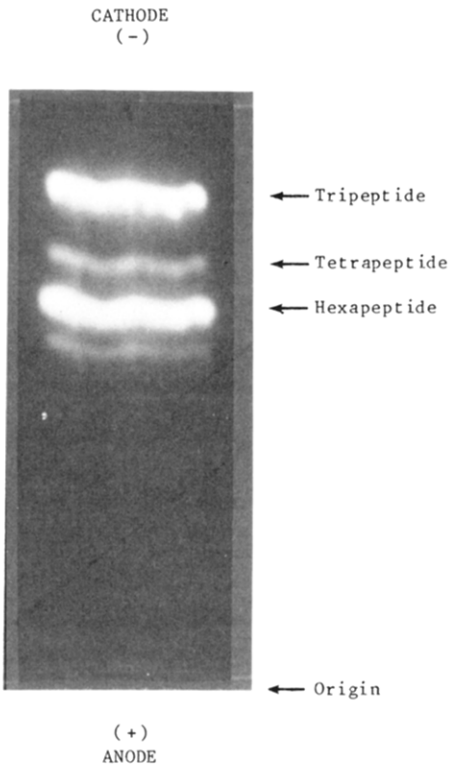


FIGURE 1: High-voltage electrophoresis on the mixture of fluorescent peptides produced by peptic hydrolysis of aspartate aminotransferase inactivated by reaction with pyridoxal 5′-sulfate. From top to bottom the bands represent tripeptide, tetrapeptide, hexapeptide, and a mixture of higher peptides.

to $^{1}/_{100}$ the original volume, diluted to $^{15}/_{1000}$ the original volume with 0.01 M formic acid, and purified further on an 0.8 × 80 cm column of Bio-Gel P-2 (minus 400 mesh). Elution with 0.01 M formic acid yielded the fluorescent Lys* with a characteristic spectrum almost identical with that of the tripeptide. Yield was about 24% of the chromophore present initially.

Results and Discussion

As was reported by Yang et al. (1974), the chromophore formed from the reaction of pyridoxal 5′-sulfate with the apoenzyme is stable at a pH below 5 for many days, even in 6 M guanidine hydrochloride, but decomposes at pH 8.2. We, therefore, incubated with pepsin at pH 1.9–2.5 at about 37 °C for 5–24 h. Only a 4% loss in the initial intensity of the absorption at 386 nm was observed after 5 h. The peak position was shifted slightly to 382 nm.

When we passed the resulting mixture of peptides through a column of Sephadex G-15, the bulk of the fluorescent chromophore emerged as a single broad band in the last peptide-containing fractions. Thin-layer electrophoresis at pH 1.9 gave four major fluorescent bands (Figure 1). The fastest moving band consisted largely of a tripeptide and the next to slowest band of a hexapeptide together with a larger amount of a tryptophan-containing peptide that could be separated by a second electrophoresis at pH 4.7.

A better procedure involved initial separation on a column of Bio-Gel P-2 followed by thin-layer chromatography and finally electrophoresis at pH 1.9. The chromophore emerged from the gel filtration column as two bands (Figure 2), the first containing mainly the hexapeptide and smaller amounts of tetrapeptide and another larger peptide. The second band contained mostly tripeptide and some tetrapeptide. Pure tri-, tetra-, and hexapeptides were obtained in good yield by using both thin-layer chromatography and electrophoresis.

Table I: Amino Acid Analyses

	amounts relative to Asx = 1.00					
				tripeptide		
amino acid	hexapeptide[b]	pentapeptide[c]	tetrapeptide	averaged[d]	from hexapeptide[e]	modified lysine (Lys*)[f]
chromophore[a]	0.84	0.51	0.97	1.05 ± 0.08	1.23	1.00
Ser	0.94	0.97	0.98	0.91 ± 0.04	0.88	
Lys	0.47	0.36	0.47	0.33 (0.11–0.54)	0.11	0.18
Asx	1.00	1.00	1.00	1.00	1.00	
Phe	0.84	0.81	0.88			
Gly	0.94	0.97		0.04 ± 0.02	0.01	
Leu	0.85					
Ala	0.18	0.03	0.02			
Glu	0.18	0.12	0.05	0.03 ± 0.01	0.03	
His	0.36		0.33			

[a] For intact peptide. Estimated from light absorption at 382 nm. Some uncertainty exists because of small amounts of turbidity and because the molar absorptivity is not known exactly. [b] Also contained 0.10 Thr, 0.11 Val, 0.08 Ile, and 0.08 Arg. [c] From degradation of hexapeptide by Pronase. The low content of chromophore could have resulted from a pipeting error (×2) or from unrecognized degradation of the very sensitive ring. [d] Average of analyses (three in duplicate) on five different samples, some of which were treated with dilute NaOH or NH_4OH at pH 10–11 before the acid hydrolysis. Two samples contained small amounts of threonine. [e] Obtained by degradation of hexapeptide with carboxypeptidase Y. Duplicate analyses. [f] Obtained by acid hydrolysis of tripeptide. Given is the ratio of lysine to the estimated amount of the chromophore.

