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Preferential production of rapamycin *vs* prolylrapamycin by *Streptomyces hygroscopicus*

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A trace of prolylrapamycin is often produced in rapamycin fermentations carried out by strains of *Streptomyces hygroscopicus*. Prolylrapamycin was produced as the major rapamycin when L-proline was added to the fermentation medium. Addition of proline plus thiazolidine-2-carboxylic acid (T2CA), a sulfur-containing proline analog, prevented rapamycin production and stimulated prolylrapamycin production, thereby resulting in an even greater selective production of prolylrapamycin. T2CA addition inhibited rapamycin production even in the presence of L-lysine which is converted into pipecolic acid intracellularly and normally stimulates rapamycin formation. Addition of the rapamycin precursor, DL-pipecolic acid, surprisingly failed to stimulate rapamycin production. However, when DL-pipecolic acid was added with L-proline, it reduced the formation of prolylrapamycin and stimulated rapamycin production; this was evident especially in the presence of T2CA. The evidence suggests that T2CA suppresses rapamycin production by inhibiting intracellular conversion of L-lysine into pipecolate. Furthermore, the data suggest that uptake of pipecolate into the cell is stimulated or induced by growth in the presence of L-proline and/or T2CA.

Keywords: biosynthesis; immunosuppressants; prolylrapamycin; rapamycin; *Streptomyces hygroscopicus*

Introduction

Rapamycin is a 31-membered macrolide antibiotic (Figure 1) produced by *Streptomyces hygroscopicus*. While originally discovered as an antifungal agent [21], rapamycin is being developed as a new immunosuppressant [20]. This versatile molecule also possesses antitumor activity [5].

Biosynthetic studies on rapamycin indicated that the precursors of the molecule are acetate, propionate, shikimate, l-methionine, and l-lysine (via pipecolate) [10,14–16]. Apparently, rapamycin biosynthesis starts with a moiety derived from shikimate; then, seven acetate and seven propionate units participate to build up a polyketide backbone in a head-to-tail fashion; finally, pipecolate attaches to the polyketide chain, followed by ring closure via lactone formation. Three methyl groups are transferred from methionine via *S*-adenosyl methionine to form the three methoxy groups. Rapamycin biosynthetic genes were found in a 110-kbp DNA region that encodes three polyketide synthase clusters and 24 additional open reading frames [11,19]. A protein encoded by *rapP* is enzymatically similar to the pipecolate-incorporating enzyme in the immunomycin-producing *S. hygroscopicus* var *ascomyceticus*. Products of *rapM* and *rapQ* and those of *rapJ* and *rapN* are structurally homologous to methyltransferases and cytochrome P-450 enzymes, respectively [11,19]. A review of rapamycin biosynthesis has been published [17].

On the basis of knowledge of rapamycin biosynthesis and nutritional studies on the rapamycin fermentation [3,4,9], we have been pursuing the generation of novel rapamycins by feeding analogs of the rapamycin precursors. In this paper, we present the effects of l-proline and its sulfur analog, thiazolidine-2-carboxylic acid (T2CA), on production of rapamycin and prolylrapamycin (Figure 1). We report that selective production of prolylrapamycin is achieved by the addition of l-proline and T2CA to the fermentation medium and that addition of dl-pipecolate to the medium has no effect unless it is accompanied by l-proline and/or T2CA; under such conditions, dl-pipecolate stimulates rapamycin production.

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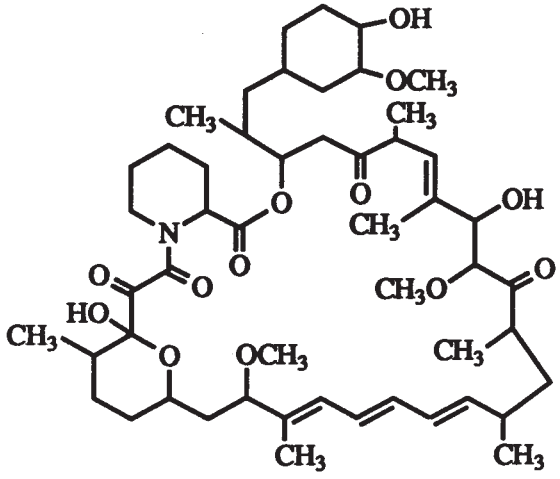
Materials and methods

