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Covalent Aspartylation of Aspartyl-tRNA Synthetase from Bakers' Yeast by Its Cognate Aspartyl Adenylate: Identification of the Labeled Residues†

Hafedh Mejdoub, Daniel Kern, Richard Giegé, Jean-Pierre Ebel, Yves Boulanger,* and Joseph Reinbolt
Institut de Biologie Moléculaire et Cellulaire du CNRS, 67084 Strasbourg Cedex, France
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ABSTRACT: Aspartyl-tRNA synthetase from bakers' yeast gives an unstable complex with the cognate adenylate, which reacts after dissociation with amino acid side chains of the protein. This leads to a covalent incorporation of aspartic acid into aspartyl-tRNA synthetase via amide or ester bonds formed between the α-carboxyl group of activated aspartic acid and accessible lysines, serines, and threonines. This property is used to label the peptides at the surface of the enzyme. The main labeled residues have been identified, and their location in the primary structure is discussed in relation to structural properties of aspartyl-tRNA synthetase.

In a previous paper (Kern et al., 1985) a peculiar property of aspartyl-tRNA synthetase from bakers' yeast was reported: in the absence of $tRNA^{Asp}$ the enzyme forms a weak complex with its cognate aspartyl-adenylate, which easily dissociates from the synthetase. The activated carboxyl group of aspartic acid will then react with any nucleophilic acceptor present in the reaction mixture, in particular with amino acid side chains of the enzyme itself. By use of a chemical labeling technique it was shown that this phenomenon led to covalent incorporation of aspartic acid residues into the protein via the formation of stable amide bonds with lysine residues, their number depending not only on the pH of the reaction mixture but also on the chemical nature of the buffer chosen. Furthermore, this covalent binding of as many as eight aspartates per enzyme subunit did not affect the maximal velocity of the overall aminoacylation of $tRNA^{Asp}$ but significantly reduced the affinity for this tRNA. This indicates that some of the aspartylated residues must play a part, directly or indirectly, in tRNA binding. Finally as discussed in this initial study, aspartylation of aspartyl-tRNA synthetase probably occurs in accessible regions of the protein so that the in situ synthesized aspartyl adenylate can be used as a structural probe to map the surface of this protein. This is of particular interest as the determination of the crystallographic structures of both aspartyl-tRNA synthetase and its complex with $tRNA^{Asp}$ is well under way (Dietrich et al., 1980; Lorber et al., 1983a; Moras et al., 1983).

In this paper we bring new data concerning the aspartylated residues: indeed, isolation and sequencing of the labeled peptides definitely establish that the activated α-carboxyl group of aspartic acid not only reacts with lysines but also forms

† This work was supported by grants from the CNRS.
* Author to whom correspondence should be addressed.

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stable ester bonds with alcohol groups of serine and threonine residues. The major aspartylated peptides have been identified, and their location within the protein structure (Amiri et al., 1985) is discussed in relation to known structural properties of the enzyme.

Experimental Procedures

General. [^{14}C]-L-Aspartic acid (200 mCi/mmol) was from the Commissariat â l'Energie Atomique (Saclay, France). Omnifluor was from New England Nuclear and L-aspartic acid from Merck (Darmstadt, FRG). Hepes,[1] Mes, and inorganic pyrophosphatase (1 unit catalyzed the formation of 1 μmol of inorganic phosphate/min at 25 °C and at pH 7.4) were from Sigma (Saint Louis, MO). Sephadex G-50, G-100, and G-200 superfine were from Pharmacia (Uppsala, Sweden), and glass-fiber disks GF/c were from Whatman (Maidstone, U.K.). For liquid scintillation counting, an Omnifluor solution was used (4 g/L of toluene) when radioactivity was precipitated on 3MM Whatman paper disks or a Bray's solution (60 g of naphthalene, 4 g of Omnifluor, 100 mL of methanol, 20 mL of ethylene glycol, and dioxane up to 1 L) when radioactivity was measured in aqueous solution. Cellulose TLC sheets (Polygram CEL 400) were from Macherey-Nagel (Dueren, FRG).

Enzymes. Aspartyl-tRNA synthetase (EC 6.1.1.12) from bakers' yeast (cytoplasmic enzyme) was purified as described by Lorber et al. (1983b). Trypsin, chymotrypsin, and carboxypeptidases A and B were from Worthington (Freehold, NJ).