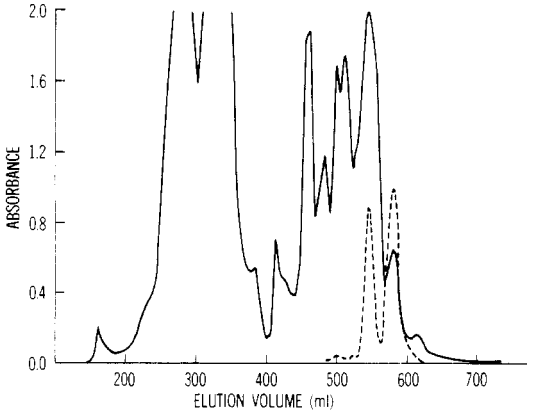


FIGURE 2: Separation of fluorescent peptides on a column of Bio-Gel P-2. A peptic digest of 215 mg (5 μmol) of apo aspartate aminotransferase treated with pyridoxal 5′-sulfate was applied to a 2.7 × 100 cm column of the gel and eluted with 0.01 M formic acid. Solid line, absorbance at 280 nm; dashed line, absorbance at 386 nm.

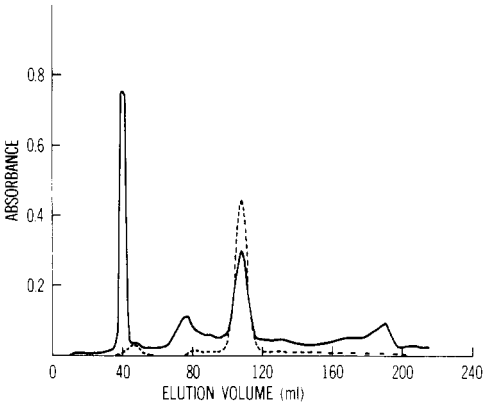


FIGURE 3: Elution diagram of the hydrolysate of pyridoxal sulfate modified peptides from the active site of apo aspartate aminotransferase chromatographed on the hydrogen form of SP-Sephadex. The sample was eluted with a gradient of ammonium formate buffer (0.007–0.4 M), pH 2.9. Solid line, absorbance at 280 nm; dashed line, absorbance at 382 nm.

From the amino acid analyses (Table I) and the end group analysis, the major modified peptides from reaction with pyridoxal sulfate were identified as the following unique sequences containing lysine-258 of the active site in a modified form (Lys*):

Ser-Lys*-Asn
Phe-Ser-Lys*-Asn
Ser-Lys*-Asn-Phe-Gly-Leu

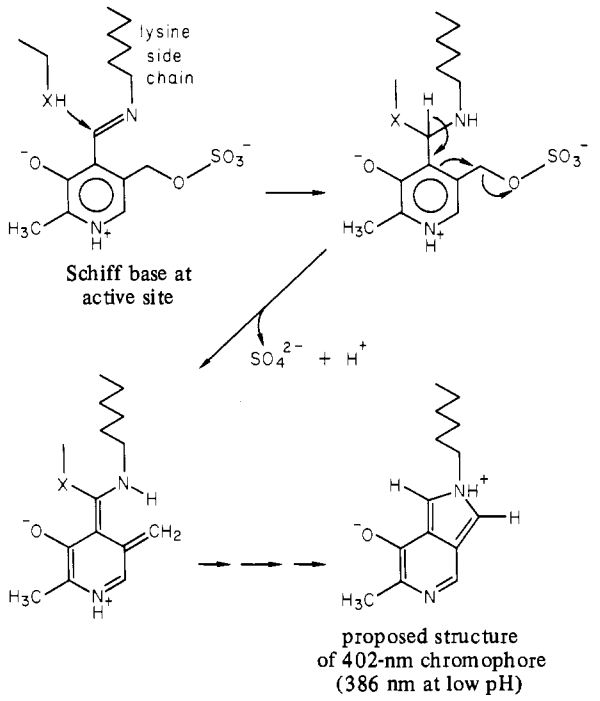
These peptides represent the sequence of residues Phe-256 through Leu-262 (Ovchinnikov et al., 1973; Doonan et al., 1974).

