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Biochemistry

Conversion of isoaspartyl peptides to normal peptides: Implications for the cellular repair of damaged proteins

(protein methylation/carboxyl methyltransferase/erythrocyte)

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ABSTRACT **The hypothesis that cellular protein carboxylmethylation reactions recognize altered aspartyl residues as part of a protein repair pathway has been tested in an *in vitro* system using tetragastrin (Trp-Met-Asp-Phe-NH_2) as a model sequence. The L-isoaspartyl form of tetragastrin, where the phenylalanine residue is linked to the side-chain carboxyl group of the aspartate residue ([*iso*-Asp^3]tetragastrin), is a substrate for the erythrocyte protein carboxyl methyltransferases, while the normal form is not. The enzymatically produced α-methyl ester of [*iso*-Asp^3]tetragastrin, [*iso*-Asp(OMe)^3]tetragastrin, is unstable at pH 7.4 and 37°C and spontaneously demethylates with a half-time of 41 min to an intermediate L-succinimide form ([Asu^3]tetragastrin) that, in turn, spontaneously hydrolyzes with a half time of 116 min to give a mixture of normal tetragastrin (20%) and [*iso*-Asp^3]tetragastrin (80%). This sequence of enzymatic and nonenzymatic reactions can be coupled in a single reaction mixture; the [*iso*-Asp^3]tetragastrin that is produced upon succinimide hydrolysis can reenter the reaction sequence by enzymatic methylation, and the net result of the process is the conversion of the isomerized peptide to the normal peptide. The efficiency of this "repair" reaction is limited by a side reaction of racemization at the α-carbon of the succinimide (half-time = 580 min). In a 24-hr time period, normal L-aspartyl-containing tetragastrin is obtained in about 50% yield from the coupled reaction mixture; other products include [D-*iso*-Asp^3]tetragastrin and [D-Asp^3]tetragastrin. The versatile chemistry of succinimide peptides suggests that methylated L-isoaspartyl sites (and possibly methylated D-aspartyl sites) in cellular polypeptides can eventually yield "repaired" normal L-aspartyl sites through succinimide intermediates.**

Of the many enzymatic methylation reactions that have been described (1), mammalian protein carboxyl-methylation reactions appear to be among the most flexible with regard to substrate specificity. For instance, over 40 distinct membrane and cytosolic polypeptides have been found to be methyl-acceptor substrates in intact human erythrocytes (2–5). The broad specificity of these reactions can be explained by studies showing that the common feature of methyl-acceptor sites is the occurrence of an altered aspartyl residue. For example, methylated D-aspartyl residues have been isolated from proteolytically digested erythrocyte proteins (3, 6). The enzymatic methylation of substrates by the brain enzyme is induced by the formation of β-isomerized L-aspartyl (isoaspartyl) sites (7), and synthetic peptides containing L-isoaspartyl (but not normal L-aspartyl) residues are stoichiometrically methylated by both the brain and erythrocyte methyltransferases (8, 9). These results suggest that proteins containing either L-isoaspartyl or D-aspartyl residues are recognized by protein carboxyl methyltransferases. It has been proposed that these residues could originate either as errors in protein synthesis or as degradation products of normal L-aspartyl and L-asparginyl residues in proteins (for a review, see ref. 5).

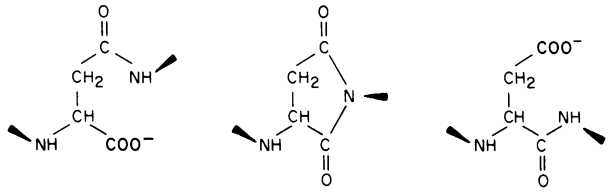

FIG. 1. Structure of aspartyl peptide derivatives.

