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Molecular Weight, Amino Acid Composition and Physicochemical Properties of the Exocellular DD-Carboxypeptidase–Transpeptidase of *Streptomyces* R39

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The exocellular DD-carboxypeptidase–transpeptidase from *Streptomyces* R39 was purified to protein homogeneity and in milligram amounts. The isolated enzyme consisted of one polypeptide chain of molecular weight about 53300. Its amino acid composition and several physicochemical properties were determined and compared with those of the exocellular DD-carboxypeptidase–transpeptidase from *Streptomyces* R61.

Previous papers have described the isolation and enzymic properties of the exocellular DD-carboxypeptidases–transpeptidases produced by *Streptomyces* strains R61 and R39 (Leyh-Bouille *et al.*, 1971, 1972; Pollock *et al.*, 1972; Nieto *et al.*, 1973*a*,*b*; Perkins *et al.*, 1973; Frère *et al.*, 1973*a*,*b*). Both enzymes exhibited different specificity profiles for peptide substrates, and these differences correlated very well with the types of peptide cross-linking which occurred in the wall peptidoglycans of the corresponding strains (Ghuysen *et al.*, 1973; Perkins *et al.*, 1973).

The present paper describes the purification of milligram amounts of the DD-carboxypeptidase–transpeptidase from *Streptomyces* strain R39, some chemical and physicochemical properties of the isolated protein and the differences which exist between this enzyme and the DD-carboxypeptidase–transpeptidase from *Streptomyces* R61.

Materials and Methods

Enzyme activity

One unit of enzyme catalyses the hydrolysis of 1 μequiv. of D-Ala-D-Ala linkage/min at 37°C when the enzyme is exposed to 8mM-Ac_2‡-L-Lys-D-Ala-D-Ala (i.e. at a concentration 10 times the K_m value) in 0.03M-Tris–HCl buffer, pH7.5, supplemented with 3mM-$MgCl_2$. In previous papers, one unit referred to the conversion of 1nmol of substrate/h.

Buffers

Concentrations refer to the concentrations of the partly ionized species.

Measurement of protein concentrations

This was determined by measuring the amount of total amino groups available to fluorodinitrobenzene after hydrolysis with 6M-HCl (100°C, 20h) or by measuring the extinction at 280 and 260nm and using Kalckar's formula (Kalckar, 1947).

Molecular-weight standards

The following compounds were used as molecular-weight standards: Dextran Blue (mol.wt. 2×10^6; Pharmacia Fine Chemicals, Uppsala, Sweden); aldolase (158000; subunit 39500), bovine serum albumin (68000), ovalbumin (44000), carbonic anhydrase (29000), chymotrypsinogen (25000) and myoglobin (17200). The proteins were purchased from Sigma Chemical Co., Kingston-upon-Thames, U.K.

Physicochemical-methods

Diffusion coefficient ($D_{20,w}$). The diffusion coefficients were determined at two different ionic strengths. In some experiments, the enzyme solution (4mg/ml) was dialysed against 0.1M-Tris–HCl buffer, pH7.7,

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‡ Abbreviation: Ac, acetyl.

containing 3mM-$MgCl_2$ and 0.2M-NaCl. In the other experiments, the enzyme concentration was decreased to 2mg/ml and the NaCl concentration was increased to 0.5M. These experiments were carried out at a speed of 13410rev./min. For other conditions see Frère *et al.* (1973*a*).

Equilibrium sedimentations. The equilibrium sedimentations were performed in 0.1M-Tris–HCl buffer, pH7.7, containing 3mM-$MgCl_2$ and either 0.2M- or 0.5M-NaCl, at 12578 rev./min for 22h at 20°C. The initial protein concentrations were 4mg/ml (in the 0.2M-NaCl buffer) and 2mg/ml (in the 0.5M-NaCl buffer). For other conditions see Frère *et al.* (1973*a*). The apparent molecular weight ($M_{app.}$) was calculated as described by O'Donnell & Woods (1962).

Spectra. The spectra were obtained by using a Cary 17 double-beam spectrophotometer with automatic slit-width adjustment. Fluorescence emissions were measured at 90° to the exciting beam in an Aminco–Bowman recording spectrofluorimeter with jacketed cell-holder at constant temperature.