Activity Measurements and Isolation of Aspartylated Aspartyl-tRNA Synthetase. $tRNA^{Asp}$ aminoacylation reaction, PP_i–ATP exchange, aspartylation, and isolation of the labeled enzyme were carried out as published by Kern et al. (1985). Like in this previous work, aspartylation was carried out at acidic, neutral, and alkaline pH: incubation mixtures contained 100 mM Na-Mes buffer, pH 6.0, or 100 mM Na-Hepes buffer, pH 7.2 or 8.6, 2 mM ATP, 2 or 3 mM $MgCl_2$, 0.3 mM L-aspartic acid, 5 or 10 units of inorganic pyrophosphatase/mL, and 4.5 μM aspartyl-tRNA synthetase. The reaction was carried out at 30 °C for 10 h. After 10 h, the medium was supplemented with fresh reactants, ATP and $MgCl_2$ (1 mM each) and L-aspartic acid (100 μM), and the reaction was continued for another 14 h. Other experimental details were given in the previous study (Kern et al., 1985). It must be stressed that all samples of aspartylated aspartyl-tRNA synthetase used in this study were pooled after chromatography on Sepharose 4B in order to separate the labeled enzyme not only from the free reactants but also from possible aggregates of high molecular weight. In other words, protein chemistry was done on labeled dimeric aspartyl-tRNA synthetase.

Protein Chemistry. The methods used were essentially as reported by Hounwanou et al. (1983) and Amiri et al. (1985): aspartylated aspartyl-tRNA synthetase was subjected to tryptic digestion, and the resulting digests were fractionated by a combination of gel filtration on Sephadex G-50 and HPLC on a reverse-phase C18 column. Sequencing of peptides was done with the manual double-coupling technique (DABITC + PITC) of Chang et al. (1978). Since radioactive [^{14}C]-L-aspartic acid was used to label the enzyme, radioactive peptides were monitored by both absorbance measurements at 220 nm and liquid scintillation counting. Occasionally the classical dinitrophenylation and dansylation methods were used to check for the presence of a free ε-amino group (lysine) or imidazole ring (histidine) in some of the aspartylated peptides. Digestions with chymotrypsin and carboxypeptidases A and B were also used to complete some sequence determinations.

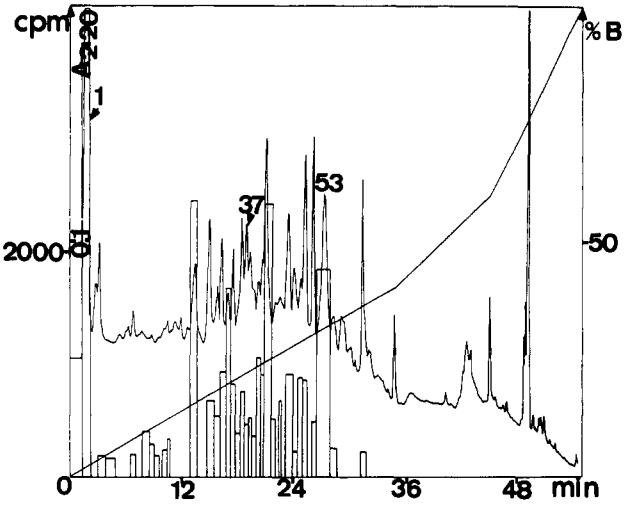

FIGURE 1: High-performance liquid chromatography of tryptic peptides arising from aspartyl-tRNA synthetase aspartylated at pH 6.0. The chromatogram corresponds to a fraction pooled after a first chromatography on a column of Sephadex G-50 (not shown). The column (Novapak C18, 15 × 0.39 cm i.d., particle size 5 μm, Waters Associates) was eluted at room temperature at a flow rate of 1 mL/min with three successive linear gradients made up of two solvents: (A) 0.05% trifluoroacetic acid (v/v) and (B) acetonitrile. Peptides were monitored at 220 nm (Waters Model 450 UV detector, absorbance scale 0.2) and automatically collected with a Gilson Model 201 fraction collector. Rectangles correspond to radioactivity measurements after scintillation counting (see Experimental Procedures). For instance, peak 53 corresponds to peptide 363–372 containing the aspartylated Lys-371 (see Table I, Results, and Discussion) whereas peak 37 corresponds to a nonasparylated peptide (476–485). On the other hand, this peptide was found to be aspartylated at pH 8.6 (see Table I and Discussion).