Microorganism

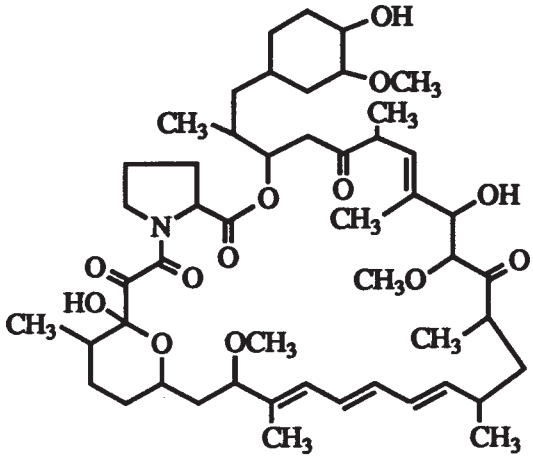
Streptomyces hygroscopicus C9, a spontaneous variant derived from strain AY-B1206, was used to prepare a spore suspension as described previously [9]. The spore suspension was stored at −80°C.

Seed cultivation

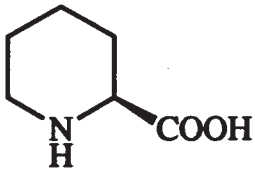
A seed culture was initiated by adding 0.4 ml of a thawed spore suspension to a 250-ml baffled Erlenmyer flask containing 25 ml of a seed medium consisting of (g L^{-1}): glucose 10; Bactopeptone (Difco Laboratories, Detroit, MI, USA) 4; yeast extract (Difco) 4; casamino acids (Difco) 1.5; $MgSO_4 \cdot 7H_2O$ 0.5; and K_2HPO_4 1.0, pH 7.0–7.3. Incubation was at 28°C for 2 days on a rotary shaker (220 rpm). The resulting culture was centrifuged at 4°C for 15 min ($5000 \times g$), and the cells were washed once with 100 mM 2-(*N*-morpholino)ethanesulfonic acid monohydrate (MES) buffer (pH 6.0) containing 0.5% NaCl and 0.05% $MgSO_4 \cdot 7H_2O$. The washed cells were resuspended in the same buffer to make a 10-ml suspension, and 0.5-ml por-



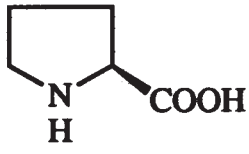
Rapamycin



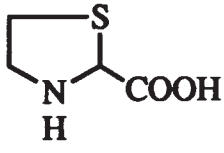
Prolylrapamycin



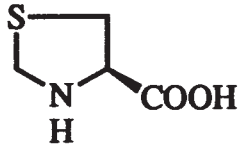
L-Pipecolic acid



L-Proline



Thiazolidine-2-carboxylic acid (T2CA)



L-Thiazolidine-4-carboxylic acid (Thiaproline)

Figure 1 Structures of rapamycin, prolylrapamycin, d1-pipecolic acid, l-proline, and sulfur analogs of l-proline.

tions of the suspension were inoculated into 250-ml baffled Erlenmyer flasks containing 25 ml of chemically-defined fermentation medium.

Fermentation

We used a basal chemically-defined medium based on Medium 3, which had been previously developed [3], with the following modifications: l-lysine was eliminated and the concentrations of $FeSO_4 \cdot 7H_2O$ and K_2HPO_4 were reduced to 100 mg L^{-1} and 0.87 g L^{-1}, respectively. For the purpose of adding precursors and/or analogs, 2-fold concentrated basal medium and the individual additive solutions were separately prepared and autoclaved. At the time of inoculation, the additives were added to the basal medium, and the total volume of the medium was adjusted with sterile deionized water. Fermentations were normally conducted at 28°C for 7 days on a rotary shaker (220 rpm). However, fermentations conducted in the presence of T2CA were performed for 9 days, since the growth of *S. hygroscopicus* was somewhat delayed. Cell growth was measured by optical means and then converted to dry cell weight (DCW: g L^{-1}) as previously described [9].

