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Transpeptidase Activity of *Streptomyces* D-Alanyl-D Carboxypeptidases

(penicillin/bacterial cell-wall synthesis/ristocetin donor and acceptor configurations)

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ABSTRACT **In the presence of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala as donor, and either D-[^{14}C]alanine, [^{14}C]-glycine, or *meso*-[^{3}H]diaminopimelic acid as acceptor, the DD carboxypeptidases from *Streptomyces* R61 and R39 catalyze a transpeptidation reaction with the release of terminal D-alanine from the donor and the formation of either N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-[^{14}C]Ala, N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly, or N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-*meso*-[^{3}H]diaminopimelic acid. The reaction appears to be a true transpeptidation, and is not simply a "reversal of hydrolysis". Transpeptidation is inhibited by penicillin at concentrations that inhibit hydrolysis (carboxypeptidase action) of the donor peptide. There are differences in the specificity profiles of the *Streptomyces* enzymes for acceptor molecules: only the R61 enzyme used [^{14}C]Gly-Gly as acceptor; transfer of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala to this acceptor resulted in the formation of N^{α},N^{ϵ}-diacetyl-Lys-D-Ala-[^{14}C]Gly-Gly, with the synthesis of a (D-Ala-Gly) peptide bond in an endoposition.**

From studies of the specificity profiles of soluble, purified DD carboxypeptidases excreted from *Streptomyces* strains (*albus* G, R61, Kll, and R39) (1–5), some of us suggested (2, 4) that these enzymes might be solubilized forms of transpeptidases which, when integrated in the plasma membrane, would catalyze the last step in the synthesis of bacterial cell-wall peptidoglycan (6, 7), and would thus function as carboxypeptidases or transpeptidases, depending upon the availability of a nucleophilic acceptor (H_2O or R-NH_2). A similar conclusion could be drawn from results obtained by Reynolds (8, 9) with a completely different system. We now present experimental evidence showing that two different *Streptomyces* carboxypeptidases can indeed perform transpeptidations in aqueous solution when the proper acceptor and donor are present.

MATERIALS AND METHODS

The substrate (donor) peptide used was N^{α}, N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala. DD carboxypeptidases from strains *albus* G, R61, and R39 were used. Enzyme units have been defined (2, 4, 5): at substrate concentrations of 10 times the K_m values, one enzyme unit catalyzes the hydrolysis of 1 neq of D-Ala-D-Ala linkage per hour at 37°. K_m values are 12 mM, 0.8 mM, and 0.33 mM for the R61, R39, and *albus* G enzymes, respectively.

Paper chromatography was done on Whatman SG-81 silica-gel loaded paper. Ascending chromatograms were developed for 5 hr in 1-butanol–acetic acid–water 3:1:1. The R_F values were 0.36 for glycine, 0.42 for D-alanine, 0.62 for N^{α}, N^{ϵ}-diacetyl-L-Lys-D-Ala-Gly and 0.72 for N^{α}, N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala. Amino acids were detected with ninhydrin, and tripeptide was detected either with the Schweppe reagent (10) or by the Rydon and Smith method (11).

Paper electrophoresis was performed in 0.2 N acetic acid (pH 2.9), in acetic acid–pyridine–water 0.33:4:1000, pH 5.6, and in acetic acid–collidine–water 2.65:9.10:1000, pH 7.

Radioactive chromatograms or electrophoretograms were quantitatively analyzed by cutting strips (3.6 cm in width) into sections of 5–10 mm (12). The paper sections were placed in counting vials, 0.5 ml of water was added, and the vials were allowed to stand overnight. Bray's (13) dioxane scintillant (11 ml) was added and the samples were counted. In some experiments, samples were eluted before addition of the scintillant.

Free D-alanine was estimated by reaction with fluorodinitrobenzene (14, 15).