Results

Fractionation and Purification of Labeled Peptides. Figure 1 shows a typical chromatogram of a tryptic digest of aspartylated aspartyl-tRNA synthetase as obtained by high-performance liquid chromatography on a reverse-phase C18 column after a first fractionation of this digest on a Sephadex G-50 column. In all cases radioactive fractions were systematically subjected to amino acid analysis: for a given peak, molar ratios close to integrate numbers were taken as a good indication of peptide purity. This was further assessed by peptide sequencing: indeed, a peak containing a unique peptide yields a unique sequence by the microsequencing technique of Chang et al. (1978). On the other hand, whenever amino acid analysis suggested a peptide mixture, the corresponding fraction was subjected to further purification steps until homogeneity was reached. This study was carried out on three series of experiments corresponding to enzyme samples aspartylated at pH 6.0, 7.2, and 8.6, respectively.

Characterization of Aspartylated Residues within Peptide Sequences. The sequences of the major aspartylated peptides at the three pH values are listed in Table I. The first striking result is that whatever the pH chosen for the aspartylation reaction, beside lysine residues (Kern et al., 1985), serine and threonine residues are also targets for covalent binding of activated aspartic acid. This means that the ester bond formed between aspartate and serine or threonine alcoholic groups is

[1] Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; TLC, thin-layer chromatography; DABITC, *p*-[[*p*-(dimethylamino)phenyl]azo]phenyl isothiocyanate; DABTH, [[[(dimethylamino)phenyl]azo]phenyl]thiohydantoin; PITC, phenyl isothiocyanate; CNBr, cyanogen bromide; HPLC, high-performance liquid chromatography.

Table I: Sequences of Labeled Peptides

peptide	sequence	amino acid aspartylated	no. of Asp fixed[a]
28–31	Pro-Leu-Ser-Lys	Ser-30	1 (a)
44–47	Gln-Arg-Lys-Lys	Lys-46	1 (a, c), 2 (c)
59–64	Glu-Ala-Arg-Glu-Lys-Lys	Lys-63	1 (b)
78–84	Leu-Pro-Leu-Ile-Gln-Ser-Arg	Ser-83	1 (a, c)
115–119	Val-His-Asn-Thr-Arg	Thr-118	1 (b)
129–130	Thr-Leu	Thr-129	1 (a)
132–142	Gln-Gln-Ala-Ser-Leu-Ile-Gln-Gly-Leu-Val-Lys	Ser-135	1 (a)
157–160	Ala-Gly-Ser-Leu	Ser-159	1 (b)
170–174	Gly-Ile-Val-Lys-Lys	Lys-173	1 (b)
194–204	Ile-Tyr-Thr-Ile-Ser-Glu-Thr-Pro-Glu-Ala-Leu		
196–213	Thr-Ile-Ser-Glu-Thr-Pro-Glu-Ala-Leu-Pro-Ile-Leu-Leu-Glu-Asp-Ala-Ser-Arg	Thr-196, Ser-198, or Thr-200	1 (c)
241–249	Thr-Val-Thr-Asn-Gln-Ala-Ile-Phe-Arg	Thr-241	1 (a, b)
263–265	Ala-Thr-Lys	Thr-264	1 (a)
267–274	Phe-Thr-Glu-Val-His-Thr-Pro-Lys	Thr-268 or -272	2 (c)
326–333	Ala-Glu-Asn-Ser-Asn-Thr-His-Arg	Ser-329	2 (a, c)
362–372	Phe-Val-Phe-Ile-Phe-Ser-Glu-Leu-Pro-Lys-Arg	Ser-367	1 (a), 2 (c)
363–372	Val-Phe-Ile-Phe-Ser-Glu-Leu-Pro-Lys-Arg	Lys-371	0.5 (a)
390–393	Lys-Leu-Pro-Lys	Lys-390	1 (c)
400–410	Leu-Thr-Tyr-Lys-Glu-Gly-Ile-Glu-Met-Leu-Arg	Lys-403	1 (b)
445–453	Leu-Asp-Lys-Phe-Pro-Leu-Glu-Ile-Arg	Lys-447	1 (b)
476–485	Gly-Glu-Glu-Ile-Leu-Ser-Gly-Ala-Gln-Arg	Ser-481	1 (c)
532–544	Val-Val-Met-Phe-Tyr-Leu-Asp-Leu-Lys-Asn-Ile-Arg-Arg	Lys-540	1 (c)