Preparation of culture extracts

Culture broth (10 ml) was centrifuged at 4°C for 15 min ($5000 \times g$) to separate supernatant fluid from cells. The supernatant was put aside and the precipitated pellet was extracted with 10 ml methanol by shaking at 30°C for 1 h. The methanol extract was centrifuged at 4°C for 15 min ($5000 \times g$), and the resulting methanol supernatant was combined with the culture supernatant, followed by extraction with 20 ml ethyl acetate. The separated organic layer was dried by the addition of anhydrous sodium sulfate, allowed to stand for about 10 h, and concentrated to dryness under reduced pressure. The resulting residue was redissolved in 0.5 ml methanol.

HPLC analysis

HPLC analysis was conducted on a Waters™ LC Module I (Millipore Corp, Milford, MA, USA). The sample (10 μl)

was loaded onto a C_{18} reversed phase column (Nova-Pak C_{18}, 3.9 × 150 mm, Millipore) and eluted isocratically with the mobile phase (1,4-dioxane/water (55/45)) at a flow rate of 1.0 ml min^{-1} for 40 min. Rapamycin and its derivatives were monitored at 287 nm. Under these conditions, rapamycin and prolylrapamycin were eluted at retention times (RTs) of *ca* 27 and 18 min, respectively. Since RTs of rapamycin and its derivatives were somewhat variable from one run to another, we calculated relative retention times (RRTs) of the individual eluted peaks as compared to the RT of rapamycin which was given an RRT of 1.00. RRT values remained constant as long as the same mobile phase was used. Concentrations of rapamycin and prolylrapamycin were calculated by measuring peak areas. In order to decide whether new peaks were derivatives of rapamycin, we examined UV absorption of the peaks using a Waters™ 996 Photodiode Array Detector (Millipore), since rapamycin and its analogs show the same specific UV absorption which originates from the triene structure in the macrolide ring.

Authentic samples and chemicals

Authentic samples of rapamycin and prolylrapamycin were obtained from Wyeth-Ayerst Research (Princeton, NJ, USA). l-Proline, l-lysine, dl-pipecolic acid, and l-thiazolidine-4-carboxylic acid (thiaproline) were from Sigma Chemical Co (St Louis, MO, USA), and thiazolidine-2-carboxylic acid (T2CA) was purchased from Lancaster Synthesis Inc (Windham, NH, USA). All chemicals were of the highest quality available.

Results

Prolylrapamycin production with exogenous L-proline

In the course of feeding studies of pipecolate analogs, we observed that l-proline addition at 10 g L^{-1} stimulated formation of a rapamycin-like peak which has a relative retention time (RRT) of 0.66 on HPLC chromatograms (Figure 2). Without l-proline addition, none or only a small amount of this compound was formed. The compound was found to be prolylrapamycin ('21-norrapamycin'; [18]) by HPLC comparison with authentic prolylrapamycin. The rapamycin peak was somewhat reduced by l-proline addition but that of prolylrapamycin was markedly stimulated (Figure 2). In the fermentation with l-proline, a minor peak of unknown composition at an RRT of 0.53 was also observed.

As l-proline concentration was increased up to 10 g L^{-1}, prolylrapamycin accumulated in a linear fashion (Figure 3a); volumetric rapamycin production was also elevated but only up to a l-proline concentration of 5 g L^{-1}. Since l-proline also stimulated cell growth (ie, final DCWs (g L^{-1}) were 0.46, 0.71, 1.33, 1.87, and 1.89 at l-proline concentrations (g L^{-1}) of 0, 2.5, 5, 10, and 20, respectively), specific production of rapamycin and prolylrapamycin were calculated (Figure 3b). Specific production of prolylrapamycin exhibited a major increase at 2.5 g L^{-1} of l-proline, followed by a moderate increase up to 10 g L^{-1}, whereas that of rapamycin showed no increase and a decline with increasing concentrations of l-proline between 2.5 and 20 g L^{-1}.