RESULTS

Transpeptidase Activity of the R61 and R39 Enzymes at High Acceptor Concentrations. Fig. 1*a* shows the rate of release of the C-terminal D-alanine from N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala by R61 and R39 DD carboxypeptidases. Incubations were at 37°C in 17 mM phosphate buffer, pH 8, for the R61 enzyme and either in the same phosphate buffer or in 34 mM veronal buffer, pH 8.5, for the R39 enzyme; the final enzyme concentrations were 27 units/μl and 4 units/μl, respectively. The concentration of tripeptide was 1.5 mM; maximal hydrolysis was reached after about 90 min.

Fig. 1 *b* and *c* show the rate of transpeptidation when either D-[*U*-^{14}C]alanine, L-[*U*-^{14}C]alanine, or [*U*-^{14}C]glycine was present in the reaction mixtures. Molar ratios of [^{14}C]amino-acids to tripeptide were either 100:1 or 10:1, at final pH values of 7.5 and 7.9, respectively. After various times of incubation, [^{14}C]aminoacids and newly-labeled tripeptide were separated and quantitated.

With both the R61 and R39 enzymes, D-alanine and glycine were found to be acceptors, while L-alanine, even at a ratio to tripeptide of 100:1, was not an acceptor. At the 100:1 ratio of D-alanine or glycine to tripeptide, formation of either N^{α},-N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]D-Ala or N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly closely paralleled hydrolysis of the donor tripeptide in the absence of amino acid acceptor (Fig. 1*b* and *c*, compare with *a*). After 90 min, at which time hydrolysis was

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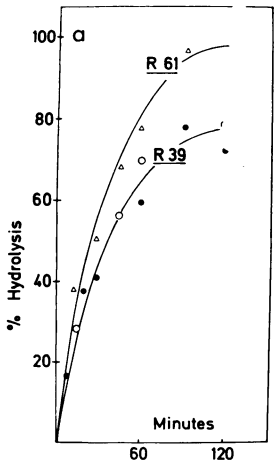
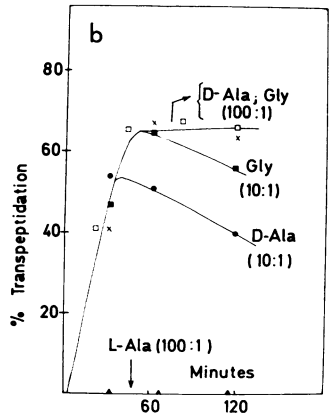
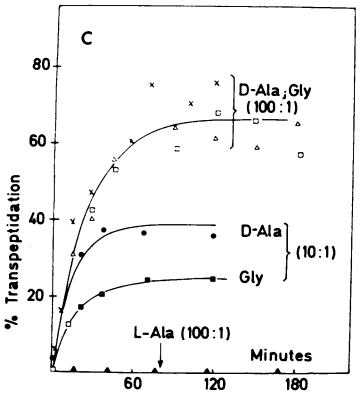


FIG. 1. (*a*) Rate of release of C-terminal D-alanine from N^{α}, N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala, by R39 and R61 DD carboxypeptidases. For conditions, see text. Δ——Δ and ●——●, 17 mM phosphate buffer, pH 8; ○——○, 34 mM Veronal buffer, pH 8.5.



(*b*) Rate of formation of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-[^{14}C]-Ala or N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly by R61 DD carboxypeptidase. Ratios of aminoacid acceptor to tripeptide donor are in parentheses. Percent of transpeptidation refers to the mole percent of unlabeled tripeptide donor converted into newly-labeled peptide. Each reaction mixture (30 μl final volume, 17 mM phosphate buffer, pH 8) contained 45 nmol of N^{α}, N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala and one of the following acceptors: 4500 nmol of D-[^{14}C]-alanine (25,000 cpm/μmol), 450 nmol of D-[^{14}C]alanine (250,000 cpm/μmol), 4500 nmol of L-[^{14}C]alanine (30,000 cpm/μmol), 4500 nmol of [^{14}C]glycine (30,000 cpm/μmol), or 450 nmol of [^{14}C]glycine (300,000 cpm/μmol).