[a] Number of Asp fixed (mol/mol of peptide) at pH 6.0 (a), 7.2 (b), and 8.6 (c).

stable enough to survive the purification procedure by gel filtration and HPLC (see Figure 1) as all serine- and threonine-containing peptides listed in Table I were isolated in good yields (as high as those containing lysine). It must be stressed that when each of these labeled peptides was subjected to Edman–Chang degradation, radioactive aspartic acid was always released as a DABTH derivative at the first step of the degradation together with the N-terminal residue. Therefore, the peptidic-like bond between the α-carboxyl group of aspartic acid and the ϵ-amino group of lysine and the ester bond with amino alcohols (serine or threonine) are both split during the cyclization reaction in trifluoroacetic acid. In some cases radioactive aspartic acid was also released at the second step together with the second residue, and this was regarded as a good evidence for two successive aspartates attached to the same residue of the protein. Indeed, the previous paper (Kern et al., 1985) had shown that two aspartic acid residues (or perhaps more) could be linked to the same side chain of aspartyl-tRNA synthetase, the α-amino group of the first one becoming a new target for the covalent binding of a second one: after dinitrophenylation of the aspartylated enzyme, amino acid analyses gave a significant increase in the aspartic acid content, which showed that some of the incorporated residues had their α-amino group protected by a second aspartylation at the very same site. Therefore, peptide sequencing brings another evidence for this possibility, particularly for the enzyme aspartylated at alkaline pH: peptides 44–47 (Lys-46), 267–274 (Thr-268 or -272), 326–333 (Ser-329), and 363–372 (Ser-367).

Another point to be stressed is the fact that Edman–Chang degradation still left some ambiguities as to the aspartylated residue whenever serine and threonine were simultaneously present in the same labeled peptide. These ambiguities could be lifted in a few instances where digestion with carboxypeptidases A and B definitely established that one of the possible targets was released as a free amino acid: by use of this approach it was shown that Ser-212 (peptide 196–213) was not aspartylated, which only leaves three possibilities for labeling, Thr-196, Ser-198, or Thr-200, while Thr-241 (peptide 241–249) and Ser-329 (peptide 326–333) were labeled. As already mentioned above, the dinitrophenylation method was also used to find out whether the ϵ-amino group of a given lysine residue was free or aspartylated. It proved that Lys-371 was partially aspartylated at pH 6.0 and not at all at pH 8.6 (Table I): indeed, at the latter pH the two incorporated aspartates were successively released after the first and second steps of Edman degradation, the ϵ-amino group of lysine could be completely dinitrophenylated, and free lysine was released by carboxypeptidases A and B. Therefore these two aspartates are attached to the same Ser-367. On the other hand, when aspartylation was carried out at pH 6.0, the molar ratio for aspartic acid was only 1.5 (instead of 2 at pH 8.6). DABTH-Asp was released only after the first step of Edman degradation, and after dinitrophenylation the molar ratio of lysine dropped down to 0.5 (and not 0). This is only possible if the peak corresponding to peptide 363–372 as isolated from the reverse-phase C18 column contains in fact two different species in equimolar amounts, one aspartylated on Ser-367 only and the other one aspartylated on both Ser-367 and Lys-371 (peak 53 in Figure 1). This interesting result proves that covalent binding of one or two aspartates does not change the hydrophilic properties of the corresponding peptides, which can coelute from the reverse-phase column. Peptide 44–47 offers another example of such a situation: indeed, from digests of aspartyl-tRNA synthetase labeled at pH 8.6 a radioactive peak could be isolated that gave two radioactive spots when subjected to electrochromatography on thin-layer cellulose plates. One of these corresponds to peptide 44–47 with one aspartic acid covalently bound to Lys-46 and the other one to the same peptide with two successive aspartates attached to Lys-46. The nonlabeled peptide was also isolated from the same digests, which again shows that some lysine residues are not fully aspartylated. The dinitrophenylation method was used as well in two cases where both histidine and serine or threonine (or the three residues) were present in the same peptide (peptides 267–274 and 326–333): in both cases the imidazole ring was able to react with 1-fluoro-2,4-dinitrobenzene, which rules out histidine as a target for aspartylation. It was important to check this point even though the previous results (Kern et al., 1985) revealed the absence of any aspartylated histidine. Furthermore, digestion of peptide 326–333 with carboxypeptidases A and B yielded free histidine and threonine together with arginine, which only leaves Ser-329 as the aspartylated residue at pH 6.0 or 8.6.