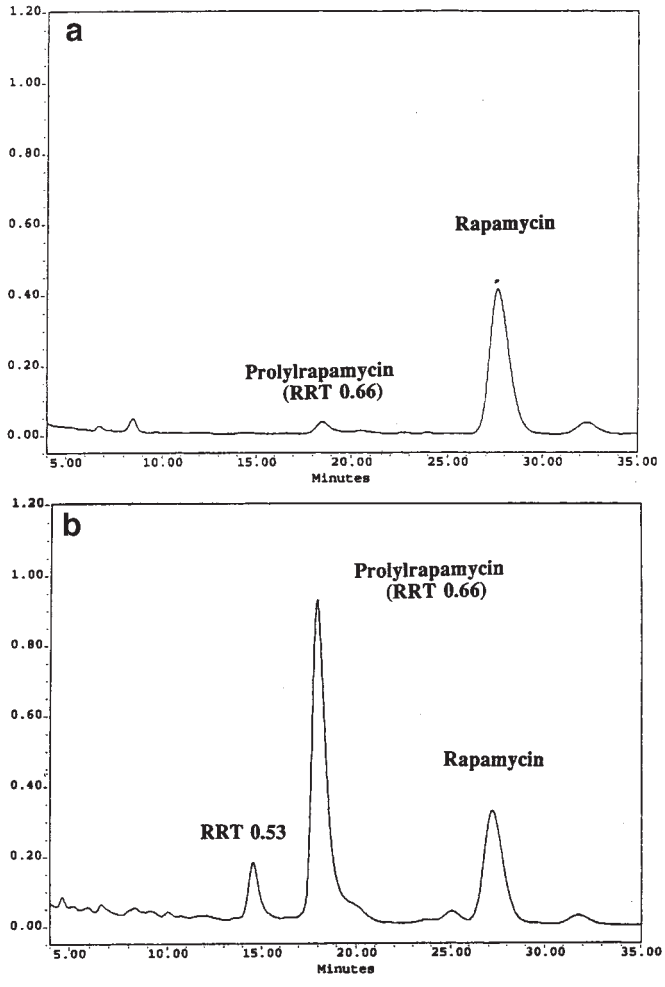


Figure 2 HPLC chromatograms of culture extracts of *S. hygroscopicus* C9 grown in the absence (a) and presence (b) of l-proline (10 g L^{-1}).

Inhibitory effect of thiazolidine-2-carboxylic acid (T2CA) on rapamycin biosynthesis

Studies involving analog addition showed that the l-proline analog T2CA (Figure 1) stimulated formation of a rapamycin-like compound with a peak at RRT 0.66 on HPLC chromatograms, which we assumed to be prolylrapamycin. Direct HPLC comparisons of the compound and prolylrapamycin with two different mobile phases showed identity. A low concentration of T2CA (0.25 g L^{-1}) stimulated formation of prolylrapamycin, while rapamycin formation was suppressed at all concentrations of T2CA tested (Table 1). Virtually no rapamycin was produced at a T2CA concentration of 2 g L^{-1} or higher. Prolylrapamycin formation was inhibited by T2CA when its concentration was increased above 0.25 g L^{-1}. Furthermore, T2CA lowered cell growth at concentrations of 2 g L^{-1} or higher. On the other hand, another sulfur-containing l-proline analog, l-thiazolidine-4-carboxylic acid (Figure 1), inhibited growth strongly at a concentration of 0.05 g L^{-1} and showed no differential effect on rapamycin *vs* prolylrapamycin production (Table 1).

