Subunit Structure of Dipeptidyl Transferase*

Robert M. Metrione,† Yoshihiro Okuda,‡ and Gordon F. Fairclough, Jr.§

ABSTRACT: In the presence of relatively low concentrations (1.0–3.0 M) of guanidinium chloride beef spleen dipeptidyl transferase (mol wt 197,000) dissociates rapidly into subunits having a sedimentation coefficient of 6 S and slowly to a component having a sedimentation coefficient of 2 S. At higher concentrations of guanidinium chloride, in the presence of 8 M urea or at pH values below 3, the enzyme dissociates into the smaller subunits having a sedimentation coefficient near 1.8 S. The molecular weight of the smaller subunits has been determined by sedimentation equilibrium, gel electrophoresis in the presence of sodium dodecyl sulfate, and gel exclusion chromatography and found to be about 24,000. The enzyme contains 7–8 sulfhydryl groups/unit of 197,000 in buffer, 6 M guanidinium chloride, 8 M urea, or 1% sodium dodecyl sulfate. Amino-terminal analysis indicates the presence of 6 amino-terminal aspartic acid (or asparagine) and of 2 amino-terminal leucine residues per unit of 197,000. The data suggest that the native enzyme is composed of 8 subunits, in the form of 2 tetramers, and that 2 different types of subunits may be present.

In a previous communication (Metrione *et al.*, 1966) the purification of beef spleen dipeptidyl transferase was reported and the enzyme was shown to undergo dissociation into subunits upon treatment with *p*-mercuribenzoate or sodium dodecyl sulfate. The finding that dipeptidyl transferase is an aggregate of subunits has suggested the possibility that the activity of the enzyme may involve the cooperative action of catalytic sites on separate subunits (Würz *et al.*, 1962; Metrione *et al.*, 1966). The effect of low concentrations of urea on the polymerase and hydrolase activities of dipeptidyl transferase (Heinrich and Fruton, 1968) has led to the suggestion that the individual subunits may be capable of catalyzing the hydrolysis of the substrate while a concerted action of more than 1 subunit may be required for polymerase activity. Studies were, therefore, undertaken to characterize the dissociation behavior of the enzyme, and to examine the relationship between its state of aggregation and its catalytic properties as a hydrolase and a polymerase.

Experimental Section

Determination of Enzyme Activity. The transamidation assay, with glycyl-L-phenylalaninamide as the substrate, was employed in the manner described previously (Metrione *et al.*, 1966).

Further Purification of Dipeptidyl Transferase. The enzyme was purified in the manner described previously to yield the CM fraction (Metrione *et al.*, 1966). Further purification to remove traces of material with a lower sedimentation coefficient which was present in some preparations was achieved by fractionation with ammonium sulfate.

METHOD I. In a typical preparation, solid ammonium sulfate (Mann Enzyme Grade) was added to 80% of saturation to an ice-cold solution of CM fraction (46 mg of protein/ml in 37.2 ml of 0.1 M KCl; specific activity 23.8 units/mg). The precipitate was centrifuged, washed with 25 ml of cold 65% saturated ammonium sulfate, and successively extracted three times with 50-ml portions of 60% saturated ammonium sulfate and three times with 50-ml portions of 55% saturated ammonium sulfate. The extracts were allowed to stand for 16 days at room temperature (*ca.* 24°) in closed vials, and the resulting precipitates were collected by centrifugation. The deposition of protein precipitate proceeded slowly, and microscopic observation showed the presence of apparently crystalline material (plates) together with less regular forms. The combined precipitates were suspended in water and dialyzed against 0.1 M KCl to yield 463 mg of AS-1 fraction, having a specific activity of 28.0 units/mg.

The enzyme was reprecipitated by the addition of a solution of saturated ammonium sulfate to incipient cloudiness (45% of saturation), centrifuged to remove colored insoluble material, and by allowing the supernatant fluid to stand at room temperature in a closed vial for 28 days. The resulting precipitate was collected by centrifugation and dissolved in water, and the solution was dialyzed against 0.1 M KCl to yield 190 mg of AS-2 fraction, having a specific activity of 28.0 units/mg.