The physiological role of this enzymatic activity is still unclear. It is known that enzymatically formed methyl esters of proteins and isoaspartyl peptides are rapidly demethylated at pH 7.4 and 37°C, probably by an intramolecular mechanism that results in the formation of a transiently stable succinimide intermediate (refs. 10–13; see structures of Fig. 1). Succinimide formation can promote further bond rearrangements, including racemization and isomerization (6, 14–16). Thus, it has been proposed that the function of protein carboxyl methylation is to chemically activate atypical aspartyl residues by ester formation (6–8). Such activation will allow succinimide formation and possibly allow a partial "repair" reaction that would result in the formation of L-aspartyl residues from D-aspartyl or L-isoaspartyl residues. Such a physiological repair function might restore function to damaged proteins.

To test this hypothesis, the L-isoaspartyl form of tetragastrin ([*iso*-Asp^3]tetragastrin; normal tetragastrin = Trp-Met-Asp-Phe-NH_2) was chosen as a model peptide. With purified carboxyl methyltransferase from bovine erythrocytes, the conversion of this peptide to normal tetragastrin has been achieved at the predicted efficiency. The main limitation on complete conversion appears to be the racemization of the succinimide residue. While this work was in progress, similar results were obtained with a methyltransferase from bovine brain (17).

MATERIALS AND METHODS

Materials. Tetragastrin (Sigma) was chemically converted to the derivative peptides [*iso*-Asp^3]tetragastrin, [D-*iso*-

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Abbreviations: [*iso*-Asp^3]tetragastrin, 3-isoaspartyl-substituted tetragastrin; [*iso*-Asp(OMe)^3]tetragastrin, α-methyl ester of [*iso*-Asp^3] tetragastrin; [Asu^3]tetragastrin, 3-succinimide derivative of tetragastrin; [Asp(OMe)^3]tetragastrin, β-methyl ester of normal tetragastrin; AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine.

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Asp[3]]tetragastrin, [D-Asp[3]]tetragastrin, and the L-succinimide derivative [Asu[3]]tetragastrin as described (9). Briefly, normal tetragastrin was purified by reversed-phase HPLC, chemically methyl-esterified to the β-methyl ester, [Asp(OMe)[3]]tetragastrin, and then deesterified for 260 min at 37°C in 0.1 M sodium phosphate (pH 7.4) to promote succinimide formation and isomerization/racemization reactions. Peptide products were then separated from the reaction mixture by reversed-phase HPLC. For a typical reaction, the following proportions of products were observed: [*iso*-Asp[3]]tetragastrin, 27.8%; [D-*iso*-Asp[3]]tetragastrin, 0.9%; normal tetragastrin, 8.3%; [D-Asp[3]]tetragastrin, 0.4%; [Asu[3]]-tetragastrin, 30.9% (containing a small proportion of [D-Asu[3]]tetragastrin); unreacted [Asp(OMe)[3]]tetragastrin, 27.4%; and unknown minor products, 4.3%. Standard solutions of these purified peptides were prepared in 0.05% trifluoroacetic acid. The α-methyl ester of [*iso*-Asp[3]]tetragastrin, [*iso*-Asp(OMe)[3]]tetragastrin, was prepared synthetically by methyl-esterification (8 nmol in 1 ml of 0.1 M HCl/MeOH for 19 hr at 23°C as described for preparation of [Asp(OMe)[3]]tetragastrin; ref. 9) and was purified by HPLC. The product had identical chromatographic behavior as the α-methyl ester produced from the enzymatic carboxylmethylation of [*iso*-Asp[3]]tetragastrin.

Protein carboxyl methyltransferase was a generous gift of J. Gilbert of this laboratory and was purified from bovine erythrocytes to a specific activity of 2900 pmol·min^{-1}·(mg of protein)$^{-1}$ by ammonium sulfate precipitation, Sephadex G-75 gel-filtration chromatography, and DEAE-cellulose chromatography. The enzyme was concentrated by ultrafiltration with a final buffer composition of 20 mM Tris·HCl/0.2 mM Na_2EDTA/10% glycerol/15 mM 2-mercaptoethanol/25 μM phenylmethylsulfonyl fluoride at pH 8.0. The DEAE-cellulose step separates the activity into two isozymes; the more acidic isozyme (II) was used in these experiments (J. Gilbert, I. M. Ota, and S.C., unpublished data). The purity of this enzyme preparation was estimated at 90% from gel electrophoresis in sodium dodecyl sulfate.