Polyacrylamide-gel electrophoresis. This was done in Tris–glycine buffer, pH8.45, as previously described (Leyh-Bouille *et al.*, 1972). Electrophoreses were also performed in 0.01M-sodium phosphate, pH7.1, in the presence of 0.1% (w/v) sodium dodecyl sulphate (Weber & Osborn, 1969) with prior incubation of the proteins for 15h at 37°C, in the same phosphate buffer containing 1% (w/v) sodium dodecyl sulphate, with or without the addition of 2-mercaptoethanol (1%, final volume).

Amino acid composition of the strain R39 protein. This was determined on several samples hydrolysed for 24, 48 and 96h at 110°C in constant-boiling HCl (made from AnalaR HCl by addition of water and redistillation), after the addition of a crystal of phenol and removal of air in a vacuum. The instrument was a Biocal automatic analyser (8032 München, Gräfelfing, Germany). The values for threonine and serine were extrapolated to zero time. The values for all the other amino acids were independent of the hydrolysis times. Cysteine was determined after performic acid oxidation (Hirs, 1956) and tryptophan was determined from the u.v. spectrum in alkali by the method of Bencze & Schmid (1957).

Growth conditions of Streptomyces R39

Streptomyces R39 was grown for 24h at 28°C with shaking in peptone medium (medium A described by Leyh-Bouille *et al.*, 1971). After two successive subcultures of increasing size, 100 litres of culture in exponential phase were used to inoculate 400 litres of the same peptone medium contained in a 500-litre tank. This culture was grown at 28°C for 96h with mechanical stirring (120rev./min) and an air-flow rate of 100 litres/min at an air pressure of 1.5×10^5 Pa. Silicone A emulsion (Dow Corning Co., Midland, Mich., U.S.A.; 20ml) was used as antifoam. After centrifugation, the enzyme activity in the culture fluid was 2.5munits/ml or 1.5munits/mg of protein.

Results

Purification of the enzyme

Step 1. The enzyme was adsorbed from 500 litres of culture fluid (see the Materials and Methods section) on 3.7kg wet wt. of DEAE-cellulose (MN2100 DEAE, Macherey, Nagel and Co., D-156, Düren, Germany) equilibrated against 0.1M-Tris–HCl, pH7.5. All subsequent steps were performed at 4°C. The enzyme was eluted from the DEAE-cellulose by two subsequent treatments with 5 litres of 0.1M-Tris–HCl buffer, pH7.5, containing 1mM-$MgCl_2$ and 0.4M-NaCl. The solution was concentrated to 1.5 litres by dialysis against Carbowax 4000, and solid $(NH_4)_2SO_4$ was added to 50% saturation. The precipitate was discarded and the $(NH_4)_2SO_4$ concentration in the supernatant was increased to 90% saturation. The precipitate was collected by centrifugation, dissolved in 240ml of 0.1M-Tris–HCl buffer, pH7.7, containing 1mM-$MgCl_2$ and the solution was dialysed against the same buffer.

Step 2. After step 1, the enzyme solution was applied to a 600ml column of DEAE-cellulose (4cm×48cm) equilibrated against 0.1M-Tris–HCl buffer, pH7.7, containing 1mM-$MgCl_2$ and 0.1M-NaCl. Some enzymically inactive proteins were eliminated by washing the column with the same buffer and others were eluted by increasing the NaCl concentration in the buffer to 0.19M. The resin was then treated with an increasing convex gradient of NaCl (mixing flask: 970ml of 0.1M-Tris–HCl buffer+1mM-$MgCl_2$+0.19M-NaCl; upper flask: 0.1M-Tris–HCl buffer+1mM-$MgCl_2$+0.28M-NaCl). The enzyme was eluted at a NaCl concentration of about 0.24M. The active fractions were pooled and concentrated to 20ml by ultrafiltration through Amicon UM10 membranes.

Step 3. After step 2, the concentrated solution was filtered through a 400ml column (3cm×45cm) of Sephadex G-100 previously equilibrated against 0.05M-cacodylate–HCl buffer, pH6.0, containing 1mM-$MgCl_2$+0.3M-NaCl. The enzyme was eluted at a K_D value of 0.21 (and well separated from a yellow and a brown pigment with K_D values of 0–0.02 and 0.72 respectively). The active fractions were pooled (90ml).