METHOD II. An alternative procedure was to add to the CM fraction (6 mg/ml in 466 ml of 0.1 M KCl, specific activity 28.0 units/mg) solid ammonium sulfate to 80% of saturation. After being kept in the cold overnight, the suspension was centrifuged, and the precipitate was dissolved in a minimal volume of phosphate buffer (0.1 M, pH 6.8). The concentration of ammonium ion was determined by Nesslerization, and the concentration of ammonium sulfate was adjusted to 45% of saturation with solid ammonium sulfate. The solution, which contained 70 mg of protein/ml, was kept in an open erlenmeyer flask for 10 days in the refrigerator. The resulting precipitate was collected by centrifugation

* From the Department of Biochemistry, Yale University, New Haven, Connecticut, and the Department of Biochemistry, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107. *Received January 7, 1970.* This work was aided by grants from the U. S. Public Health Service (GM-06452) and from the National Science Foundation (GB-6212X and GB-7879).

† To whom inquiries should be addressed at Jefferson Medical College.

‡ Present address, Nakamura Gakuen College, Fakuoka, Japan.

§ Present address: Biophysics Research Laboratory, Peter Bent Brigham Hospital, Boston, Mass. 02115.

to yield 680 mg of AS-3 fraction, having a specific activity of 30.0 units/mg.

It has also been found that the dipeptidyl transferase activity can be separated from cathepsin B activity by applying the 40–70% ammonium sulfate fraction to a Sephadex G-200 column equilibrated with 0.9% NaCl under conditions which were otherwise identical with those reported earlier (R. M. Metrione, unpublished data; Metrione *et al.*, 1966). The fractions containing the dipeptidyl transferase activity were then pooled, precipitated with 80% saturated ammonium sulfate, and centrifuged. The precipitate was dissolved in a small amount of 0.9% NaCl and dialyzed against 0.9% NaCl. The protein concentration was adjusted to approximately 25 mg/ml and the heat treatment and subsequent steps of the purification (Metrione *et al.*, 1966) were carried out. The addition of the gel filtration step for the separation of the two catheptic activities does not appear to affect the properties of the enzyme in that the sedimentation coefficient and molecular weight of the native enzyme were in agreement with those of earlier preparations.

Physical Studies. The ultracentrifuge experiments were performed with a Spinco Model E instrument equipped with a phase plate as a schlieren diaphragm, ultraviolet-scanning optics, and a rotor temperature indicating control. For sedimentation velocity experiments the conventional 12-mm cell with an aluminum centerpiece was used. The double-sector filled Epon cell was used for sedimentation equilibrium experiments which were conducted according to Yphantis (1964) with ultraviolet-scanning optics at 280 mμ, or the low-speed method (Van Holde and Baldwin, 1958) with Rayleigh interference optics.

The urea used in the sedimentation studies was Ultra Pure grade (Mann Research Laboratories) from which ammonia had been removed by storage in a vacuum desiccator over sulfuric acid for 3 days. Guanidinium chloride was prepared from the corresponding carbonate (Eastman Organic Chemicals) and was crystallized from water and recrystallized from methanol or was purified according to Nozaki and Tanford (1967).

The partial specific volume was determined by means of density measurements according to Linderstrom-Lang and Lanz (1938), using the gradient forming device suggested by Riggsby and Rappaport (1965). The determination of the partial specific volume of the enzyme in 2.5 M guanidinium chloride was performed in a gradient column prepared from suitable mixtures of *m*-xylene and bromobenzene saturated with 2.5 M guanidinium chloride and found to be 0.75 ml/g as compared with 0.73 ml/g for the native enzyme (Metrione *et al.*, 1966). For runs in guanidinium chloride and urea, the density and viscosity data of Kawahara and Tanford (1966) were used in the calculations. The temperature was controlled at 20°. Unless otherwise stated, the sedimentation experiments were conducted in the presence of 0.1 M sodium phosphate buffer (pH 6.8); the concentration of enzyme was 4–6 mg/ml in the sedimentation velocity experiments and 2.5–3.2 mg/ml in the sedimentation equilibrium studies with interference optics and 0.05–0.52 mg/ml with absorption optics. After a sufficient amount of a concentrated solution of urea or guanidinium chloride in the buffer had been added to give the desired concentration, ultracentrifugation was begun as soon as possible after mixing, unless otherwise indicated.

Studies of the effect of pH on the sedimentation behavior of the enzyme were performed in sodium formate buffers at pH 2.5, 3.0, 3.5, and 4.0 (0.1 μ); the concentration of enzyme (CM fraction) was 5 mg/ml. The ultracentrifugation was begun immediately after the acidic buffer had been added.