S-Adenosyl-L-methionine (AdoMet) hydrogen sulfate was obtained from Boehringer Mannheim, stock solutions were prepared in 10 mM HCl, and their concentration and purity were monitored by HPLC (254-nm detection). These preparations contained <1% *S*-adenosyl-L-homocysteine (AdoHcy). AdoHcy hydrolase was provided by B. Matuszewska and R. Borchardt (Department of Pharmaceutical Chemistry, University of Kansas). The preparation (0.07 mg of protein per ml) was purified from *Alcaligenes faecalis* to a specific activity of 0.77 μmol of inosine formed per mg of protein per min. Adenosine deaminase (Sigma type I, calf intestinal mucosa; 2 μmol of adenosine deaminated per min per μl of enzyme preparation) was transferred to incubation mixtures as an ammonium sulfate suspension.

Reversed-Phase HPLC. A Waters dual-pump system, Alltech (Dearfield, IL) Econosphere 5-μm C_{18} reversed-phase columns (4.6 mm × 250 mm), and solvent system [solvent A, 0.1% trifluoroacetic acid; solvent B, 0.1% trifluoroacetic acid/90% (vol/vol) acetonitrile] were used as described (9). Tetragastrin peptides were detected by absorbance at 214 nm, assuming an extinction coefficient of 35,000 $M^{-1}\cdot cm^{-1}$ for each species. Peak elution times varied depending upon whether a guard column was included in the separation system; standard peptides were used to calibrate the separation system during each experiment.

D/L-Aspartic Acid Determination. Lyophilized samples of HPLC-purified peptides were acid-hydrolyzed *in vacuo* (in 6 M HCl for 1 hr at 150°C or 6 hr at 108°C using a Waters Pico Tag Work Station) and were derivatized with an *o*-phthalaldehyde/*N*-acetyl-L-cysteine reagent. The resulting fluorescent D- and L-aspartyl adducts were chromatographed and quantified by the method of Aswad (18) as modified by Murray and Clarke (13). Pure standards of L-form tetragastrin peptides were tested to measure racemization due to the acid hydrolysis (2% D-aspartyl formation at 108°C; 7.5% D-aspartyl formation at 150°C), and the results were corrected for this background racemization.

RESULTS

[*iso*-Asp[3]]Tetragastrin has been shown (9) to be a high-affinity substrate for the erythrocyte protein carboxyl methyltransferase. The product of the methylation reaction is [*iso*-Asp(OMe)[3]]tetragastrin and was found in preliminary experiments to demethylate spontaneously to form a series of rearranged peptide intermediates and products at pH 7.4. Thus, it seemed possible that the enzymatic methylation reaction and the nonenzymatic demethylation reactions could be coupled in a single incubation mixture. We performed this experiment by enzymatically methylating [*iso*-Asp[3]]tetragastrin at pH 7.4, where the nonenzymatic formation of intermediate and rearranged peptides is promoted.