Step 4. After step 3, the solution was applied to a 30ml column (2cm×10cm) of DEAE-Sephadex A-50, previously equilibrated against 0.05M-cacodylate buffer, pH6.0, containing 1mM-$MgCl_2$+0.3M-NaCl. Enzymically inactive proteins were eliminated by washing the column first with 300ml of the same cacodylate–$MgCl_2$ buffer containing 0.4M-NaCl and

then with 300ml of the same buffer containing 0.47M-NaCl. The enzyme was eluted with a convex gradient of NaCl (mixing flask: 500ml of cacodylate buffer +1mM-$MgCl_2$+0.47M-NaCl; upper flask: cacodylate buffer+1mM-$MgCl_2$+0.5M-NaCl). A single peak of protein was obtained which closely correlated with the activity. The active fractions were pooled and concentrated to 20ml by ultrafiltration. The concentrated solution was filtered through the 400ml column of Sephadex G-100, in 0.05M-cacodylate buffer, pH6.0, containing 1mM-$MgCl_2$ and 0.4M-NaCl. The most active fractions of the eluted peak exhibited the same high specific activity. They were pooled, concentrated by ultrafiltration and dialysed against 0.1M-Tris–HCl buffer, pH7.7, containing 0.2M-NaCl and 0.05M-$MgCl_2$ (fraction A, Table 1). The other fractions of the eluted peak had lower specific activities. They were pooled, concentrated and dialysed (fraction B, Table 1). Fraction B (specific activity 13 units/mg of protein) was stored as it was and used for various preliminary experiments. Fraction A (specific activity 17 units/mg of protein) behaved as a homogeneous protein in several analytical tests (see below). However, benzylpenicillin binding occurred at a ratio of 0.9mol of benzylpenicillin/mol of enzyme (Frère *et al.*, 1974), suggesting that some impurities were still present in the preparation.

Step 5. After step 4, 1ml of acetone previously cooled to −20°C was added to fraction A (1ml; 1.6mg of protein). The mixture was stirred for 30min at −20°C, then the precipitate was collected by centrifugation at −5°C and redissolved in 1ml of 0.1M-Tris–HCl buffer, pH7.7, containing 0.2M-NaCl and 0.05M-$MgCl_2$. At this stage, benzylpenicillin binding occurred at a ratio of 1.04±0.03mol of benzylpenicillin/mol of enzyme (Frère *et al.*, 1974). When compared with fraction A, the final preparation had a specific activity increased by about 11%. The enzyme recovery was about 90%. Table 1 gives the total recoveries and enrichments in specific activity after each step of the purification procedure. The final preparation (after step 5) was used for the determination of amino acid composition and for penicillin binding, whereas preparation A, of about 90% purity (after step 4), was used for most of the physical tests.

Transpeptidase activity of the purified R39 enzyme

During its purification, the enzyme was monitored by testing its DD-carboxypeptidase activity on Ac_2-L-Lys-D-Ala-D-Ala. The enzyme preparations of lower specific activity (3.5 units/mg of protein) which had been obtained previously (Leyh-Bouille *et al.*, 1972) catalysed transpeptidation reactions when the reaction mixtures contained a suitable acceptor in addition to the above tripeptide donor. The highly purified preparation (after step 5) was also able to catalyse such transpeptidation reactions. Ac_2-L-Lys-D-Ala-D-Ala (5mM) and *meso*-diaminopimelic acid (8mM) were incubated at 37°C with 2.2pmol (0.12μg) of *Streptomyces* R39 enzyme in 40μl of 0.03M-Tris–HCl buffer, pH7.5, containing 0.1M-NaCl and 0.02M-$MgCl_2$. After 30min, 17.7 and 12.4% of the tripeptide donor were converted into Ac_2-L-Lys-D-Ala (hydrolysis product) and Ac_2-L-Lys-D-Ala-(D)-*meso*-diaminopilemic acid (transpeptidation product) respectively.