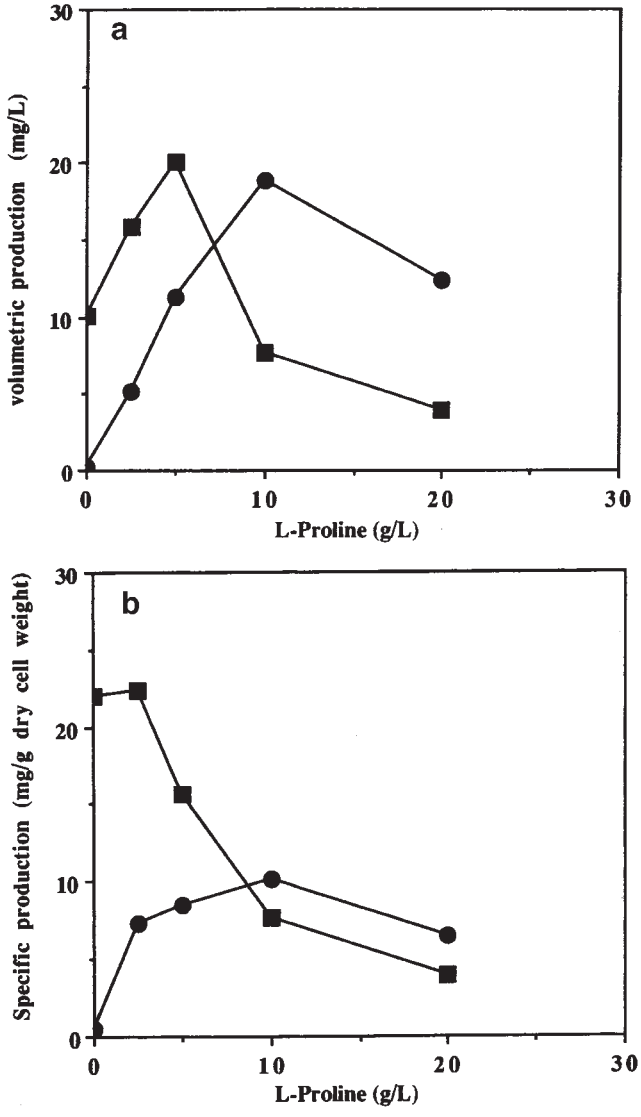


Figure 3 Effect of exogenous l-proline concentration on volumetric (a) and specific (b) production of rapamycin (■) and prolylrapamycin (●).

Table 1 Effect of thiazolidine-2-carboxylic acid (T2CA) and l-thiazolidine-4-carboxylic acid (thiaproline) on production of rapamycin and prolylrapamycin

Additive concentration (g L^{-1})	DCW (g L^{-1})	Production (mg L^{-1})	
		Rapamycin	Prolylrapamycin
None	0.49	10.9	0.5
T2CA			
0.25	0.46	2.1	2.4
0.5	0.60	0.9	1.9
1	0.43	0.5	1.2
2	0.34	0	0.2
3	0.31	0	0
Thiaproline			
0.025	0.48	11.6	0.7
0.05	0.10	6.1	0
0.1	0	0	0

DCW, dry cell weight.

Selective production of prolylrapamycin with exogenous L-proline in the presence of T2CA

Since a low concentration of T2CA suppressed rapamycin production and slightly stimulated formation of prolylrapamycin (Table 1), we examined its effect on prolylrapamycin formation brought about by adding l-proline at four concentrations. The concentration of T2CA chosen was 2 g L^{-1} at which little or no rapamycin accumulates but a small amount of prolylrapamycin may be formed (Table 1).

As shown in Figure 4a, in the absence of l-proline, prolylrapamycin accumulated to a small extent (2.7 mg L^{-1}), and only a trace of rapamycin was produced. As the concentration of added l-proline was increased, formation of prolylrapamycin was continuously stimulated and reached 35 mg L^{-1} at an l-proline concentration of 20 g L^{-1}, whereas rapamycin production was very low. Specific production of prolylrapamycin was also stimulated by l-proline (Figure 4b). Cell growth was enhanced by l-proline to