Fig. 2 shows the results of such a preliminary experiment

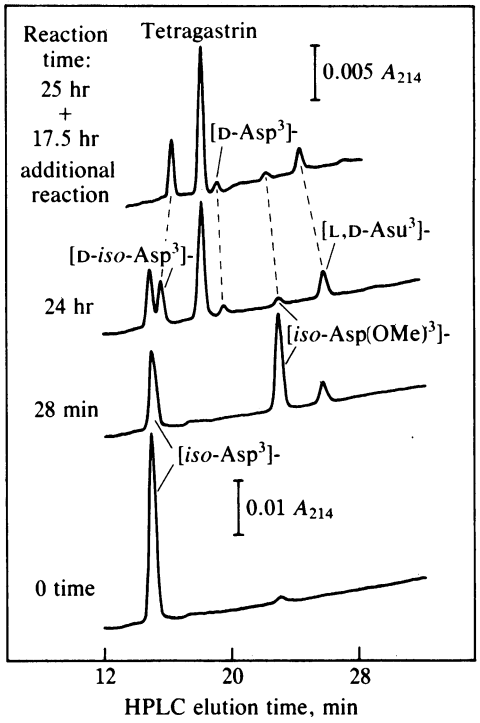

FIG. 2. HPLC analysis of tetragastrin peptides formed by enzymatic methylation and nonenzymatic demethylation reactions. [*iso*-Asp[3]]Tetragastrin was incubated with AdoMet and protein carboxyl methyltransferase at pH 7.4 and 37°C, and the peptide reaction products were separated and quantified by HPLC. The incubation mixture (final volume, 66 μl) contained bovine erythrocyte protein carboxyl methyltransferase II (20 μl; 1330 pmol·min^{-1}·ml^{-1}), AdoMet (3 μl, 5.2 mM), [*iso*-Asp[3]]tetragastrin (10 μl; 160 μM), and 0.1 M sodium phosphate (pH 7.4; 33 μl). After incubation at 37°C, aliquots (20 μl) of the reaction mixture were analyzed by reversed-phase HPLC (1 ml per min flow rate; 20–40% solvent B linear gradient for 40 min), with detection at 214 nm. For the uppermost chromatograph, a 20-μl reaction mixture identical in composition was incubated for 25 hr at 37°C and was then supplemented with additional methyltransferase (5 μl) and additional AdoMet stock solution (2 μl). The incubation was continued at 37°C for an additional 17.5 hr to drive the reaction to completion. An aliquot of the mixture (13.5 μl) was then analyzed by reversed-phase HPLC as above. For this chromatogram, removal of the guard column resulted in small shifts in the peptide elution times. The identity of the peaks was established by experiments in which standard peptides were cochromatographed with the reaction mixtures (data not shown).

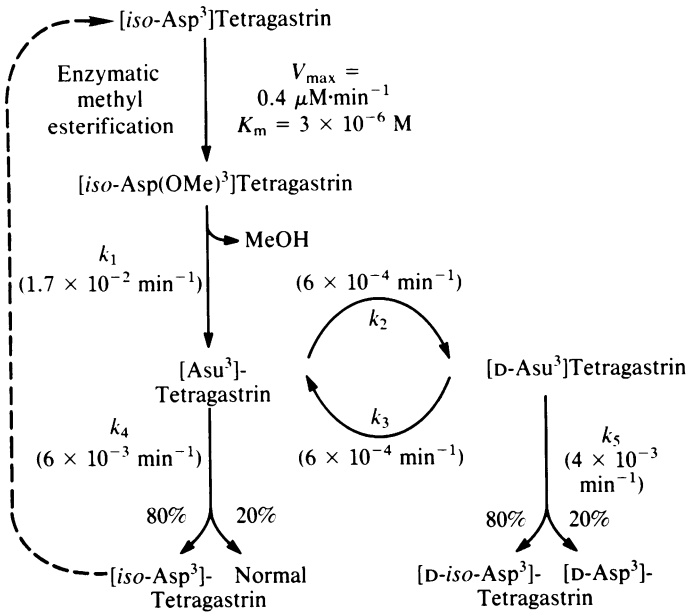

FIG. 3. Enzymatic and nonenzymatic reaction pathways for the conversion of [*iso*-Asp³]tetragastrin to normal tetragastrin and other products. In this scheme, the only peptide recognized by the carboxyl methyltransferase is [*iso*-Asp³]tetragastrin. This is the only peptide that can reenter the reaction sequence, and the products that build up are normal tetragastrin and its D-aspartyl and D-isoaspartyl derivatives. Rate constants for the individual steps were determined from the data described below. The V_{max} for the methyltransferase was calculated for the particular amount of enzyme used in these experiments.

as analyzed by reversed-phase HPLC. At zero reaction time, the [*iso*-Asp³]tetragastrin was eluted at its characteristic position in the chromatogram. At an early reaction time (28 min), the formation of the methylated product ([*iso*-Asp(OMe)³]tetragastrin) was seen as well as [Asu³]tetragastrin. As will be shown below, the succinimide derivative was the immediate product of [*iso*-Asp(OMe)³]tetragastrin demethylation. Fig. 2 also shows a later time point (24 hr) in the reaction sequence. Three new peaks not observed at earlier times were present as major components. These new species were determined to be [D-*iso*-Asp³]tetragastrin, [D-Asp³]tetragastrin, and normal L-aspartyl-containing tetragastrin. Thus, at some stage of the reaction, epimerization and isomerization at the aspartyl center had occurred. The major product of this reaction is the normal peptide, which represents a net "repair" of L-isoaspartyl-containing tetragastrin to the normal form under these conditions.