The tripeptide, for which the largest number of different samples was isolated and analyzed, contained the chromophore together with serine and asparagine (analyzed as aspartate) in a 1:1:1 ratio. In addition, a variable amount of lysine was obtained. In some samples there was a small amount of an extra peak traveling ahead of lysine and near the histidine position in the elution diagram from the amino acid analyzer. End group analysis by dansylation showed that the N terminus of both the tripeptide and the hexapeptide is serine. However, the tetrapeptide has an N-terminal phenylalanine.

Pronase cleaved leucine from the C terminus of the hexapeptide (Table I), giving a band of $R_f = 0.05$ but had no effect on the tripeptide. Carboxypeptidase Y at pH 5.0 acted on the hexapeptide to convert it to the tripeptide that was recovered in 60–70% yield and analyzed (Table I). Aminopeptidase M appears to cleave both peptide bonds in the tripeptide. Migration in two thin-layer chromatography systems [acetic acid–1-butanol–water (1:4:1 v/v) and chloroform–methanol–17% ammonium hydroxide (2:2:1 v/v)] of the fluorescent product was the same as that of both modified lysine-258 (Lys*) produced by acid hydrolysis of modified peptides and a nonenzymatically produced Lys*. Since serine and asparagine were not well separated, the results are ambiguous. It is possible that the expected dipeptide Lys*-Asn may not have separated from Lys*.

Treatment with 6 M HCl at 35 °C for 20 min gave two new peptides of $R_f = 0.06$ and 0.10 in the acetic acid–butanol–water system. The peptide of $R_f = 0.06$ must be Ser-Lys* as judged by amino acid analysis. Treatment with HCl for 14 h gave the fluorescent Lys*, which retains the characteristic light-absorbing properties of the peptides. This derivative was isolated by chromatography on SP-Sephadex (Figure 3) in a yield of 24%. Its structure, whose proof is described in the preceding paper (Scott et al., 1982), is that of lysine whose ε-amino group has been incorporated into a substituted 2*H*-pyrrolo[3,4-*c*]pyridine ring (Scheme I).

Scheme I



The cause of the variable lysine analyses can probably be traced to the complex reactivity of the pyrrolopyridine chromophore that is quite stable in acid but is decomposed slowly in neutral solutions and rapidly at high pH (Scott et al., 1982). A number of products are formed, some of which may be converted by oxidative processes into substances that release lysine. Peptides treated with base or decomposed by sunlight before acid hydrolysis gave more lysine than did untreated samples. The isolated Lys* gave about 11% of the stoichiometric amount of lysine when subjected to the usual conditions of hydrolysis and amino acid analysis. When it was treated before analysis with NaOH or NH_4OH at a pH of 10–11 until the fluorescence disappeared, the yield increased to about 24 and 46%, respectively. Prior irradiation with sunlight raised the yield to about 55%.

When the isolated Lys* was passed through the amino acid analyzer without treatment with acid, about 7% was apparently converted to lysine. The remainder emerged as at least three ninhydrin-positive bands ahead of lysine and a small peak near the histidine position. Two peaks had light absorption characteristic of 3-hydroxypyridine derivatives, but none had the absorption or fluorescence of the original pyrrolopyridine. Thus, although the chromophore is rather stable in acid and can withstand chromatography on SP-Sephadex in formic acid, it is decomposed in the amino acid analyzer.

All of the fluorescent peptides have the spectral properties shown in Figure 4. Especially characteristic is a sharp spike at 282 nm in the low-pH form. As in the modified enzyme prior to digestion with pepsin, the spectra are pH dependent. Although there were no isosbestic points in the spectra of the enzyme between pH 5.0 and pH 8.2, the spectra of the peptides have sharp isosbestic points and a pK value of 6.4, as well as a higher one above 11. The instability of the chromophore at high pH is illustrated in Figure 5, which shows the decay of the absorption band of the tripeptide at pH 10.4. The half-life is about 20 min at 25 °C.