(*c*) Rate of formation of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-[^{14}C]Ala and N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly by R39 DD carboxypeptidase. ×——× and Δ——Δ, ratio D-Ala to tripeptide = 100:1; □——□, ratio Gly to tripeptide = 100:1. Experiments were done in 17 mM phosphate buffer, pH 8, except those of curve Δ——Δ, which were in 34 mM Veronal buffer, pH 8.5.

maximal, yields of transpeptidation product, expressed as percent of tripeptide donor present in the reaction mixture, were about 65–75% (Fig. 1*b* and *c*). Relative to the amount of hydrolysis, however, transpeptidation was virtually complete with the R39 enzyme, but only about 65% complete with the R61 enzyme (Fig. 1*b* and *c*, compare with *a*). At the 10:1 ratio of D-alanine or glycine to tripeptide, transpeptidation decreased more markedly with R39 enzyme (Fig. 1*c*) than with R61 enzyme (Fig. 1*b*).

When [^{14}C]glycine was used as the acceptor, at a molar ratio of acceptor to donor of 10:1, the amount of unlabeled D-alanine released from donor during the formation of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly was identical with both enzymes to that observed during hydrolysis of the tripeptide in the absence of glycine (Fig. 1*a*).

Isolation and Characterization of N^{α},N^{ϵ}-Diacetyl-L-Lys-D-Ala-D-[^{14}C]Ala. N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala (1.215 μmol) was incubated at 37°C in 17 mM phosphate buffer, pH 8, with R39 DD carboxypeptidase (3500 units) in the presence of 121.5 μmol of [^{14}C]D-alanine (25,000 cpm/μmol), in a final volume of 810 μl. After 100 min, the reaction mixture was chromatographed and the area corresponding to the tripeptide was eluted with 0.1 M phosphate buffer, pH 8. The isolated material was incubated with the R39 DD carboxypeptidase in the absence of acceptor, under conditions that allowed complete hydrolysis of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala. Released D-[^{14}C]alanine was isolated by paper chromatography and paper electrophoresis at pH 2.9 and pH 5.6. The radioactivity recovered both in the tripeptide region (after transpeptidation) and in the D-[^{14}C]alanine area (after hydrolysis of labeled tripeptide with fresh enzyme) indicated that formation of diacetyl-L-Lys-D-Ala-D-[^{14}C]Ala had occurred to the extent of 80% based on the amount of expected transpeptidation after 100 min of incubation (see Fig. 1*c*).

Test for Reversal of Hydrolysis. The tripeptide N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala was first hydrolyzed (in phosphate buffer) completely to the dipeptide N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala and D-alanine by R61 enzyme, under the same conditions as in Fig. 1*a*. The mixture was then boiled for 10 min and lyophilized. The freeze-dried material was dissolved in water and incubated at 37° with either fresh R39 or fresh R61 enzyme in the presence of D-[^{14}C]alanine, at a ratio of D-alanine to dipeptide of 100:1. Analyses of the reaction mixtures showed that no labeled tripeptide was formed during the second incubation, demonstrating that the enzymes were unable to synthesize N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-[^{14}C]Ala from the dipeptide N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala and D-[^{14}C]-alanine.

Transpeptidase Activity of the R61 and R39 Enzymes at Low Acceptor Concentrations. N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala (4.75 mM) and [2-^{14}C]glycine (0.3 mM or 0.03 mM) were