Effects of ionic strength on aggregation of the R39 protein

The apparent molecular weight of the *Streptomyces* R39 enzyme, measured by filtration on a 1.5cm×65cm column of Sephadex G-100 in various

Table 1. *Purification of Streptomyces R39 DD-carboxypeptidase–transpeptidase*

The protein concentration was determined by measuring the extinction at 260 and 280nm. The concentration of the final preparations (step 4, A and B, and step 5) was determined by measuring the amount of total amino groups available to fluorodinitrobenzene. For further details see the Materials and Methods section. Step 5 was performed with only 1.6mg of preparation A. The preparations after steps 4 and 5 were devoid of β-lactamase activity.

Step	Total protein (mg)	Total activity (units)	Yield (%)	Specific activity (units/mg of protein)	Enrichment
Culture supernatant	8.4×10^5	1250	100	0.0015	1
Step 1	2.9×10^3	1050*	84	0.36	240
Step 2	166	830	66	5.0	3300
Step 3	65	720	58	11.0	6700
Step 4 A	21.8	375	30	17.1	11400
B	11.4	147	12	13.0	8700
Step 5	1.4			19.8	12700

* The total activity obtained after $(NH_4)_2SO_4$ fractionation by adding up the total units of all fractions (0–50%, 50–90% and >90%) was always equal to about 130% of the total activity of the original solution. It is possible that an inhibitor was eliminated during this step.

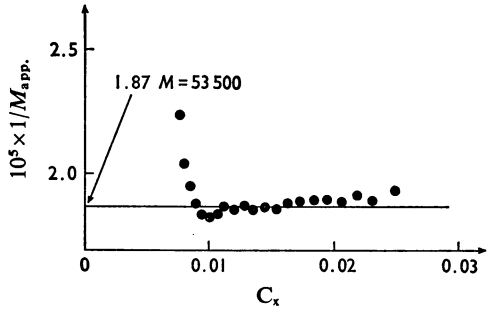


Fig. 1. *Reciprocal of the apparent molecular weight of Streptomyces R39* DD-*carboxypeptidase–transpeptidase as a function of the concentration in the column (C_x) during sedimentation equilibrium*

For conditions see the text and Frère *et al.* (1973*a*). Experiments were carried out in 0.1M-Tris–HCl buffer, pH7.7, containing 3mM-$MgCl_2$ and 0.5M-NaCl at initial concentration of 2mg of protein/ml. C_x is expressed in arbitrary units.

Tris–HCl–NaCl buffers (with Dextran Blue, ovalbumin, myoglobin, bovin serum albumin and chymotrypsinogen as standards), decreased as the ionic strength of the buffer increased. Ionic strengths equal to or higher than 0.07M, and ionic strengths lower than 0.07M, were obtained with 0.1M- and 0.01M-Tris–HCl buffers, pH7.7, respectively, supplemented with the appropriate amount of NaCl. The apparent molecular weight was over 100000 at $I = 0.008$M, 86000 at $I = 0.025$M, 70000 at $I = 0.070$M and seemed to stabilize at 55000–60000 at $I > 0.20$M.

Equilibrium sedimentation and diffusion coefficient

Fig. 1 shows the plots of $1/M_{app.}$ versus concentration for preparation A. The experiment was carried out in 0.1M-Tris–HCl buffer, pH7.7, containing 3mM-$MgCl_2$ and 0.5M-NaCl. A molecular weight of 53500 was obtained by extrapolation. The slightly increased values of $1/M_{app.}$ at high concentrations probably resulted from the non-ideality of the solution, owing to its high ionic strength.

The enzyme exhibited a $D_{20,w}$ value of 7.88×10^{-7}cm^2·s^{-1}. Since a spherical non-hydrated protein of mol.wt. 53500 has a diffusion constant D_0 of 8.48×10^{-7}cm^2·s^{-1}, the enzyme had a frictional ratio ($f/f_0 = D_0/D_{20,w}$) of 1.07. Equilibrium sedimentation was also carried out in the same Tris–$MgCl_2$ buffer as above except that the NaCl concentration was 0.2M instead of 0.5M. At this low ionic strength, the plot of $1/M_{app.}$ versus concentration gave a straight line with a slope of -1.1×10^{-4} (with the same co-ordinates as in Fig. 1). This observation again indicated some aggregation of the *Streptomyces* R39 protein.