It is also worth noticing that no tyrosine-containing peptides were isolated during the course of this study, which confirms

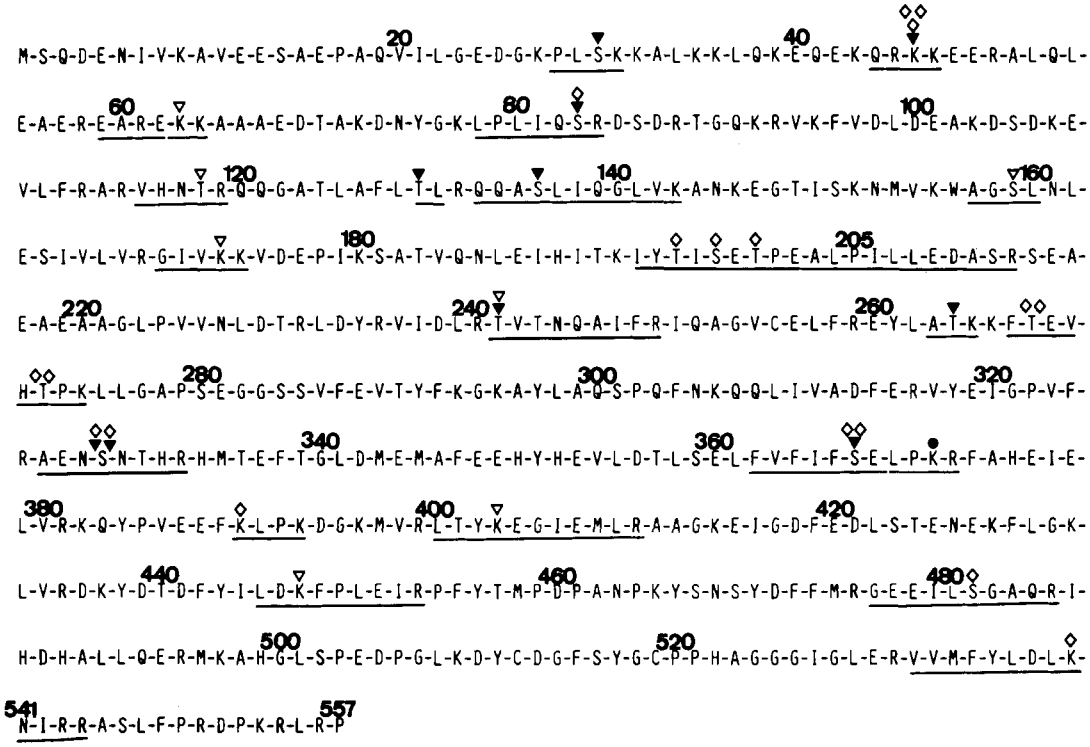

FIGURE 2: Location of the major aspartylated residues within the protein sequence. Peptide aspartylated at pH 6.0: (▼) monoaspartylated, (▼▼) diaspartylated, or (●) half-aspartylated (see text). Peptide aspartylated at pH 7.2: (▽) monoaspartylated. Peptide aspartylated at pH 8.6: (◇) mono- or (◇◇) diaspartylated. Underlined sequences correspond to isolated aspartylated peptides.

the previous results. Indeed, the only possibility could have been Tyr-195 (peptide 194–204), but the combined tryptic–chymotryptic digestions gave the two overlapping peptides 194–204 and 196–213 both bearing one radioactive aspartic acid residue; since an aspartylated phenol ring of Tyr-195 would prevent chymotryptic cleavage of the adjacent bond, this only leaves three possibilities corresponding to Thr-196, Ser-198, or Thr-200 as already pointed out. Finally, under the conditions used in this study, no labeled cysteines could be identified although this residue is a potential target for aspartyl adenylate.