With the intent of driving the reaction to completion, more methyltransferase and AdoMet were added to the reaction after 24 hr, and the incubation was continued for an additional 17.5 hr. The uppermost chromatogram in Fig. 2 shows that the additional incubation did deplete the remaining [*iso*-Asp³] tetragastrin and increased the final yields of [D-*iso*-Asp³]-, [D-Asp³]-, and normal tetragastrins. Presumably, further incubation would have led to a complete disappearance of the remaining [*iso*-Asp(OMe)³]- and [Asu³]tetragastrins seen in the uppermost chromatogram (Fig. 2). We hypothesize that the combination of enzymatic and nonenzymatic steps shown schematically in Fig. 3 results in a net "repair" reaction with a significant build-up of epimerized by-products. We next examined the intermediate stages of this overall process.

The First Step of the "Repair" Reaction: Enzymatic Methylation of the Isoaspartyl Peptide. The enzymatic methylation of [*iso*-Asp³]tetragastrin is described by simple Michaelis–Menten kinetics. The K_m for the bovine erythrocyte enzyme of this peptide was determined here to be $3 \pm 1 \times 10^{-6}$ M at pH 7.4 using assay conditions similar to those described in Fig. 2 (data not shown). This K_m value is similar to the value of 5×10^{-6} M measured for the human erythrocyte methyltransferase and is comparable to values for other L-isoaspartyl-containing peptides (9). The V_{max} of the purified bovine enzyme (at pH 7.4) was measured at 2900 pmol·min^{-1}·(mg of protein)$^{-1}$.

The Second Step of the "Repair" Reaction: Spontaneous Demethylation. To measure the kinetics of demethylation, synthetic [*iso*-Asp(OMe)³]tetragastrin was incubated in 0.1 M sodium phosphate at pH 7.4 and 37°C, and the disappearance of the ester was monitored by HPLC (data not shown). The decay of ester was consistent with a first-order process with a half-time of 41 min ($k_1 = 1.7 \times 10^{-2}$ min^{-1}). HPLC analysis confirmed that the α-methyl ester was converted to [Asu³]tetragastrin as the immediate product of demethylation, with identical kinetics of ester decay and succinimide formation. This measured stability for this ester is within the range of that observed for the protein methyl esters of intact red cells (11).

The Third Step of the "Repair" Reaction: Succinimide Opening with Competing Succinimide Epimerization. To measure the stability of the succinimide derivative of tetragastrin, a standard of this material was prepared by chemical methods and was incubated at pH 7.4 and 37°C. The time-dependent loss of succinimide and the accumulation of products formed by the ring-opening reaction were measured by HPLC (Fig. 4). As the ring-opening proceeded, the four expected products ([*iso*-Asp³]-, [D-*iso*-Asp³]-, [D-Asp³]-, and normal tetragastrins) formed at rates proportional to the remaining amounts of [Asu³]tetragastrin and [D-Asu³]tetragastrin, suggesting that no other intermediates were involved.

The measured kinetics of the reaction (Fig. 4) were consistent with a model in which the [Asu³]tetragastrin and the [D-Asu³]tetragastrin interconvert by a first-order epimerization reaction with a half-time of 580 min, and the D- and L-succinimide residues open by first-order hydrolytic processes with half-times of 173 min and 116 min, respectively. The hydrolytic opening of succinimides can occur at either the α-carbonyl group to yield an isoaspartyl residue or at the β-carbonyl to yield a normal aspartyl residue. As has been generally noted (8, 9, 14, 15), the product containing the isoaspartyl residue predominated and accounted for 80% of the product. In this model we have assumed that the rate of epimerization measured for the conversion of [Asu³]tetra-