Electrophoresis of the Streptomyces R39 enzyme

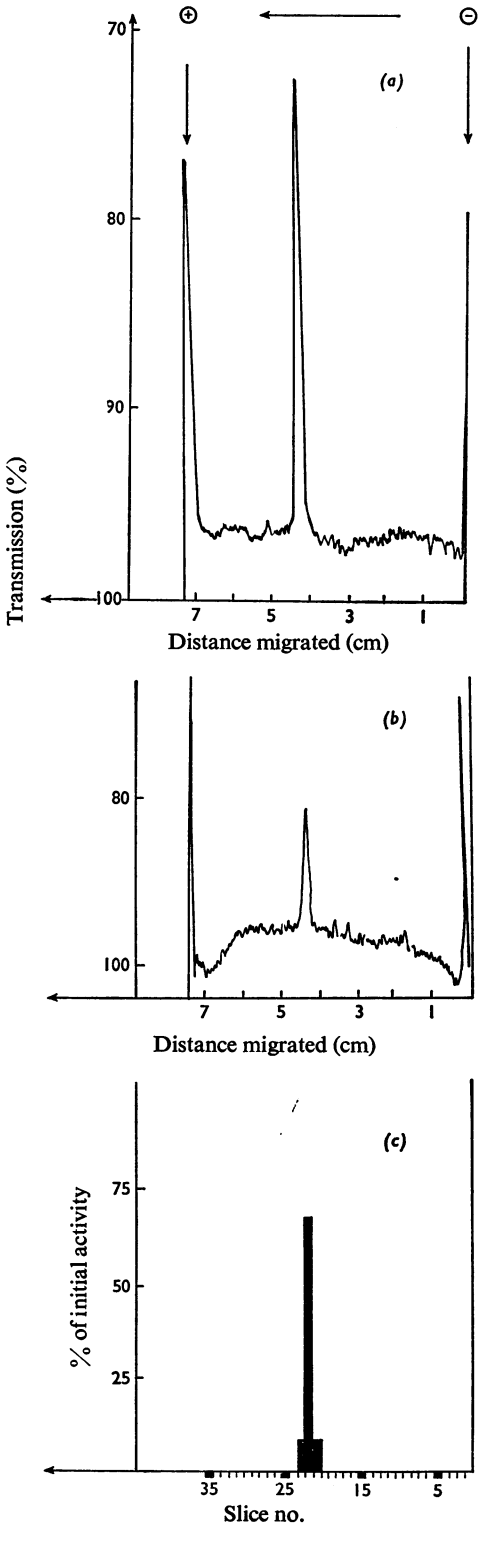
Polyacrylamide-gel electrophoresis. When submitted to gel electrophoresis at pH8.45 under standard conditions (see the Materials and Methods section) the enzyme preparation of lower specific activity (3.5 units/mg of protein) which had been obtained previously (Leyh-Bouille *et al.*, 1972) gave rise to a single protein band which migrated toward the anode with the buffer discontinuity. In the present experiments, the *Streptomyces* R39 enzyme (fraction A) was analysed on gels that had been submitted to prior electrophoresis for 3h at 4mA/gel (pre-runs). Experiments were carried out in quartz tubes so that the same gel could be scanned at 280nm and then removed from the tube and sliced into sections. These sections were then eluted and assayed for activity. Parallel gels were stained with Coomassie Blue as usual. As shown in Fig. 2 a single protein band was detected by direct scanning at 280nm, by staining with Coomassie Blue and by assaying the enzyme activity.

Gel electrophoresis in the presence of sodium dodecyl sulphate. The mercaptoethanol-treated *Streptomyces* R39 enzyme (see the Materials and Methods section) migrated as a single band. Its mobility, when compared with that of similarly treated protein standards (chymotrysinogen, carbonic anhydrase, aldolase, ovalbumin and bovin serum albumin), indicated a molecular weight of 56000 ± 3000 (7 determinations). The mobility of the *Streptomyces* R39 enzyme was unaltered when mercaptoethanol was omitted during the pretreatment of the protein with sodium dodecyl sulphate. This observation suggested that the protein probably consisted of a single polypeptide chain.