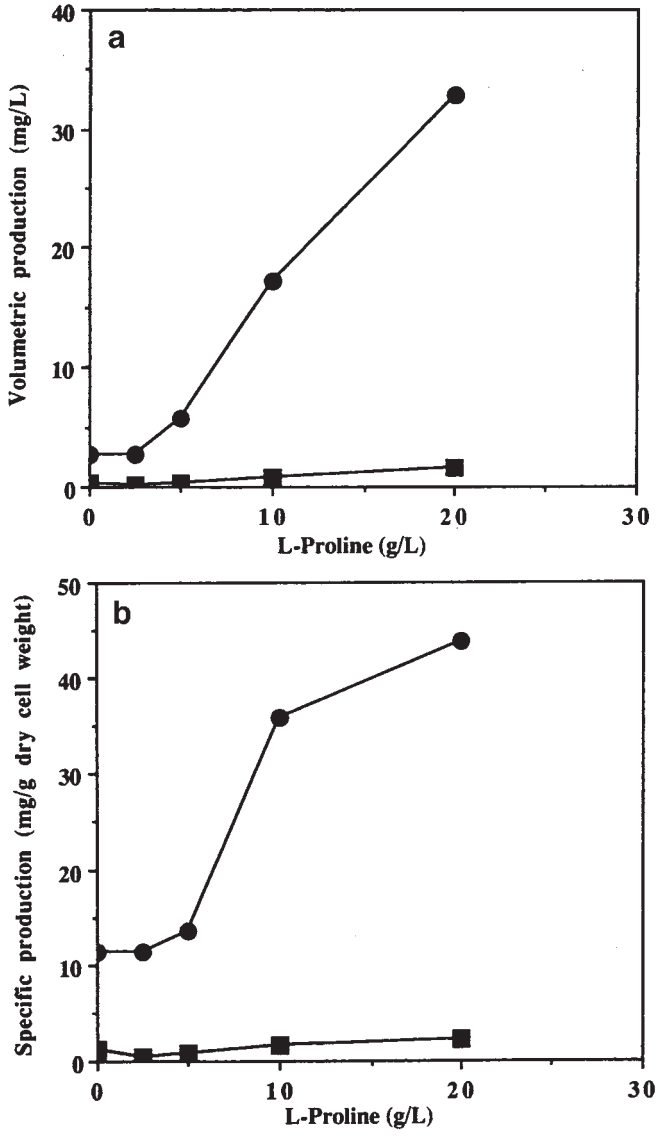


Figure 4 Effect of exogenous l-proline concentration on volumetric (a) and specific (b) production of rapamycin (■) and prolylrapamycin (●) in the presence of thiazolidine-2-carboxylic acid (2 g L^{-1}).

a lesser extent than in the absence of T2CA. DCWs (g L^{-1}) were 0.24, 0.34, 0.43, 0.48, and 0.75 at proline concentrations (g L^{-1}) of 0, 2.5, 5, 10, and 20, respectively. This is understandable since in the presence of growth-inhibitory concentrations of T2CA, more l-proline would be expected to be available for prolylrapamycin production as opposed to protein synthesis. These results show that T2CA exerts a differential effect in favor of prolylrapamycin over rapamycin when no other additives are present with the exception of l-proline.

The effect of DL-pipecolic acid and L-proline addition on formation of rapamycin and prolylrapamycin

At the late stage of the rapamycin biosynthetic pathway, dl-pipecolic acid is thought to be activated and incorporated by the pipecolate-incorporating enzyme into the polyketide chain to form the macrolide ring of rapamycin [11,19]. The l-proline-enhanced production of prolylrapamycin indicates that l-proline is recognized in the rapamycin producer as a substrate for activation and incorporation, as had been shown with the purified enzyme from the immunomycin producer [12].

In an attempt to further understand the relationship(s) between dl-pipecolic acid and l-proline in rapamycin and prolylrapamycin production, *S. hygroscopicus* C9 was cultivated with the addition of 5 g L^{-1} dl-pipecolic acid or 5 g L^{-1} l-proline. As shown in Table 2, dl-pipecolic acid, when added alone, surprisingly failed to stimulate rapamycin production. As expected, addition of l-proline stimulated formation of prolylrapamycin and suppressed that of rapamycin. However, simultaneous addition of dl-pipecolic acid and l-proline resulted in selective production of rapamycin. These results indicate that external dl-pipecolic acid has no effect when added alone but is preferentially incorporated into rapamycin when it and l-proline are added together.

Further studies on the effect of T2CA addition in the presence of added L-proline, L-lysine and/or DL-pipecolic acid

T2CA was shown above (Table 1, Figure 3) to inhibit rapamycin formation but stimulate prolylrapamycin production, particularly in the presence of exogenous l-proline. Normally, for the production of rapamycin, l-lysine is added to the medium [4]. l-Lysine is thought to be intracellularly converted into pipecolic acid and then incorporated into the rapamycin moiety, thus enhancing the titer of rapamycin [4,16]. In order to examine the effect of T2CA on rapamycin production in the presence of l-lysine or dl-pipecolic acid, *S. hygroscopicus* C9 was cultivated in the presence of these compounds (5 g L^{-1}) together with 2 g L^{-1} T2CA.

As expected, l-lysine addition increased the rapamycin titer, and T2CA decreased it (Table 3). In the absence of exogenous l-lysine, inhibition of rapamycin formation by T2CA was 100%. In the presence of l-lysine, the rapamycin titer was reduced 60% by T2CA. dl-Pipecolic acid alone failed to increase production of rapamycin. In a separate experiment, addition of the l-isomer of pipecolic acid (5 g L^{-1}) likewise failed to enhance rapamycin production (data not shown). In the presence of dl-pipecolic acid, T2CA failed to inhibit rapamycin production; indeed, formation was increased (Table 3). Thus, dl-pipecolate stimulates rapamycin production in the presence of T2CA (Table 3) or of l-proline (Table 2), but not when added alone.