Molecular Weight Determination by Gel Electrophoresis. The molecular weight of the subunits of dipeptidyl transferase was determined essentially by the method of Shapiro *et al.* (1967) with several modifications. The electrophoresis was carried out in 7% cyanogum 41 (Fisher Scientific Co.) in 0.01 M sodium phosphate (pH 7.1), containing 0.1% sodium dodecyl sulfate. An apparatus was employed which allows one to cast the gels in a slab 6 mm × 14 cm × 10 cm and to cool the gel on both sides with circulating tap water. There were seven sample slots in the 10-cm axis so that the standard protein solutions (bovine serum albumin, ovalbumin, α-chymotrypsinogen A, and cytochrome *c*) and the sample under study could be run simultaneously. Electrophoresis was at 70 V for 4 hr.

Molecular Weight Determination by Gel Filtration. A molecular weight determination was made on a Sephadex G-75 column 0.9 × 95 cm by the method of Whitaker (1963) except that all solutions contained 2.5 M guanidinium chloride. The column was calibrated with bovine serum albumin, ovalbumin, α-chymotrypsinogen A, and cytochrome *c*. The enzyme was mixed with sufficient concentrated guanidinium chloride solution to give a final concentration of 6 M guanidinium chloride, 0.05 M sodium phosphate (pH 6.8), and 5 mg of enzyme/ml. After dialyzing against 2.5 M guanidinium hydrochloride in 0.05 M sodium phosphate (pH 6.8) for 72 hr, 0.2 ml of the solution was applied to the column and the column was run using 0.05 M sodium phosphate (pH 6.8) containing 2.5 M guanidinium chloride as the eluting buffer. The molecular weight of native dipeptidyl transferase was determined on a 1.5 × 90 cm column of Bio-Gel A 1.5 m, 200–400 mesh (Bio-Rad Laboratories), in 0.05 M sodium phosphate (pH 6.8) calibrated with the same standard proteins as were used for the subunits plus human γ-globulin (E. R. Squibb and Sons).

Amino-Terminal Amino Acid Analysis. The nature of the amino-terminal residues in dipeptidyl transferase was determined by means of the fluorodinitrobenzene method (Fraenkel-Conrat *et al.*, 1955) and of the dansyl chloride method (Morse and Horecker, 1966). Fluorodinitrophenylamino acids, used as standards, were purchased from Mann Research Laboratories. Approximately 8 mg of the AS-2 fraction was used, and thin-layer chromatography of the dinitrophenyl derivatives derived from the enzyme was performed on Eastman silica gel coated plastic sheets (K301R2) with solvent systems 1, 3, and 4 of Brenner *et al.* (1961). In the experiments with dansyl chloride (Pierce Chemical Co.), approximately 7 mg of the AS-2 fraction in 0.6 ml of 0.1 M sodium bicarbonate was mixed with an equal volume of a solution of dansyl chloride in acetone (3 mg/ml). After the reaction mixture had been kept at room temperature for 3 hr, it was dialyzed against 0.05 M NaCl in the cold room and concentrated to dryness *in vacuo* at 55°. The residue was dissolved in 1.5 ml of 6 M HCl and heated at 105° for 10.5 hr. After removal of the acid *in vacuo*, the residual solid was dissolved in 0.5 ml of acetone–acetic acid (3:2, v/v) and thin-layer chromatography was performed using as solvents chloroform–methanol–acetic acid (75:20:5, v/v)

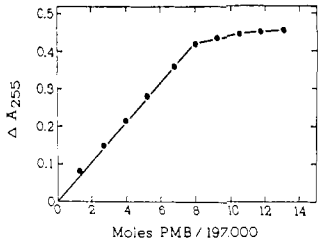

FIGURE 1: Titration of dipeptidyl transferase with *p*-mercuribenzoate. To a solution containing 2.3 mg of AS-3 fraction were added aliquots (10 μl) of a solution of *p*-mercuribenzoate (1.43 mM) in 1 M Tris–1 M sodium perchlorate buffer (pH 7.5) and the change in absorbance at 255 mμ was measured.