Gel isoelectrofocusing. Because of the high tendency of the protein to aggregate at low ionic strength, the isoelectric point of the *Streptomyces* R39 enzyme could not be determined. In normal gel electrophoresis at pH8.45, however, this enzyme migrated to the anode faster and hence was more anionic than the DD-carboxypeptidase–transpeptidase from *Streptomyces* R61 (isoelectric point pH4.8; Frère *et al.*, 1973*a*).

Amino acid composition of the Streptomyces R39 enzyme

Amino acids were determined (see the Materials and Methods section) from the purest preparation obtained (after step 5). Results are given in Table 2 and are compared with those obtained previously with the exocellular DD-carboxypeptidase–transpeptidase from *Streptomyces* R61 (Frère *et al.*, 1973*a*). The molecular weight of 53300 ± 1700 assigned to the R39 enzyme was the average value obtained by equilibrium sedimentation, polyacrylamide-gel electrophoresis in sodium dodecyl sulphate (see above) and by penicillin titration (50500; Frère



et al., 1974). The analyses showed the occurrence of two half-cystine residues (for a total number of 528) in the *Streptomyces* R39 enzyme. Thiol groups were not detected with 5,5'-dithiobis-(2-nitrobenzoic acid) even in the presence of 5M-guanidinium chloride (Frère *et al.*, 1974). These results strongly suggested the occurrence of one disulphide bridge in the *Streptomyces* R39 enzyme.

Spectra of the Streptomyces R39 enzyme

Absorption and fluorescence emission spectra are shown in Fig. 3 and can be contrasted with results for the enzyme from *Streptomyces* strain R61 (see the Discussion section). The *Streptomyces* R39 enzyme exhibited a $E^{1\%}_{1cm}$ value at 280nm of 9.7.

Comparison between the exocellular DD-*carboxypeptidases–transpeptidases from Streptomyces R39 and R61*

Table 3 summarizes the main chemical, physicochemical and enzymic properties of the two *Streptomyces* enzymes.

Discussion

The exocellular DD-carboxypeptidase–transpeptidase of *Streptomyces* R39 appeared to be one single polypeptide chain containing one disulphide bridge, with a molecular weight of 53300±1700. Strikingly, six residues (aspartate, glutamate, glycine, alanine, valine, leucine) represented nearly 70% of the total residues of the protein. The turnover number was

Fig. 2. *Polyacrylamide-gel electrophoresis at pH8.45 of Streptomyces R39* DD-*carboxypeptidase–transpeptidase*

Preparation A obtained after step 4 was used. Gels (6mm×71mm; 7% acrylamide) were polymerized in quartz tubes and submitted to pre-runs for 3h at 4mA/gel. The enzyme (11.3μg for Fig. 2*a*; 22.3μg for Figs. 2*b* and 2*c*) was deposited and the electrophoreses were performed for 135min at room temperature and at 3mA/gel. Under these conditions, Bromophenol Blue migrated 6.4cm toward the anode. (*a*) After electrophoresis, the gel was stained with Coomassie Blue and scanned at 580nm. (*b*) After electrophoresis, the gel in the quartz tube was scanned at 280nm. For this purpose, the cell compartment of a Zeiss spectrophotometer was replaced by a scanning attachment as described by Frère (1969). (*c*) The same gel as in (*b*) was removed from the tube and sliced into 2mm-thick discs. Each disc was eluted with 0.2ml of 0.1M-Tris–HCl buffer, pH7.5, containing 1mM-$MgCl_2$ for 16h at 4°C with occasional shaking and the eluates were assayed for DD-carboxypeptidase activity (see the Materials and Methods section). The activity recovery was 87% of the original value.

Table 2. *Amino acid composition of the* DD-*carboxypeptidases–transpeptidases from Streptomyces strains R39 and R61*

For details see the text. Half-cystine was measured as cysteic acid after performic acid oxidation (Hirs, 1956) of 200 μg of enzyme. The value for strain R61 was similarly checked.