Apoenzyme prepared by our procedure is known to contain an equivalent amount of inorganic phosphate in each coenzyme-binding site (Martinez-Carrion, 1975; Verge et al., 1979). It seemed possible that this phosphate might interfere

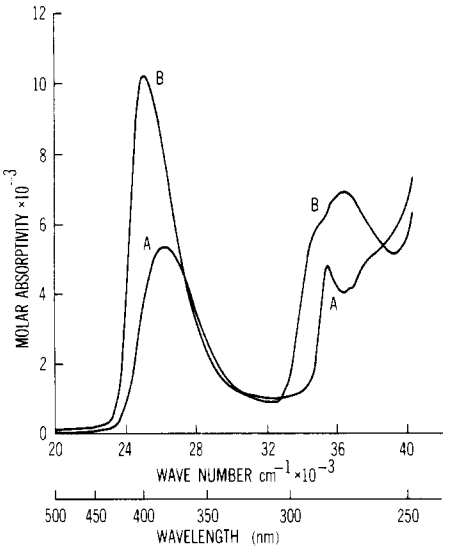


FIGURE 4: Absorption spectra of the fluorescent tripeptide: (A) low-pH form; (B) high-pH form. The molar absorptivities were computed by assuming 5.3×10^3 M^{-1} cm^{-1} for the low-pH form at 385 nm (Yang et al., 1974). This value is also in agreement with the quantitative amino acid analysis for asparagine (as aspartic acid, Table I).

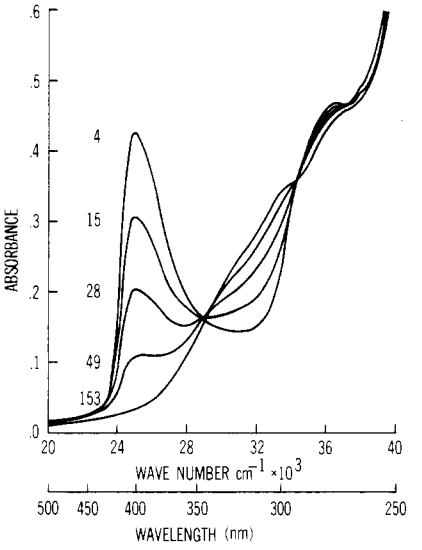


FIGURE 5: Decay of the tripeptide chromophore formed from pyridoxal sulfate at pH 10. The numbers beside the curves indicate the times in minutes after addition of NH_4OH to a 1.1×10^{-4} M solution of the peptide to raise the pH from 3 to 10.

with the proper binding of pyridoxal sulfate and induce the observed abnormal reaction. However, apoenzyme prepared by the procedure of Wada & Snell (1962) using only ammonium sulfate and no phosphate buffer reacted with pyridoxal sulfate in the same way.

Because the SH group of cysteine-390 of aspartate aminotransferase becomes reactive in the presence of substrates (Birchmeier et al., 1973), we thought initially that it might enter into the reaction with pyridoxal 5′-sulfate. To test this possibility we treated the enzyme with N-ethylmaleimide to block Cys-45 and Cys-82 after which the SH group of Cys-390 was converted to –SCN as described by Birchmeier et al. [1973; see Schmidt (1980) for details]. Pyridoxal phosphate was removed from the modified enzyme, and 1 equiv of pyridoxal sulfate was added to the resulting apoenzyme. It reacted to give a product absorbing at the same peak position and with the same distinctive shape as was observed for re-

action of pyridoxal sulfate with the unmodified apoenzyme. Thus, a free SH group on Cys-390 is not essential for the reaction.

Apoenzyme was allowed to react with the 5-carboxyethenyl analogue of pyridoxal phosphate as described under Experimental Procedures. When the resulting modified enzyme was digested with pepsin, a series of fluorescent peptides that exactly paralleled those obtained from pyridoxal sulfate were obtained. Pure samples of the tetrapeptide and the tripeptide were obtained and analyzed. Molar ratios for the tripeptide were as follows: chromophore 1.13; Asx 1.00; Lys 0.35; Ser 0.88; Glu 0.03; Gly 0.04.

The results presented here establish that the chromophores formed by the reaction of pyridoxal 5′-sulfate or the 5-carboxyethenyl analogue of pyridoxal phosphate with cytosolic aspartate aminotransferase are attached to the active site lysine-258. There is no cross-linking to other parts of the protein. Results presented in the preceding paper (Scott et al., 1982) show that for pyridoxal sulfate the chromophore formed is a substituted 2*H*-pyrrolo[3,4-*c*]pyridine as is shown in Scheme I.

Acknowledgments

We are grateful to Victor Chen and others in the laboratory for help in the isolation of the enzyme and to Cathy Achenbach for preparation of the manuscript.