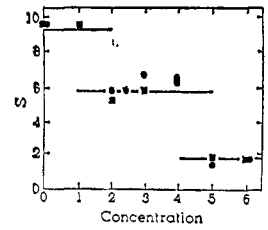

FIGURE 2: The effect of guanidinium chloride concentration on the sedimentation coefficient of dipeptidyl transferase. The ultracentrifugation was conducted at 59,780 rpm, 20.0°, in potassium phosphate buffer (μ = 0.2; pH 7.3) using AS-2 fraction at 6 mg/ml (●); or at 56,000 rpm, 20.0°, in sodium phosphate buffer (0.1 M; pH 6.8) using the CM fraction at 4 mg/ml (■). The sedimentation coefficients are $s_{20,w}$ values. No values are included at the ends of the lines since the schlieren peaks for these regions were too small to allow accurate determinations of $s_{20,w}$.

and chloroform–*t*-amyl alcohol–acetic acid (75:20:3, v/v). The dansyl-amino acids used as standards were prepared in the manner described by Morse and Horecker (1966).

Quantitative analysis of the amino-terminal residues was performed by the cyanate procedure of Stark and Smyth (1963), using 23 mg of the AS-2 fraction. Guanidinium chloride was the denaturing agent employed. In view of the results obtained by the fluorodinitrobenzene and dansyl methods, showing aspartic acid (or asparagine) and leucine to be the detectable amino-terminal residues, only "fraction A" (Stark and Smyth, 1963) containing neutral and acidic amino acids was collected, and subjected to amino acid analysis in the Beckman 120B amino acid analyzer. The glutamic acid found was assumed to be a normal contaminant since no terminal glutamic acids were found by the dansyl or dinitrophenyl methods and no attempt was made to hydrolyze the pyrrolidonecarboxylic acid formed. A blank was prepared by dissolving 2.73 mg of carbamylated enzyme (from the above preparation) in 1 ml of 50% acetic acid, and precipitating the protein with 0.33 ml of 20% trichloroacetic acid. After the mixture had been kept at 0° for 20 min, the precipitate was washed with two 0.5-ml portions of cold trichloroacetic acid, and the supernatant fluids were heated to 105° for 10 hr in the presence of 6 M HCl. The hydrolysate was subjected to the Stark and Smyth separation procedure, and the resulting values were used as correction factors for the data obtained in the manner described above.

Titration of Sulfhydryl Groups. The sulfhydryl groups of dipeptidyl transferase were titrated with *p*-mercuribenzoate (Sigma Chemical Co.) by the method of Boyer (1954), as modified by Coombs *et al.* (1964). In a typical experiment the reaction mixture contained 2.26 mg of CM fraction in 1.2 ml of 1 M Tris–1 M sodium perchlorate buffer (pH 7.5). The titration was performed by the addition of 10-μl samples of 10^{-3} M *p*-mercuribenzoate solution (1.43 mM) in Tris–perchlorate buffer to the enzyme solution in a 1-cm cuvet at 255 mμ in a Beckman DU spectrophotometer. The actual concentration of *p*-mercuribenzoate was determined by use of the extinction coefficient given by Boyer (1954). A typical titration curve is shown in Figure 1.

The sulfhydryl content of the enzyme was also determined by the method of Alexander (1958), using *N*-ethylmaleimide (Mann Research Laboratories). The reaction mixture contained 4.6 mg of the AS-2 fraction and 0.84 mM reagent in phosphate buffer (0.1 M, pH 6.8). The absorbance was read at 310 mμ, as suggested by Liener (1961). The molar extinction coefficient of *N*-ethylmaleimide at this wavelength was assumed to be 589.

Results

Further Purification of Dipeptidyl Transferase. The purification procedure described previously (Metrione *et al.*, 1966), when performed on a laboratory scale, yields a preparation (CM fraction) that approaches homogeneity in its sedimentation behavior, with a single component of $s_{20,w}$ = 9.7 S. Through the cooperation of the New England Enzyme Center, large-scale preparations of the enzyme became available, but in several cases, the CM fraction so obtained contained appreciable amounts of an impurity of lower sedimentation coefficient which had to be removed before the dissociation behavior of dipeptidyl transferase could be studied in the ultracentrifuge. The procedures described in the Experimental Section, involving fractional precipitation of the CM fraction with ammonium sulfate, were effective in achieving this end. The two methods employed were equally effective.