	Strain R39 (amount of enzyme hydrolysed, 31 μg; one determination)			Strain R39 (average values from four determinations)			Strain R61 (from Frère *et al.*, 1973*a*)		
	nmol	Wt. (μg)	Residues/ enzyme molecule ($M = 53500$)	Residues/ enzyme molecule ($M = 53300$)	Total mass	% (in number)	Residues/ enzyme molecule ($M = 38000$)	Total mass	% (in number)
Asp	27.10	3.12	53	50	5750	9.5	38	4370	10.9
Thr	17.20	1.73	33	34	3434	6.4	38	3838	10.9
Ser	19.30	1.68	38	38	3306	7.2	29	2523	8.3
Glu	28.60	3.69	56	57	7353	10.8	28	3612	8.0
Pro	15	1.46	29	25	2425	4.7	11	1067	3.1
Gly	32.50	1.85	63	66	3762	12.5	32	1824	9.1
Ala	41	2.91	80	82	5822	15.5	34	2414	9.7
Cys | Val	25.20	2.49	49	51	5049	9.7	30	2970	8.6
Met	2.54	0.33	5	3†	393	0.57	6	786	1.71
Ile	5.50	0.62	11	10	1130	1.9	9	1017	2.6
Leu	25.30	2.86	49	50	5650	9.5	33	3729	9.4
Tyr	4.90	0.80	10	10	1630	1.9	13	2119	3.7
Phe	5.50	0.81	11	11	1617	2.1	12	1764	3.4
His	4.17	0.57	8	9	1233	1.7	8	1096	2.3
Lys	2.22	0.28	4	5†	640	0.95	8	1024	2.3
Arg	9.90	1.53	19	19	2945	3.6	14	2170	4.0
Trp	—	—	—	6‡	1122	1.1	4	748	1.14
Total		26.73*		528	53483§		350	37395§	

* When the values for tryptophan and half-cystine found by other methods were taken into account, the total recovery was 27.4 μg, i.e. 89% of the nominal amount hydrolysed.

† These values were determined from 200 μg of hydrolysed enzyme.

‡ Estimated from the u.v. spectrum in alkali (Bencze & Schmid, 1957).

§ Taking into account a mass of 18 for H_2O.

1050mol of D-alanine liberated from Ac_2-L-Lys-D-Ala-D-Ala/min per mol of enzyme. With N^{α}-Ac-L-Lys-D-Ala-D-Ala, which is a better substrate for this enzyme (Leyh-Bouille *et al.*, 1972), the turnover number should be about 1900. There were many similarities between the *Streptomyces* R39 enzyme and the exocellular DD-carboxypeptidase–transpeptidase of *Streptomyces* strain R61 (Frère *et al.*, 1973*a*,*b*; Nieto *et al.*, 1973*a*,*b*; Leyh-Bouille *et al.*, 1971, 1972) (Table 3). Both enzymes were single polypeptide chains in which the acidic residues greatly outnumbered the basic ones. This property explained the negatively charged character of the proteins. A high proportion of the residues had hydrophobic side chains and the content of sulphur-containing residues was low. Both proteins showed a strong tendency to aggregate under various conditions. These properties appeared to be particularly enhanced in the case of the *Streptomyces* R39 enzyme. There were also major differences between the *Streptomyces* R39 and R61 enzymes. The former was a larger polypeptide than the latter. There were no free thiol groups in the *Streptomyces* R39 enzyme, but at least one occurred in the *Streptomyces* R61 enzyme. Maximum fluorescence emission was at 339–340nm with the *Streptomyces* R39 enzyme (maximum is at 347nm with free tryptophan and at 337nm with lysozyme), and at 318–320nm with the *Streptomyces* R61 enzyme (i.e. at an unusually low wavelength). Finally, the turnover number of the *Streptomyces* R39 enzyme with N^{α}-Ac-L-Lys-D-Ala-D-Ala was high and that of the *Streptomyces* R61 enzyme was low. The exocellular DD-carboxypeptidases–transpeptidases from *Streptomyces* R39 and R61 are the first and thus far the only purified enzymes able to carry out transpeptidation reactions that are similar to or identical with the physiological ones involved in peptidoglycan synthesis. Blumberg & Strominger (1972) and Umbreit & Strominger (1973) reported the purification of a DD-carboxypeptidase from *Bacillus subtilis*.