In order to determine whether T2CA can interfere with preferential rapamycin production which occurs in the presence of dl-pipecolic acid plus l-proline (Table 2), *S. hygroscopicus* C9 was incubated with various concentrations of l-proline (2.5, 5, 10, and 20 g L^{-1}) together with 2 g L^{-1} T2CA and 10 g L^{-1} dl-pipecolic acid. As seen in Table 4, although dl-pipecolic acid alone failed to enhance rapamycin production, dl-pipecolate added together with increasing concentrations of l-proline stimulated rapamycin production preferentially, even in the presence of T2CA. Only a small amount of prolylrapamycin was observed at any concentration of l-proline. Thus rapamycin production is markedly enhanced by dl-pipecolate but only when it is added with T2CA and/or l-proline.

Discussion

In addition to rapamycin [21], other naturally-occurring immunosuppressants such as FK506 and immunomycin (ascomycin) have been isolated from streptomycetes, and their structures found to resemble that of rapamycin [1,2,6,8]. Biosynthetic studies conducted on rapamycin [14–16] and immunomycin [2,12] revealed that shikimic acid, acetate, propionate, l-methionine, and dl-pipecolic

Table 2 Effect of l-proline and dl-pipecolic acid on prolylrapamycin and rapamycin production

Additive concentration (g L^{-1})	DCW (g L^{-1})	Production (mg L^{-1})	
		Rapamycin	Prolylrapamycin
None	0.42	16.1	0
l-Proline (5 g L^{-1})	1.58	11.3	7.2
dl-Pipecolic acid (5 g L^{-1})	0.80	15.2	0
l-Proline (5 g L^{-1}) + dl-pipecolic acid (5 g L^{-1})	1.34	19.3	1.0

DCW, dry cell weight.

Table 3 Effect of thiazolidine-2-carboxylic acid (T2CA) on rapamycin production in the presence of l-lysine or dl-pipecolic acid

Additive concentration (g L^{-1})	DCW (g L^{-1})	Production (mg L^{-1})	
		Rapamycin	Prolylrapamycin
None	0.29	10.9	0
T2CA (2 g L^{-1})	0.43	0	0
l-Lysine (5 g L^{-1})	0.75	37.1	0
T2CA (2 g L^{-1}) + l-lysine 95 g L^{-1}	1.07	14.6	1.8
dl-Pipecolic acid (5 g L^{-1})	1.02	8.8	0
T2CA (2 g L^{-1}) + dl-pipecolic acid (5 g L^{-1})	0.53	11.7	0

DCW, dry cell weight.

Table 4 Effect of thiazolidine-2-carboxylic acid (T2CA) on the preferential production of rapamycin in the presence of d1-pipecolic acid and l-proline

Additive concentration (g L^{-1})	DCW (g L^{-1})	Production (mg L^{-1})	
		Rapamycin	Prolylrapamycin
None	0.39	8.3	0
d1-Pipecolic acid (10 g L^{-1})	1.31	7.0	0
T2CA (2 g L^{-1}) + d1-pipecolic acid (10 g L^{-1})	0.44	7.7	0
T2CA (2 g L^{-1}) + d1-pipecolic acid (10 g L^{-1}) + l-proline (2.5 g L^{-1})	0.48	13.5	0.4
+ l-proline (5 g L^{-1})	0.53	17.7	0.9
+ l-proline (10 g L^{-1})	0.61	17.0	1.1
+ l-proline (20 g L^{-1})	0.73	26.0	2.4

DCW, dry cell weight.

acid are precursors of both compounds. The pipecolate-activating/incorporating enzyme from *S. hygroscopicus* var *ascomyceticus*, which is thought to be enzymatically equivalent to that in rapamycin-producing *S. hygroscopicus*, was purified and its pyrophosphate-ATP-exchange activity was measured with a variety of amino acids and pipecolate analogs [12]. Of all the amino acids examined, only l-proline served as substrate; d-proline did not. We confirmed in the present work that addition of l-proline to cultures of *S. hygroscopicus* C9 results in formation of prolylrapamycin [18] in a manner similar to the production of prolylrapamycin by *Actinoplanes* sp [13] and prolyl derivatives of FK506 and immunomycin by *S. tsukubaensis* [7] and *S. hygroscopicus* var *ascomyceticus* [12], respectively.