Discussion

First of all it must be emphasized that this work was performed on enzyme samples aspartylated at levels that did not yield any decrease in their catalytic activities. Besides as already mentioned under Experimental Procedures, these samples correspond to the aspartylated dimeric enzyme as they were separated from possible high molecular weight aggregates by chromatography on Sepharose 4B.

Identification of the Labeled Peptides and Their Location in the Sequence. This study confirms the involvement of lysine residues in the covalent binding of aspartic acid to aspartyl-tRNA synthetase via a chemical reaction with its cognate aspartyl adenylate, thus giving rise to amide bonds. This was first supported by amino acid analyses of the aspartylated enzyme (Kern et al., 1985); herein, modified lysine residues were identified within peptides that could be completely purified and unambiguously sequenced. In addition, this work demonstrates that serine and threonine residues are also targets for aspartyl adenylate (Table I). It was at first striking to find them since ester bonds are generally considered as fairly unstable. However, in our case these survived the purification scheme developed for peptide isolation.

It was previously shown that aspartylation could occur in a rather large range of pH values and in the presence of various buffers (Kern et al., 1985). Here three different buffers were selected, namely, Na-Mes buffer, pH 6.0, and Na-Hepes buffers, pH 7.2 and 8.6. The affinity for $tRNA^{Asp}$ is maximal in the first one whereas the catalytic activity is the highest at pH 8.6. Also, the isoelectric point of aspartyl-tRNA synthetase (pH_i 6.4–7.2; Lorber & Giegé, 1983) lies between the two extreme values. Anyhow, whatever the pH used for the aspartylation reaction, all identified aspartylated residues are evenly distributed along the sequence (Figure 2) and there does not seem to be any privileged domain in this respect. Figure 3 shows a hydrophilicity plot vs. the sequence as calculated according to the program of Hopp and Woods (1981). It is interesting to note that most labeled residues coincide with peaks of high hydrophilicity, which is in good agreement with the assumption that aspartylated residues are mostly if not exclusively located at the surface of the protein. But of course the possibility that some labeled residues lie in more hydrophobic regions cannot be entirely ruled out at this stage. On the other hand, the three cyteine residues seem to lie in rather hydrophobic areas, and this could explain the absence of any aspartylated cysteine-containing peptides in our digests. But one must of course keep in mind that a thio ester bond between the thiol group of cysteine and the α-carboxyl group of aspartic acid might be too unstable to survive during the course of peptide purification. In other words, cysteine cannot be entirely ruled out as a possible target. However, it must be emphasized that so far it has not been possible to label any of the three cysteine residues of aspartyl-tRNA synthetase with iodoacetic acid under nondenaturing conditions, which suggests that they are not readily accessible to chemical reagents (unpublished results).

The Presence of Two Successive Aspartic Acid Residues Attached to the Same Side Chain. As shown in Table I, two radioactive aspartic acid residues can be successively attached to the same amino acid side chain of the enzyme, the α-amino group of the first incorporated aspartate becoming a new target for the activated α-carboxyl group of the second one. When aspartylation of aspartyl-tRNA synthetase was carried out at