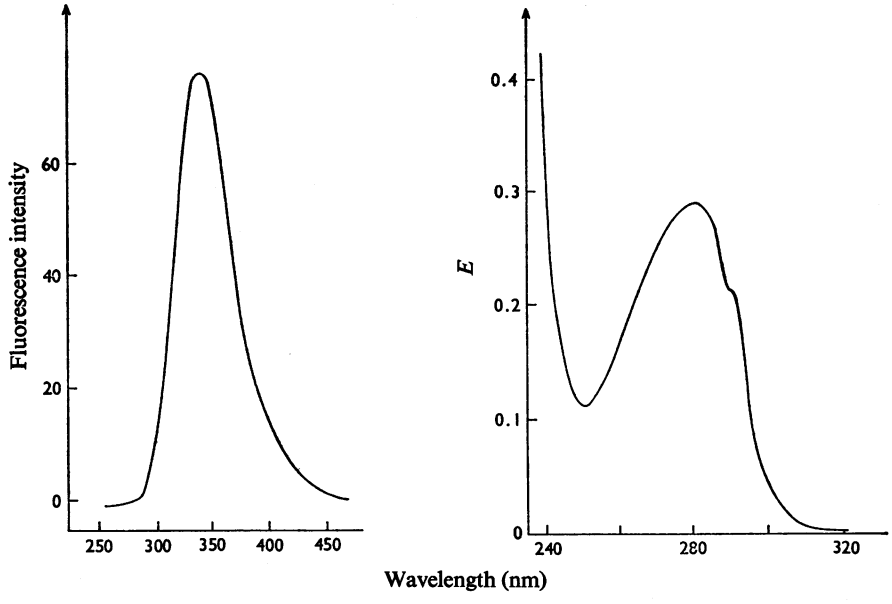


Fig. 3. *Absorption and fluorescence emission spectra of the Streptomyces R39* DD-*carboxypeptidase–transpeptidase*

The preparation obtained after step 5 was used. The spectra were determined on solutions containing either 0.298 mg of protein/ml (absorption spectrum) or 0.3 mg of protein/ml (fluorescence spectrum) in 0.1 M-Tris–HCl buffer, pH 7.7, containing 0.05 M-$MgCl_2$ and 0.2 M-NaCl. For the emission spectrum, excitation was at 285 nm. Fluorescence intensity was in arbitrary units. The fluorimeter was set at sensitivity 20, meter multiplier 0.03.

Table 3. *Summary of some physicochemical and chemical properties of the* DD-*carboxypeptidases–transpeptidases from Streptomyces strains R39 and R61*

	R39	R61
Mol.wt.	53300 ± 1000	38000 ± 1000
Frictional ratio (f/f_0)	1.07	1.12
No. of polypeptide chains	1	1
No. of disulphide bridges	1	1 or 0
No. of free thiol groups	0	1 or 3
% of acidic residues*	20.3	18.9
% of basic residues	7.4	9.7
Acidic residues* minus basic residues	12.9	9.2
% of hydrophobic residues	56	46
% of sulphur-containing residues	1.0	2.5
Molar activity (min^{-1})		
on Ac_2-L-Lys-D-Ala-D-Ala	1050	3300
on N^{α}-Ac-L-Lys-D-Ala-D-Ala	1900	15
Aggregation	At low ionic strength	On thawing (Nieto *et al.*, 1973*b*)
Maximum of fluorescence emission spectrum	339–340 nm	318–320 nm

* No account has been taken of possible asparagine and glutamine.

This enzyme did not perform transpeptidation reactions and seemed to exhibit a much lower rate constant of about $0.05 s^{-1}$ as compared with the values of $16–50 s^{-1}$ for the *Streptomyces* enzymes. Moreover, the *Bacillus* enzyme, which was purified from the plasma membrane, was not a true soluble entity but behaved as an aggregate of enzyme proteins and detergent molecules. Finally, a comparison between

the *Streptomyces* and *Bacillus* enzymes with regard to their amino acid composition was not possible, since the total amount of amino acid residues found in hydrolysates of the *Bacillus* enzyme (Umbreit & Strominger, 1973) only represented about 0.2% of the nominal amount of protein used.

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