References

Birchmeier, W., Wilson, K. J., & Christen, P. (1973) *J. Biol. Chem. 248*, 1751.
Doonan, S., Doonan, H. J., Hanford, R., Vernon, C. A., Walker, J. M., Bossa, F., Barra, D., Carloni, M., Fasella, P., Riva, F., & Walton, P. L. (1974) *FEBS Lett. 38*, 229.
Furbish, F. S., Fonda, M. L., & Metzler, D. E. (1969) *Biochemistry 8*, 5169.
Gray, W. R. (1972) *Methods Enzymol. 25*, 121.
Jenkins, W. T., Yphantis, D. A., & Sizer, I. W. (1959) *J. Biol. Chem. 234*, 51.
Martinez-Carrion, M. (1975) *Eur. J. Biochem. 54*, 39.
Martinez-Carrion, M., Turano, C., Chiancone, E., Bossa, F., Giartosio, A., Riva, F., & Fasella, P. (1967) *J. Biol. Chem. 242*, 2397.
Miura, R., & Metzler, D. E. (1976) *Biochemistry 15*, 283.
Ovchinnikov, Yu. A., Egorov, C. A., Aldonova, N. A., Feigina, M. Yu., Lipkin, V. M., Abdulaev, N. G., Grishin, E. V., Kiselev, A. P., Modyanov, N. N., Braunstein, A. E., Polyanovsky, O. L., & Nosikov, V. V. (1973) *FEBS Lett. 29*, 31.
Scardi, V., Scotto, P., Iaccarino, M., & Scarano, E. (1963) *Biochem. J. 88*, 172.
Schmidt, J. (1980) Ph.D. Dissertation, Iowa State University.
Schmidt, J., Scott, R. D., & Metzler, D. E. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 498.
Scott, R. D., Ueno, H., & Metzler, D. E. (1982) *Biochemistry* (preceding paper in this issue).
Verge, D., Tenu, J.-P., & Arrio-Dupont, M. (1979) *FEBS Lett. 100*, 265.
Wada, H., & Snell, E. E. (1962) *J. Biol. Chem. 237*, 127.
Yang, I.-Y., Khomutov, R. M., & Metzler, D. E. (1974) *Biochemistry 13*, 3877.

Structure–Function Relationships in *Escherichia coli* Translational Elongation Factor G: Modification of Lysine Residues by the Site-Specific Reagent Pyridoxal Phosphate†

Alfonso Giovane,‡ Ciro Balestrieri,‡ and Claudio Gualerzi*

ABSTRACT: Translational elongation factor G (EF-G) of *Escherichia coli* was modified with the selective, site-specific lysine reagent pyridoxal phosphate (PLP). The reaction results in the modification of a maximum of 12 lysine residues, one of which is essential for guanosine 5′-triphosphate (GTP) binding and whose modification is inhibited by the presence of GTP. Formation of a reversible adduct between 2,3-butanedione and an essential arginine similarly located in the GTP binding site [Rohrbach, M. S., & Bodley, J. W. (1977) *Biochemistry 16*, 1360–1363] also protects EF-G from PLP inactivation, suggesting that these two residues are spatially close to each other in the native factor. The essential lysine residue was found in the trypsin-resistant fragment T4 (M_r 41 000). In addition to the lysine essential for GTP binding, at least one further lysine was found to be important for EF-G function, since GTP-protected, PLP-modified EF-G molecules fully competent in binding to 50S ribosomal subunits showed decreased activity in 50S- and 70S-dependent GTP hydrolysis. It is likely that a PLP-modified lysine impairs the interaction of the factor with 30S ribosomal subunits and/or a conformational change of the factor required for the hydrolysis of GTP.

We are carrying out a systematic investigation of the phosphate-binding regions of various components of the protein synthetic machinery (Ohsawa & Gualerzi, 1981a,b) by use of the selective and site-specific lysine reagent pyridoxal phosphate (Rippa et al., 1967; Feeney et al., 1975; Glazer, 1976). In the present paper we report on the identification of an essential lysine residue in the guanine nucleotide binding site of elongation factor G (EF-G).

EF-G is one of the three protein factors required for protein biosynthesis and endowed with ribosome-dependent GTPase activity. [The others are IF2 and EF-Tu; for a recent review see Parmeggiani & Sander (1981).] The relationship between structure, mechanism of action, topographical location, and

† From the Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, D-1000 Berlin 33, West Germany. *Received January 5, 1982; revised manuscript received June 17, 1982.* C.B. was supported by a travel grant from the Italian C.N.R. This work was supported by an S.F.B. grant (S.F.B. 9) from the Deutsche Forschungsgemeinschaft.

‡ Present address: Institute of Biological Chemistry, 1st School of Medicine, University of Naples, Naples, Italy.

0006-2960/82/0421-5224$01.25/0