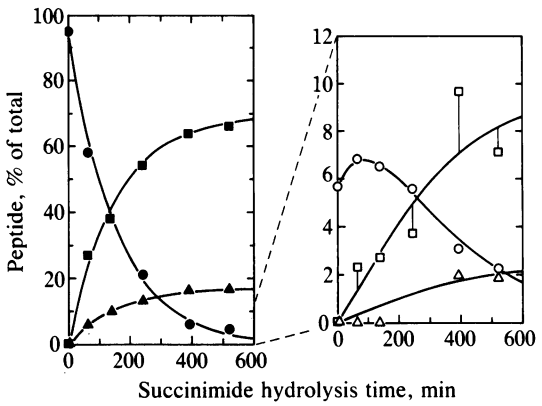

FIG. 4. HPLC analysis of tetragastrin succinimide hydrolysis and epimerization at pH 7.4. Tetragastrin succinimide (1 mM in 0.05% trifluoroacetic acid; containing 94.4% [Asu^3]tetragastrin and 5.6% [D-Asu^3]tetragastrin) was diluted with 4 vol of 0.1 M sodium phosphate (pH 7.4). After various times at 37°C, aliquots were removed and frozen on dry ice until analysis by HPLC. (*Left*) L-Stereoisomers: [Asu^3]tetragastrin (●), [*iso*-Asp^3]tetragastrin (■), and tetragastrin (▲). (*Right*) D-Stereoisomers: [D-Asu^3]tetragastrin (○), [D-*iso*-Asp^3]tetragastrin (□), and [D-Asp^3]tetragastrin (△). The [Asu^3]- and [D-Asu^3]tetragastrin forms were not separated by HPLC; the configuration of this material was determined from the D/L-aspartate ratio of hydrolysates of the peak fractions. The total amount of peptide at each time point equals 100%; all of the peptide at the beginning of the time course could be accounted for at later times. The data were fit (curves) with a kinetic scheme (Fig. 3) in which it is assumed that the L-succinimide (*Left*) and D-succinimide (*Right*) derivatives interconvert by a first-order process ($k_2 = k_3 = 6 \times 10^{-4}$ min^{-1}); that [Asu^3]tetragastrin opens by first-order hydrolysis ($k_4 = 6 \times 10^{-3}$ min^{-1}) to yield a mixture of [*iso*-Asp^3]tetragastrin (80%) and normal tetragastrin (20%) products, and that the [D-Asu^3]tetragastrin opens by first-order hydrolysis ($k_5 = 4 \times 10^{-3}$ min^{-1}) to give the same proportion of [D-*iso*-Asp^3]tetragastrin and [D-Asp^3]tetragastrin. Calculations were performed iteratively at short-time intervals by using an electronic spread sheet program (Lotus Jazz) on a MacIntosh 512K microcomputer. Changing the values of these kinetic constants by more than 10% markedly diminished the empirical fit of the kinetic scheme to the measured data points.

gastrin to [D-Asu^3]tetragastrin is equal to that of the reverse reaction.

Isoaspartyl "Repair" by Coupled Enzymatic/Nonenzymatic Steps. Reaction mixtures containing [*iso*-Asp^3]tetragastrin, carboxyl methyltransferase, and AdoMet were analyzed over a time course exceeding 24 hr (Fig. 5). The amount of methyltransferase activity present was sufficient to convert [*iso*-Asp^3]tetragastrin to [*iso*-Asp(OMe)^3]tetragastrin at a velocity of 0.4 μM per min. Experiments were performed with and without an enzymatic scavenging system (AdoHcy hydrolase and adenosine deaminase) to deplete AdoHcy and to relieve the end-product inhibition of the methylation reaction by this species.