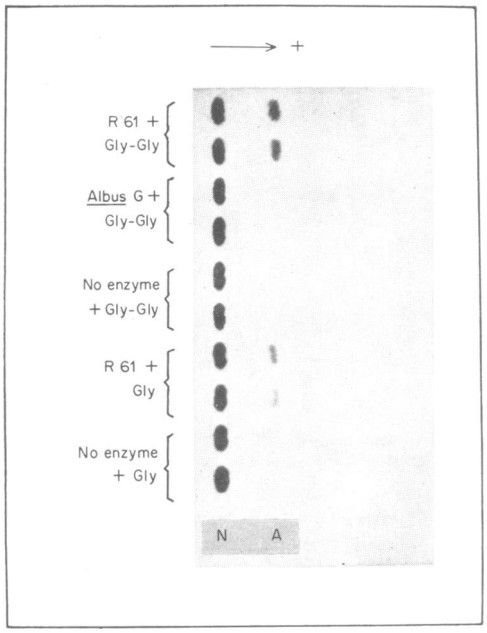


FIG. 2. Autoradiogram of an electrophoretogram showing transpeptidation of the dipeptide Gly-Gly and free Gly by the R61 enzyme, and the lack of action of the *albus* G enzyme. N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala (50 nmol) and either [1-^{14}C]-Gly-Gly or [2-^{14}C]Gly (50 nmol) were incubated for 60 min at 37°, in 30 μl of 17 mM phosphate, pH 8, in the presence of 135 units of R61 enzyme. The incubation mixtures were subjected to paper electrophoresis at pH 7, for 2 hr, at 14 V/cm. After autoradiography, radioactive areas were eluted and used to quantitate the formation of labeled N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly-Gly and N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly. The yields of transpeptidation, expressed as conversion of tripeptide donor, were 18 and 14%, respectively. Similarly, N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala (50 nmol) and [1-^{14}C]Gly-Gly (50 nmol) were incubated for 60 min at 37° in 30 μl of 0.02 M Tris·HCl buffer (pH 8)–2 mM $MgCl_2$, in the presence of 100 units of *albus* G enzyme. Transpeptidation could not be detected. *N* = unchanged acceptor. *A* = transpeptidated product.

incubated with R61 enzyme (30 units/μl) for 60 min at 37° in 0.01 M Tris·HCl buffer, pH 7.5. The amount of newly formed, labeled tripeptide showed that, in both cases, 5% of the acceptor had undergone transpeptidation. Hence, transpeptidation can occur at a molar ratio of glycine acceptor to tripeptide donor as low as 1:125. If we assume that the concentration of water in the active-site region of the enzyme is 55 M, transpeptidation is observed even at molar ratios of glycine acceptor to water of $1:1.8 \times 10^6$.

As indicated in Fig. 1*c*, R39 enzyme exhibited lower transpeptidase efficiency at lower concentrations of glycine acceptor. With equimolar (1.5 mM) tripeptide and [2-^{14}C]Gly and 5 enzyme units/μl, in 17 mM phosphate buffer (pH 8) only 3.9% of the glycine acceptor was incorporated after 60 min of incubation.

Transpeptidase Activity of R61 and R39 Enzymes with Meso-[^{3}H]Diaminopimelic Acid as Acceptor. N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala (50 nmol) and *meso*-[^{3}H]diaminopimelic acid (50 nmol or 500 nmol) were incubated for 60 min at 37° in 30 μl of 17 mM phosphate buffer, pH 8, with R61 enzyme (27 units/μl) or R39 enzyme (4 units/μl). The material transpeptidated to *meso*-diaminopimelic acid was eluted from electrophoresis strips and counted. With R61 enzyme the yields, expressed as conversion of the donor tripeptide, were 18% and 48% for ratios of *meso*-diaminopimelic acid to tripeptide of 1:1 and 10:1. With R39 enzyme, the yields were 7% and 45%, respectively. The nature of the newly-formed peptide bond, i.e., either D-alanyl-D-*meso*-diaminopimelic acid or D-alanyl-L-*meso*-diaminopimelic acid (or a mixture of both) was determined as follows: (*i*) A sample of the isolated labeled peptide was incubated with R61 enzyme for 5 hr. *meso*-[^{3}H]-diaminopimelic acid was liberated; the radioactivity recovered indicated that 94% of the N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-*meso*-[^{3}H]diaminopimelic acid had been hydrolyzed into dipeptide and free *meso*-[^{3}H]diaminopimelic acid. Since R61 enzyme does not cleave a C-terminal D-alanyl-L-alanyl linkage (4), and therefore apparently requires a C-terminal D-alanyl-D linkage for its hydrolytic activity, complete hydrolysis of the labeled tripeptide suggests that the peptide bond formed by transpeptidation was D-alanyl-D-*meso*-diaminopimelic acid. (*ii*) Isolated labeled peptide was mixed with the antibiotic ristocetin, at a molar ratio of tripeptide to ristocetin of 1:2. After paper electrophoresis, the major portion of the counts appeared to be linked to ristocetin, which is known to combine only with those peptides having a C-terminal D-D sequence (16). This result is in accord with the formation of a D-Ala-D-*meso*-diaminopimelic acid linkage by transpeptidation.