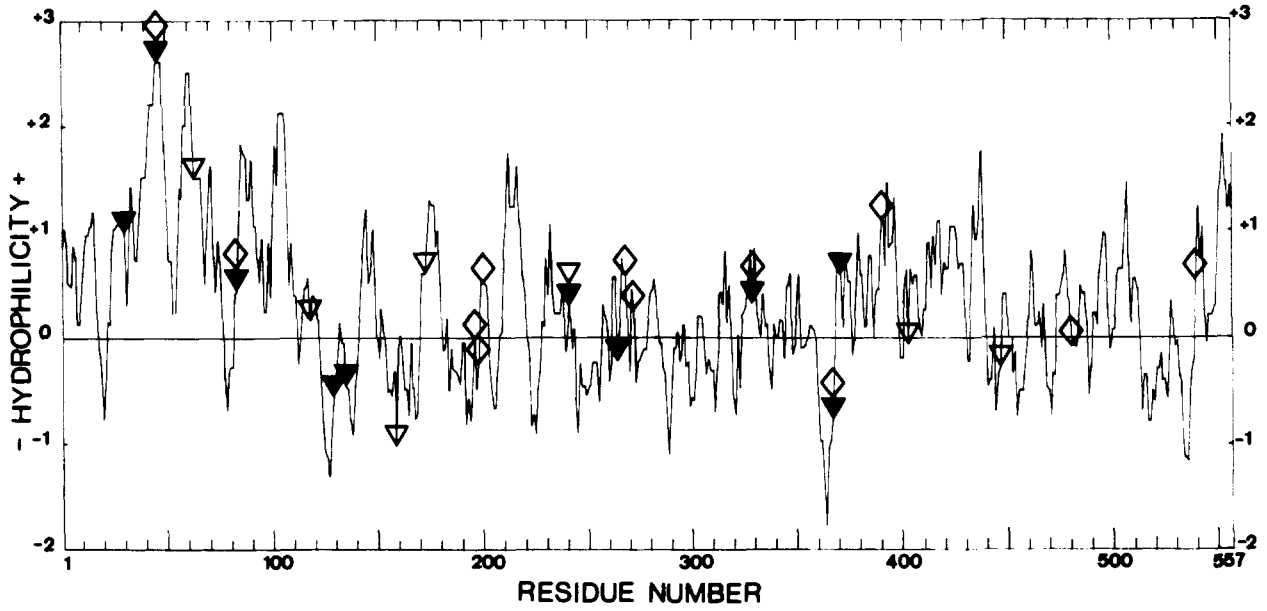

FIGURE 3: Hydrophilicity curve of aspartyl-tRNA synthetase plotted according to Hopp and Woods (1981) with hydrophilicity indexes of the various aspartylated amino acid residues. The various symbols for aspartylated amino acids are as in Figure 2: (▼) pH 6.0, (▽) pH 7.2, and (◇) pH 8.6.

acidic pH, only one peptide was found to bear two aspartates, namely, that containing Ser-329. When this reaction was performed at alkaline pH, four sites of diaspartylation were found: Lys-46, Thr-268 or Thr-272, Ser-329, and Ser-367.

The last peptide (363–372) is interesting in that it was found after reaction at both acidic and alkaline pH. As shown under Results, after aspartylation at pH 6.0 two peptides were actually contained in the same radioactive fraction from the reverse-phase column, one labeled on Ser-367 and the other one on both Ser-367 and Lys-371. In other words, Lys-371 is only partially aspartylated at acidic pH (ca. 50% yield) and not at all at alkaline pH as emphasized by the results of Edman–Chang degradation, dinitrophenylation, and carboxypeptidase A and B digestion.

Isolation of this labeled peptide (363–372 or 362–372) (peak 53, Figure 1) also had another interest in that it allowed filling of a gap in the enzyme sequence that was determined previously (Amiri et al., 1985). This gap corresponds to the stretch 354–371 for which no peptides could be isolated, probably due to high hydrophobic interactions with the reverse-phase C18 column used for peptide purification. Indeed, Figure 2 shows that Ser-367 is preceded by six hydrophobic amino acids. However, this section could be deduced from the nucleotide sequence of the structural gene (Sellami et al., 1986) and was further supported by the isolation of a CNBr fragment (residues 346–397). Unfortunately this fragment could not be completely sequenced, but its amino acid composition fitted the translated DNA sequence. Unambiguous assignment could be made after isolation of more hydrophobic peptides, bearing one or two aspartic acid residues linked to Ser-367 and Lys-371, which were easily eluted from the reverse-phase C18 column. A direct application of this work in the field of protein chemistry is the use of this aspartylation reaction as a tool for purifying highly hydrophobic peptides inasmuch as they contain a target for aspartyl adenylate.

Number and Distribution of Aspartylated Residues as a Function of pH. Another puzzling picture that emerged from these results (Table I) is that the number of the major aspartylated sites and their distribution along the enzyme sequence significantly vary with the pH: indeed, ten, seven, and nine major labeled peptides were characterized after aspartylation in the three different buffers, Na-Mes, pH 6.0, and Na-Hepes, pH 7.2 and 8.6, respectively. These numbers must be compared to the corresponding plateau values previously measured for aspartate incorporation into the enzyme (Kern et al., 1985): eight, six, and eight, respectively. The fact that the number of labeled peptides is always slightly higher than the plateau value is most probably due to an incomplete aspartylation of most if not all targets. This could be established for Lys-46 and Lys-371 since in these two cases a nonlabeled peptide could be identified together with the labeled one. A similar result would be obtained for other aspartylated sites if a systematical analysis was carried out in order to find out whether the enzymatic digest contains both the labeled and the nonlabeled species. This would be the only way to accurately determine the yield of aspartylation for each of the major sites. However, such a study would remain incomplete as each digest contains several minor radioactive peptides that were present at too low a level to allow purification and unambiguous assignment to a given sequence.