Effect of Dissociating Agents on the Sedimentation Behavior of Dipeptidyl Transferase. In solutions of guanidinium chloride dipeptidyl transferase displays sedimentation coefficients smaller than those observed for the native enzyme, indicative of dissociation into subunits (Figure 2). Three different rates of sedimentation are observed as the concentration of guanidinium chloride is increased. Significantly, at some concentrations of dissociating agent two components are seen to coexist. Material having the same sedimentation rate as the native enzyme is present at 1 and 2 M guanidinium chloride but not at higher concentrations. Further at 1 and 2 M guanidinium chloride, a component having a sedimentation rate of about 6 S also is present. This latter component is observed at concentrations of up to 5 M guanidinium chloride and is the only one observed between 2.5 and 3 M. At 4 M guanidinium chloride a new component is observed which has a sedimentation rate of 1.8 S. At concentrations above 5 M dissociating agent this is the only component observed.

The effect of time on the dissociation of dipeptidyl transferase in the presence of 2.5 M guanidinium chloride is shown in Figure 3. The 6.0S component appears immediately after mixing but exhibits a time-dependent dissociation

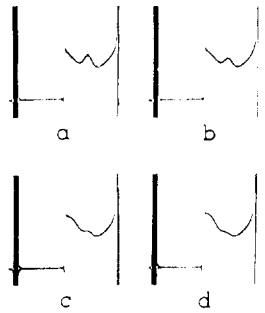

FIGURE 3: The effect of time on the sedimentation coefficient of dipeptidyl transferase at 2.5 M guanidinium chloride. The ultracentrifugation was at 56,000 rpm, 20.0°, in 0.1 M sodium phosphate (pH 6.8) at a concentration of 4 mg/ml. The ultracentrifugation was begun at the following times after mixing: a, 6 hr; b, 24 hr; c, 48 hr; d, 120 hr. The pictures were taken 64 min after attaining full speed.

into the 1.8S component which is present at all times after 24 hr.

In the presence of 8 M urea, the ultracentrifuge pattern of dipeptidyl transferase shows 1 component of $s_{20,w}$ = 2.0 S. At urea concentrations up to 4 M and protein concentrations near 6 mg/ml, there is no evidence of dissociation, the only component observed sedimenting at a rate near that for the native enzyme. However, when a much lower enzyme concentration (0.052 mg/ml) was employed taking advantage of the greater sensitivity of the ultraviolet absorption optics, dissociation appeared to occur at considerably lower urea concentrations. At 0, 0.5, and 1.0 M urea the native enzyme appears to be the only component present. At 2.0 M urea, a slower moving material appeared to be present at very low concentrations. It must be pointed out that at this protein concentration a sensitivity must be used on the ultracentrifuge which results in considerable noise in the recording. The presence of the minor component in 2 M urea must, therefore, be considered tentative. At 4.0 M urea, the intermediate form ($s_{20,w}$ = 5.6 S) is the prevalent form. At 6.4 M urea, the subunits (2.1 S) are the main species. These low enzyme concentration experiments were performed in the presence of 0.002 M dithiothreitol and 0.1 M sodium phosphate (pH 7.5).

At all pH values tested in the range 3.5–7.5, the enzyme sediments at a rate comparable with that of the native enzyme. At pH 3.0, the enzyme activity is rapidly lost and a portion of the protein precipitates. Examination of the supernatant fluid in the ultracentrifuge indicated dissociation to a component sedimenting at 2.0 S. Attempts to prevent the precipitation at pH 3, by the use of buffers other than formate or by dialysis against the acid buffer, were unsuccessful.

The molecular weight of the enzyme under a variety of conditions was determined by sedimentation equilibrium. The molecular weight of the native enzyme was reinvestigated using absorption optics. Six experiments were performed at speeds from 10,000 to 20,000 rpm, and protein concentrations of from 0.052 to 0.26 mg per ml, in 0.1 M sodium phosphate (pH 6.8). The molecular weight was determined to be 197,000 ± 10,000.

The molecular weight in 6 M guanidinium chloride, 0.052 mg/ml of protein, at 36,000 rpm, with absorption optics was 26,000. An experiment under the same conditions but with 8 M urea instead of the guanidinium chloride was performed and a molecular weight of 27,000 was determined. These experiments can be considered as mutually confirming determinations of the molecular weight of the subunits.