Transpeptidase Activity of R61 and R39 Enzymes with [1-^{14}C]Glycyl-Glycine Dipeptide as Acceptor. In the above experiments, peptide bonds formed by transpeptidation were located at the C-terminal position in the newly synthesized

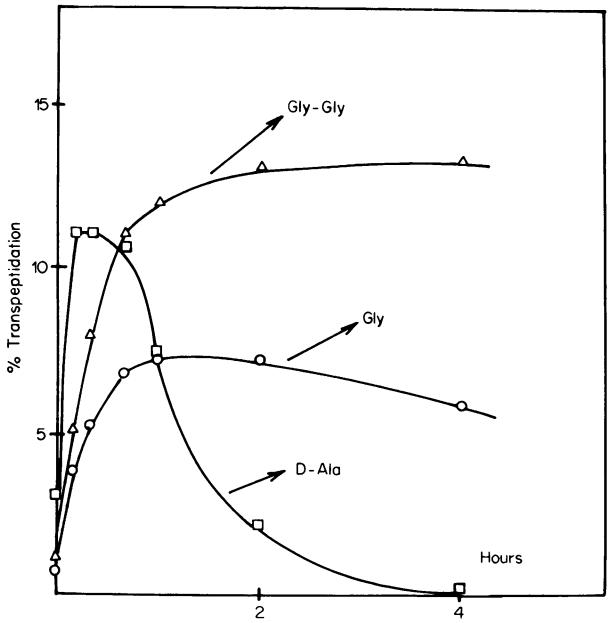


FIG. 3. Rate of transpeptidation by R61 enzyme with an acceptor to donor ratio of 1:1. In a total volume of 150 μl, there were 25 μl of 0.1 M phosphate buffer, pH 8, 675 units of R61 enzyme, 250 nmol of tripeptide donor and of acceptor, [2-^{14}C]-glycine, [1-^{14}C]glycyl-glycine, or D-[*U*-^{14}*C*]alanine. Incubation was at 37°, and 20-μl samples were taken at intervals for paper electrophoresis. After electrophoresis at pH 7 for 2 hr (14 V/cm), strips were dried and radioactive areas were counted.

peptide. The ability of R61 enzyme to catalyze the formation of a D-alanyl-glycyl peptide bond in an endoposition was also tested. The conditions used were analogous to those of Fig. 1*b* and *c*, except that [1-^{14}C]glycyl-glycine was used as acceptor. After 60 min of incubation, N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[1-^{14}C]Gly-Gly was quantitated. Yields, expressed as conversion of donor tripeptide, were about 50, 20, and 1% for ratios of Gly-Gly to tripeptide of 100:1, 1:1, and 1:10, respectively. Fig. 2 shows the formation of the labeled tetrapeptide N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[1-^{14}C]Gly-Gly, as revealed by autoradiography after paper electrophoresis. Formation of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly by the same enzyme is also shown in Fig. 2.

With R39 enzyme under the same conditions, no transfer to [^{14}C]Gly-Gly acceptor could be detected.