At present it is difficult to explain why different peptides were isolated in the three sets of experiments, all the more as the isolation procedure was exactly the same in all three cases. Indeed, of the ten major sites identified after aspartylation at pH 6.0 only four, namely, Lys-46, Ser-83, Ser-329, and Ser-367, were found after labeling at pH 8.6, and none of these was labeled at neutral pH. Besides, only one of the seven residues aspartylated at pH 7.2, Thr-241, was found to be labeled at acidic pH as well. These differences are quite intriguing, and one can only speculate that when pH varies, small conformational changes in the tertiary structure or solvent effect occur that may change accessibility and reactivity of potential targets at the surface of the protein. Indeed, the crucial effect of pH on enzyme solubility was often noticed: solutions of aspartyl-tRNA synthetase can be dialyzed against alkaline (pH 8.6) or acidic (pH 6.0) buffers with no significant losses while a rapid decrease in the pH value (within the range 8.6–6.0) almost invariably causes partial precipitation. Another likely explanation could be that aspartylation of a given target causes local alterations in enzyme properties, thus hampering the labeling of a neighboring potential site. In other words, the four above sites aspartylated at both pH 6.0 and

pH 8.6 might well be labeled at pH 7.2 but at too low a level to allow unambiguous identification. It is worth mentioning that aggregation cannot account for these differences between the three pH values since all protein chemistry experiments were performed on the dimeric enzyme after separation from aggregates as stated above. Anyhow, this odd behavior of aspartyl-tRNA synthetase with regard to the nature and pH of the buffer chosen for the labeling reaction should be kept in mind if this labeling method by the enzymatically synthesized aspartyl adenylate is to be used to probe the surface of any protein.

Structural Interpretation of the Aspartylation Data. As already pointed out, most aspartylation sites seem to belong to highly hydrophilic areas of the protein. It is also worth mentioning that they are never located in the predicted random coils according to the secondary structure predictions made from the sequence (Amiri et al., 1985); in other words, they would rather be part of ordered sequences, α-helices, β-strands, or turns.

That some of these aspartylated residues lie within highly accessible domains of aspartyl-tRNA synthetase could be further assessed by the results of limited proteolysis, which will be described in a forthcoming paper: indeed, the first 50–52 and last 17–20 residues could easily be removed by limited tryptic or chymotryptic digestions. Besides, it should be recalled that the purified enzyme has a frayed N-terminal sequence as it contains several types of subunits starting at or near Gln-19 (Amiri et al., 1985). In other words, the first 19 residues (starting from the initiating Met-1) are practically absent from highly purified samples due to limited proteolysis during enzyme isolation. All these results emphasize the accessibility of the N-terminal section 1–50 to various probes, and indeed, it contains two of the main targets, Ser-30 and Lys-46, the latter being diaspartylated at pH 8.6 (Table I). Similarly, Lys-540, another aspartylated residue, is at or near a site of limited tryptic or chymotryptic cleavage as the last 17–20 residues are easily chopped off by these two proteases as well.

In conclusion, it is now worth focusing on those residues important for tRNA–enzyme interaction. Such residues will be investigated with various chemical labeling techniques on both the enzyme and the enzyme–tRNA complex. Among these, aspartylation of the complex can be tried provided a two-cell compartment system is used as already studied (Kern et al., 1985): one containing the adenylate generating system and the other one the tRNA–enzyme complex. Such a system is absolutely necessary since in the presence of $tRNA^{Asp}$ only aminoacylation of this tRNA by the activated aspartic acid will occur and no covalent binding of the amino acid to the synthetase will take place. All these possibilities are being explored. From a different standpoint, the identification of these aspartylated residues, most likely located on the enzyme surface, will provide independent and useful structural informations when interpreting electron-density maps of both free and complexed enzyme.

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