Fig. 5 shows that the behavior of the coupled reaction system could be described on the basis of the behavior of the individual steps of the reaction studied above. [*iso*-Asp^3]Tetragastrin was quickly consumed, and the methylated product was gradually converted to the succinimide derivative. As the reaction proceeded, the nonmethylatable products (normal, [D-Asp^3], and [D-*iso*-Asp^3]tetragastrins) continued to build up. When the experimental data were compared to theoretical predictions based on the kinetic constants determined above (K_m, V_{max}, k_1, k_2, k_3, k_4, k_5; see Fig. 3), the major deviation was that the predicted, nearly complete conversion of [*iso*-Asp^3]tetragastrin did not occur. It is possible that this was a result of the partial inactivation of the methyltransferase during the incubation. This may result from the buildup of the methyltransferase inhibitor AdoHcy. Including a scavenger system for AdoHcy substantially lowered the levels of [*iso*-Asp^3]tetragastrin at intermediate times and concomitantly increased the intermediate levels of [*iso*-Asp(OMe)^3]tetragastrin and [Asu^3]tetragastrin. It also was noted that the experimental recovery of normal tetragastrin was somewhat below the theoretical efficiency of this process in both cases. At 1600 min of reaction time, approximately 60% of the total peptide was predicted to be in the normal configuration, while (by extrapolation) the experimental yield would not quite be 50%. This lowered yield of "repaired" normal product could be due to methyltransferase denaturation or a selective degradation of a small amount of normal peptide by a contaminating protease. It seems clear, however, that the experimental behavior of the system is very close to the predicted model.

DISCUSSION

The substoichiometry of mammalian protein carboxyl-methylation reactions has made it difficult to follow the chemical events following the spontaneous loss of the methyl ester (11). The availability of peptide substrates for this enzyme (7–9) has recently made possible the observation that demethylation can occur via an intramolecular reaction leading to succinimide formation (12, 13). In the experiments described here, we have used tetragastrin peptides to follow the further spontaneous epimerization and hydrolysis of the succinimide derivative, and the entire sequence of enzymatic and nonenzymatic reactions (Fig. 3) has been coupled in a single mixture (Fig. 5).

The formation of normal tetragastrin from [*iso*-Asp^3]-tetragastrin suggests that an L-isoaspartyl residue in a protein could potentially be converted to a normal L-aspartyl residue. These experiments have been performed under physiological conditions of pH and temperature and with concentrations of protein carboxyl methyltransferase similar to those found in intact erythrocytes. In these cells, which lack the capacity for protein synthesis, the accumulation of potentially nonfunctional isoaspartyl proteins may limit their life span in the circulation. The presence of the methylation system may help to insure that erythrocytes continue to function for their 120-day existence.

It should be stressed that the sequences of reactions observed in this study with purified methyltransferase and peptide substrates may not describe the net effect of the methylation reaction in proteins. Although L-isoaspartyl-containing peptides are now firmly established as stoichiometric substrates for mammalian protein carboxyl methyltransferase (7–9, 19), no evidence has yet been presented for the existence of a methylated L-isoaspartyl residue in a cellular protein. Furthermore, even though the rates of protein demethylation are compatible with succinimide formation, these succinimide derivatives have also not yet been directly observed in proteins. Finally, the participation of other enzymes that may act directly on the protein methyl ester (or succinimide derivative) to effect repair or degradation have not been ruled out.

L-Isoaspartyl residues may form in proteins either by spontaneous degradation reactions of L-aspartyl and L-asparginyl residues (5, 20) or by the misincorporation of L-aspartyl residues in protein synthesis (8). If an isoaspartyl residue is derived from an asparaginyl residue, then the coupled enzymatic/nonenzymatic pathway described here would be adequate only for "repairing" the residue to the normal L-aspartyl configuration; repair back to L-asparagine would require the incorporation of an amide group.