Rate of Transpeptidation at Acceptor to Donor Ratios of 1:1. In the experiments in Fig. 1*b* with R61 enzyme, saturating or near-saturating concentrations of acceptor were present, so that differences in the ability of amino acids to serve as acceptors in transpeptidation were difficult to detect. However, at lower (nonsaturating) concentrations of acceptor, any differences should be observable from an analysis of the rate of the transpeptidation reaction. The R61 enzyme was incubated with donor and [^{14}C]glycine, [^{14}C]glycyl-glycine, or D-[^{14}C]alanine as acceptor (acceptor to donor ratio, 1:1), and samples were taken at intervals to measure the amount of transpeptidation product (Fig. 3). With D-[^{14}C]alanine as acceptor, the product was chemically the same as the donor; after initial rapid transpeptidation, it was destroyed by hydrolysis over the next 4 hr. With [^{14}C]glycyl-glycine as acceptor, N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly-Gly was less rapidly formed than the corresponding D-alanine product, but the maximal amount of glycyl-glycine product was greater since it was not a substrate for hydrolysis by the enzyme and loss of product did not occur. With [^{14}C]glycine as acceptor, the product was relatively stable, although, in comparison to D-alanine or glycyl-glycine transpeptidation, the initial rate of product formation was slower. Apparently, D-alanine was a better acceptor than glycyl-glycine, which in turn was a better acceptor than glycine.

Effect of Penicillin G on Transpeptidase Activity of R61 and R39 Enzymes. Both R39 and R61 DD carboxypeptidases are inhibited by low concentrations of penicillin (4, 5). The effect of penicillin on both the release of D-alanine from the tripeptide donor and the formation of N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-[^{14}C]Gly was studied. 1.5 mM Tripeptide and 15 mM [*U*-^{14}C]glycine were incubated at 37° for 60 min, in 17 mM phosphate buffer (pH 8) with R61 enzyme (27 units/μl) or R39 enzyme (4 units/μl) in the absence of penicillin (see conditions of Fig. 1*b* and *c*) and in the presence of various amounts of the antibiotic. Release of free D-alanine and formation of diacetyl-L-Lys-D-Ala-[^{14}C]Gly are shown in Table 1. Under the experimental conditions used, the R39 enzyme was slightly more sensitive to the action of penicillin than was the R61 enzyme. A parallel loss of transpeptidation activity and of hydrolytic activity for both enzymes was observed.

Transpeptidase Activity of the Albus G Enzyme. In contrast to the R61 and R39 enzymes, the *albus* G enzyme exhibited little transpeptidase activity. At ratios of glycine to tripeptide of 10:1 and 100:1, the yield of transpeptidation product expressed as conversion of donor tripeptide (in one experiment) was 1.4 and 1.6%. In another experiment, transpeptidation of D-alanine, glycine, or glycyl-glycine could not be detected (Fig. 2). Incubations were in 20 mM Tris buffer (pH 8)–2 mM $MgCl_2$ for 1 hr, at which time the hydrolysis of donor tripeptide was complete.

DISCUSSION

The hydrolytic action and penicillin sensitivity of the soluble DD carboxypeptidases of several strains of *Streptomyces* have been studied on peptides with C-terminal DD dipeptide linkages (1–5). The present experiments show that in the presence of a suitable carboxyl donor, diacetyl-L-Lys-D-Ala-D-Ala, and a proper amino acceptor, the purified R61 and R39 enzymes are able to catalyze transpeptidation with release of the terminal D-alanine residue of the donor peptide. The rate of transpeptidation (at saturating D-alanine or glycine acceptor concentrations) paralleled that of hydrolysis of donor peptide, with water as the only acceptor (Fig. 1*a*, *b*, and *c*). When penicillin was added to the transpeptidation assay, inhibition paralleled the results observed in the carboxypeptidase assay (Table 1). These findings strongly suggest that a single enzyme is responsible for both carboxypeptidase and transpeptidase activities.