A value of 30,000 ± 1,000 was determined in 2.5 M guanidinium chloride in two runs at 15,000 rpm, with interference optics. This value most likely reflects incomplete dissociation of the 6.0S component into 1.8S subunits, even though ultracentrifugation was begun 24 hr after the mixing of enzyme and reagent and terminated 48 hr after mixing and can be considered an upper limit for the 1.8S component. A value of 53,000 was obtained by mixing enzyme with guanidinium chloride in potassium phosphate buffer (pH 7.3), μ = 0.2, beginning ultracentrifugation immediately and terminating the experiment after 24 hr. This value probably is lower than the true molecular weight of the 6.0S component since although this intermediate form is the main component under these conditions, considerable contamination with 1.8S component exists.

Molecular Weight Determination on Gels. The molecular weight of the subunits was determined by electrophoresis in polyacrylamide gel according to Shapiro *et al.* (1967), and found to be 23,000. When a sample of the enzyme in 2.5 M guanidinium chloride was applied to a calibrated Sephadex G-75 column which had been equilibrated with 2.5 M guanidinium chloride a molecular weight of 22,000 was found. The molecular weight of the native enzyme was 193,000 ± 7,000.

Amino-Terminal Amino Acid Residues of Dipeptidyl Transferase. The application of the fluorodinitrobenzene and dansyl chloride methods showed aspartic acid (or asparagine) to be the predominant amino-terminal residue in dipeptidyl transferase, but thin-layer chromatography reproducibly showed a weak spot corresponding to the leucine derivative in addition to the strong spot for the aspartic acid derivative.

Quantitative amino-terminal amino acid analysis by the cyanate method of Stark and Smyth (1963), as modified for the present work (see Experimental Section), gave the following values (corrected for blank) for the acidic and neutral amino acids per unit weight of 197,000: Asp, 5.6; Leu, 2.1; Thr, 0.2; Ser, 0.1; (Glu, 2.1); Pro, <0.1; Gly, 0.3; Ala, 0.3; Val, <0.1; Ile, <0.1; Tyr, <0.1; Phe, <0.1. These results indicate the presence of 6 amino-terminal aspartic acid (or asparagine) residues and 2 amino-terminal leucine residues per 197,000 g of dipeptidyl transferase.

Sulfhydryl Groups of Dipeptidyl Transferase. It has long been known that dipeptidyl transferase requires activation by sulfhydryl compounds, suggesting the involvement of a sulfhydryl group in the catalytic mechanism of the enzyme (Fruton and Mycek, 1956). Direct determination of the number of SH groups by titration of native dipeptidyl transferase with *p*-mercuribenzoate gave a value of 7.6/unit of 197,000. In the presence of 6 M guanidinium chloride or 8 M urea, the corresponding values were 7.3 and 6.9, respectively. Use of the spectrophotometric method involving reaction with *N*-ethylmaleimide gave a value of 7.3 SH groups/unit of 197,000 of the native enzyme; this value was unchanged in the presence of 1% sodium dodecyl sulfate. These results indicate that dipeptidyl transferase has 7 to 8 sulfhydryl groups in the native enzyme and that no additional

sulfhydryl groups are made available for reaction upon treatment with guanidinium chloride, urea, or sodium dodecyl sulfate, apparently ruling out any buried sulfhydryl groups.

Discussion

The molecular weight of native dipeptidyl transferase has been shown by ultracentrifugation to be 197,000 ± 10,000. Ultracentrifugation in 6 M guanidinium chloride and 8 M urea indicate that the molecular weight of the subunits is 26,500 ± 500. The molecular weight determined by gel electrophoresis in sodium dodecyl sulfate is 23,000 and by gel filtration in 2.5 M guanidinium chloride is 22,000. These results indicate that the molecular weight of the subunits is between 22,000 and 27,000 and that there are 7.3–9.0 subunits/unit of 197,000. An averaging of the molecular weights of the subunits as determined by the 4 methods gives a molecular weight of 24,500 and 8 subunits/197,000 unit weight. These data are summarized in Table I.

Amino-terminal analysis indicates the presence of 7.7 amino-terminal residues/197,000 molecular weight (5.6 aspartic acid or asparagines and 2.1 leucines). The sulfhydryl content has been shown to be between 6.9 and 7.6 sulfhydryls per 197,000 and if it is assumed that there is 1 sulfhydryl/subunit this would indicate 7 or 8 subunits per native molecule.