Another form of aspartyl damage is reflected by the finding of D-aspartic acid in hydrolysates of aging proteins (21–23). Methylated D-aspartyl residues have been isolated previously from erythrocyte proteins (3, 6), suggesting that enzymatic

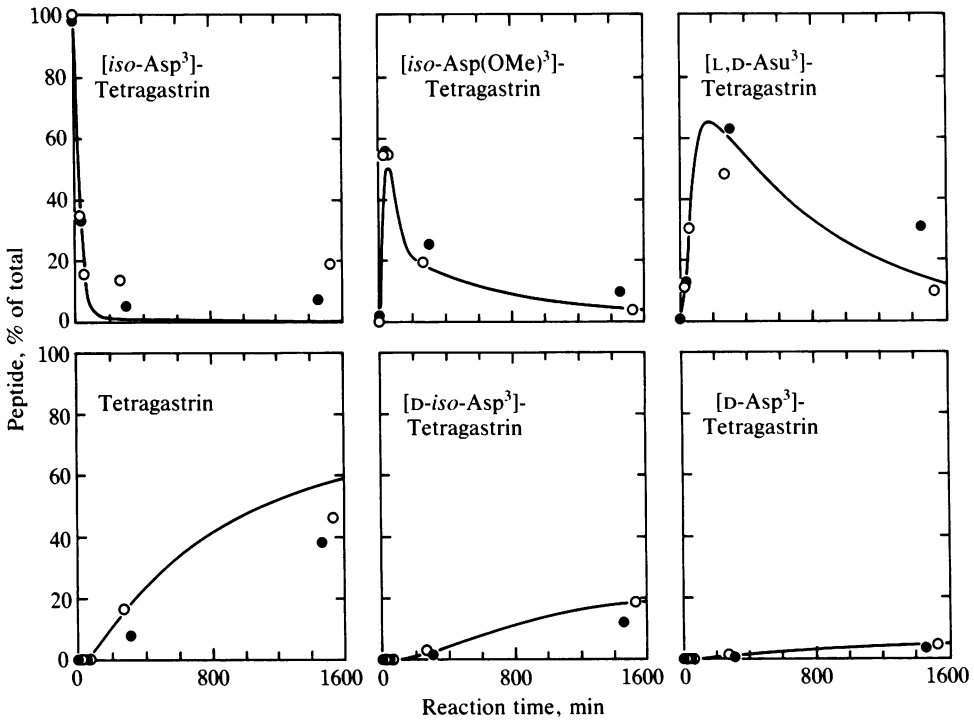

FIG. 5. Time course of the overall conversion of [*iso*-Asp³]tetragastrin to normal tetragastrin and side products catalyzed by the protein carboxyl methyltransferase. [*iso*-Asp³]Tetragastrin was treated with bovine protein carboxyl methyltransferase II and AdoMet at 37°C and pH 7.4, and the peptide products were analyzed at various times by reversed-phase HPLC. Two sets of reaction conditions were used. Under the first set of conditions, giving open-symbol data, 30 μl of [*iso*-Asp³]tetragastrin (133 μM) was incubated (37°C) with 60 μl of bovine methyltransferase (0.46 mg of protein per ml), 10 μl of 5.2 mM AdoMet, and 100 μl of 0.1 M sodium phosphate (pH 7.4). The amounts of each derivative were quantified from the UV absorbance of the HPLC peaks. Under the second set of conditions, giving closed-symbol data, AdoHcy hydrolase and adenosine deaminase were included in the incubation mixture to remove the product inhibitor AdoHcy [30 μl of 1.7 mM AdoMet, 5 μl of AdoHcy hydrolase, 2 μl of adenosine deaminase, and 73 μl of pH 7.4 buffer (0.1 M sodium phosphate)]. The measured accumulation of products was compared to that predicted for the reaction scheme shown in Fig. 3. The smooth curves shown here were calculated from the experimentally determined enzymatic and nonenzymatic rate constants (Fig. 3) by a computer-aided iteration process.

methylation of infrequent D-aspartyl sites occurs *in vivo*. However, no model peptides containing D-aspartyl residues have been found to act as *in vitro* substrates for the erythrocyte or brain methyltransferases (8, 9). Such methylated residues would be expected to demethylate spontaneously to the D-succinimide residue, which could then epimerize to the L-succinimide residue and yield normal and L-isoaspartyl sites as products. Thus, rapid epimerization of succinimides may enable D-aspartyl damage to be at least partly repaired. This pathway could not be very efficient, however, since D-succinimide hydrolysis would be expected to yield substantial amounts of the D-isoaspartyl derivative, and no evidence has been presented for the methylation of such a product.

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