The transfer of the dipeptidyl residue to a proper acceptor molecule is a true transpeptidation reaction and cannot be simply a reversal of hydrolysis for the following three reasons: (*i*) When the product of the hydrolytic action (i.e., N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala dipeptide) was incubated with fresh R39 or R61 enzyme in the presence of a large excess of D-alanine, no conversion to tripeptide was observed. (*ii*) At very low acceptor concentrations (i.e., when the proportion of donor to acceptor was as much as 100:1), the R61 enzyme still catalyzed a measurable transpeptidation. Under such conditions, a straight-forward reversal of hydrolysis would hardly be detected. (*iii*) Gly-Gly was a good acceptor for the R61 enzyme, and the reaction product diacetyl-L-Lys-D-Ala-Gly-Gly appeared not to be a substrate for the enzyme

TABLE 1. *Effect of penicillin G on hydrolysis (release of* D-*Ala from* N^{α},N^{ϵ}-*diacetyl-*L-*Lys-*D-*Ala-*D-*Ala) and on transpeptidation (formation of* N^{α},N^{ϵ}-*diacetyl-*L-*Lys-*D-*Ala-*[^{14}C]-*Gly] by* DD *carboxypeptidases–transpeptidases from R61 and R39*

	Percent Inhibition			
	R61 enzyme		R39 enzyme	
Penicillin concentration μM	Hydrolysis	Transpeptidation	Hydrolysis	Transpeptidation
100	100	94	95	90
50		92	95	90
10	91	88	95	87
5		58	86	83
1	9	10	31	
0.5	0	8	23	7
0.1	0	4	10	0
0.01	0	0	0	0

Values are calculated on the basis of a control experiment without penicillin. For comparison, the values for hydrolysis and transpeptidation obtained in the control were set to 100%.

(Fig. 3). Especially in this latter case, transpeptidation cannot be the reversal of hydrolysis.

The synthesis of a C-terminal D-alanyl-D-*meso*-diaminopimelic acid linkage (from N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala and *meso*-diaminopimelic acid) by the R61 and R39 enzymes, and the synthesis of a D-alanyl-glycyl bond in an endoposition (from N^{α},N^{ϵ}-diacetyl-L-Lys-D-Ala-D-Ala and Gly-Gly) by the R61 enzyme, are of prime interest in elucidation of the peptide-crosslinking functions played by these enzymes in peptidoglycan synthesis of the bacterial cell wall. In *Escherichia coli* and *Bacillus megaterium* KM, peptide crosslinking involves a D-alanyl-D-alanine sequence in the donor peptide and a *meso*-diaminopimelic acid residue in the acceptor peptide, resulting in the formation of a C-terminal D-alanyl-D-*meso*-diaminopimelic acid interpeptide bond (17, 18). The presence of a transpeptidase in a particulate preparation from *B. megaterium* that catalyzes the incorporation of *meso*- or DD-diaminopimelic acid has been briefly reported (19). In *Streptomyces* species, analysis of the peptidoglycan layer (20) indicates that peptide crosslinking in these organisms involves a D-alanyl-D-alanine donor peptide sequence and a glycyl-LL-diaminopimelic acid sequence in the acceptor peptide to yield a D-Ala-Gly interpeptide bond in an endoposition.

Both the R61 and R39 enzymes show specificity in their requirements for acceptors. Whereas D-alanine and glycine were good acceptors, L-alanine was not an acceptor and glycylglycine was an acceptor only for the R61 enzyme. Although Gly-Gly was not an acceptor for the R39 enzyme in our transpeptidase system, it does not follow that Gly-LL-diaminopimelic acid would also be unsuitable as an acceptor. The Gly-LL-diaminopimelic acid, i.e., the sequence that occurs in *Streptomyces albus* G (20), and probably also in the R39 strain, is considerably larger than Gly-Gly, and could conceivably be suitably oriented to permit transpeptidation. The *albus* G enzyme did not catalyze transpeptidation reactions with any of the donor–acceptor systems used. It is possible that, in this instance, the natural *Streptomyces* donor or acceptor activities, or both, are required for effective transpeptidation. It is also noteworthy that although all DD carboxypeptidases isolated from *Streptomyces* hydrolyze dimers and oligomers of the cell-wall peptide if the interpeptide bond is mediated through a C-terminal D-alanyl-D-linkage (see chart I, ref. 1), the *albus* G enzyme is unique among the *Streptomyces* DD carboxypeptidases in several respects. It is the only DD carboxypeptidase that lyses bacterial cells and walls, and the only one that is a basic protein (1, 5).

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