Taken together these data indicate that there are 7–9 subunits/native dipeptidyl transferase molecule. There is no evidence that enzymes with molecular weights in the vicinity of 200,000 contain an odd number of subunits (Klotz and Darnall, 1969). Conceptually the association of subunits by even numbers is more easily understood than by odd numbers (Monod *et al.*, 1965). It therefore seems reasonable to assume that dipeptidyl transferase is made up of 8 subunits.

When dipeptidyl transferase is mixed with guanidinium chloride at a final concentration of 2.5 M and immediately subjected to sedimentation equilibrium centrifugation for 24 hr, a molecular weight of 53,000 is obtained. Under these conditions dipeptidyl transferase has been shown to be in the process of dissociation from an intermediate 6.2S form to the subunits. This suggests that a higher molecular weight would exist for the intermediate form. A molecular weight in the neighborhood of 100,000 would be consistent with the sedimentation coefficient (Yue *et al.*, 1967; Morino and Snell, 1967). The finding that the concentration of guanidinium chloride must be raised to 6 M before the dissociation is complete suggests that the binding which holds the intermediate form together is relatively strong. The data would thus suggest that as the concentration of guanidinium chloride is increased, the initial dissociation is a rapid one to two components (tetramers) of molecular weight near 100,000. The further dissociation occurs with time or with higher guanidinium chloride concentration to a subunit of molecular weight near 24,000. These data are summarized in Table I. The amino-terminal composition reported here (6 aspartic acids or asparagines and 2 leucines per 197,000) suggests the possibility of 2 types of subunits. The possibility of heterogeneity not detected by the physical studies described here cannot be excluded, nor can the possibility that this composition has resulted from partial degradation of the amino end of some of the chains. The component of intermediate molecular weight may be composed of three aspartyl-type subunits and one leucyl-type subunit but there is no direct evidence to support this hypothesis at present.

TABLE I: Some Properties of Dipeptidyl Transferase.

Property	Value
Molecular weight by sedimentation equilibrium	
In 0.1 M phosphate pH 6.8	197,000
In 6 M guanidinium chloride	26,000
In 8 M urea	27,000
Molecular weight by gel filtration	
In 2.5 M guanidine	22,000
Molecular weight by acrylamide gel electrophoresis	
In 0.1% sodium dodecyl sulfate	23,000
SH content per 197,000 *p*-mercuribenzoate titration in	
1 M Tris–1 M $NaClO_4$ (pH 7.5)	7.6
6 M Guanidinium chloride	7.3
8 M Urea	6.9
N-Ethylmaleimide titration	
0.1 M Sodium phosphate (pH 6.8)	7.3
1% Sodium dodecyl sulfate	7.3
Amino-terminal content per 197,000	
5.6 aspartic acid and 2.1 leucine	7.7

The presence of 8 sulfhydryl groups and 8 subunits suggests the existence of 1 sulfhydryl group per subunit and the possibility of 1 active site per subunit. This would tend to oppose the suggestion that 2 types of subunits are present. The types of amino terminal would then be an artifact.

It has been suggested (Würz *et al.*, 1962; Metrione *et al.*, 1966) that the polymerase activity of dipeptidyl transferase may involve the cooperative interaction of catalytic centers on adjacent subunits of the native enzyme. Heinrich and Fruton (1968) presented evidence which indicated that as the urea concentration is increased from 0 to 2 M the per cent of the reacted substrate which was hydrolyzed increased and the per cent of reacted substrate which was polymerized decreased. The suggestion that the intact enzyme is required for polymerization while dissociated subunits are capable of catalyzing hydrolysis is given tentative support by the ultracentrifuge data, under the same conditions, reported in this paper. It should be pointed out that the sensitivity of the ultracentrifuge does not allow the accurate determination of the few percentages differences up to 2.0 M. The distinction should be more pronounced at higher concentrations of urea since by 4 M urea the intermediate form predominates and by 6.4 M the subunits predominate. Unfortunately, data on the relative formation of the products of hydrolysis and polymerization at these concentrations are not available. It may be that the trend toward partition of the two activities shown at low urea concentrations will continue at concentrations greater than 2 M.

The results reported here should indicate that if conclusions between molecular weights and activity are to be drawn the molecular weights and activity measurements must be performed at the same protein concentration.

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