In the immunomycin-producing streptomycete, the addition of l-thiazolidine-4-carboxylic acid (thiaproline) resulted in the accumulation of prolylimmunomycin, although thiaproline was a poor substrate *in vitro* for the incorporating enzyme [12]. In our study, thiaproline at 0.05 g L^{-1} exhibited strong growth inhibition of *S. hygroscopicus* C9. On the other hand, thiazolidine-2-carboxylic acid (T2CA) stimulated formation of prolylrapamycin particularly when added in the presence of exogenous l-proline. This analog inhibited growth only at high concentrations (eg, 2 g L^{-1}). Thus, thiaproline stimulates formation of prolylimmunomycin in the immunomycin-producing *S. hygroscopicus* var *ascomyceticus*, while T2CA increases prolylrapamycin production in *S. hygroscopicus*. It is interesting that T2CA addition leads to prolylrapamycin and not to a T2CA-derivative of rapamycin (G Carter, personal communication).

In the rapamycin-producing *Actinoplanes* sp, l-proline addition was found to generate simultaneous formation of prolylrapamycin and 32-*O*-desmethylprolylrapamycin* [13]. We observed that *S. hygroscopicus* C9 produces a rapamycin derivative with an RRT of 0.53 together with prolylrapamycin at concentrations of l-proline such as 10 g L^{-1} or higher (Figure 2). This derivative is not 32-desmethylrapamycin (RRT = 0.91) or 32-desmethoxyrapamycin (RRT = 1.27), but it is possible that it might be rapamycin lacking a methyl or methoxy group at C7 or C41. In the presence of T2CA, the formation of the RRT 0.53 compound was also stimulated by l-proline addition (data not shown).

Simultaneous addition of l-proline and d1-pipecolic acid resulted in preferential formation of rapamycin with only a trace amount of prolylrapamycin. This suggests that the pipecolate-incorporating enzyme favors pipecolic acid over l-proline as a substrate, in agreement with results obtained with the pure enzyme from the immunomycin-producer [2,12], T2CA did not inhibit this preferential rapamycin production. Furthermore, although T2CA suppressed rapamycin production in the presence of l-lysine, it did not when d1-pipecolic acid was added instead of l-lysine. In summary: (i) T2CA stimulates prolylrapamycin formation when it alone is added separately or when added with l-proline; and (ii) T2CA inhibits rapamycin production when added separately or with l-lysine but not when added with d1-pipecolic acid.

We propose (Figure 5) the following hypothetical scenario to explain the observations made in this work: (i) production of rapamycin and prolylrapamycin depends on endogenous pools of pipecolic acid and l-proline, respectively; (ii) the pipecolate-incorporating enzyme favors pipecolate, but under a deficiency of intracellular pipecolate, it incorporates l-proline to produce prolylrapamycin; (iii) T2CA inhibits the intracellular conversion of l-lysine into pipecolate, thereby lowering titers of rapamycin when added alone or with l-lysine; (iv) T2CA does not interfere with activation and incorporation of internal pipecolic acid to make rapamycin or of l-proline to make prolylrapamycin; (v) since T2CA brings about pipecolate deficiency by inhibiting conversion of internal l-lysine to pipecolic acid especially in the absence of exogenous l-lysine, and even in the presence of added l-lysine, T2CA addition results in the efficient incorporation of l-proline by the enzyme into prolylrapamycin; and (vi) l-proline and/or T2CA stimulate uptake of d1-pipecolate into the cell.

Addition of d1- or l-pipecolic acid failed to enhance rapamycin production, whereas exogenous l-lysine did. This was unexpected, since labeled pipecolate was shown to be incorporated preferentially over l-lysine into rapamycin in our earlier studies [16]. Our observation contrasts with the elevated production of rapamycin and its derivatives by the *Actinoplanes* strain upon addition of 7.8 mM (1 g L^{-1}) l-pipecolic acid [13]. *S. hygroscopicus* C9 may not take up exogenous d1-pipecolic acid efficiently enough to compete with the endogenous conversion of l-lysine to pipecolate. However, in the presence of T2CA, exogenous pipecolate does stimulate rapamycin production. The poor competition between external pipecolate and internal pipecolate would be expected to shift towards the former when

* In the original paper [13], the compound was called 27-*O*-desmethylprolylrapamycin, but the new numbering system (Chemical Abstracts Index Guide, 1994) refers to the C atom as No. 32.

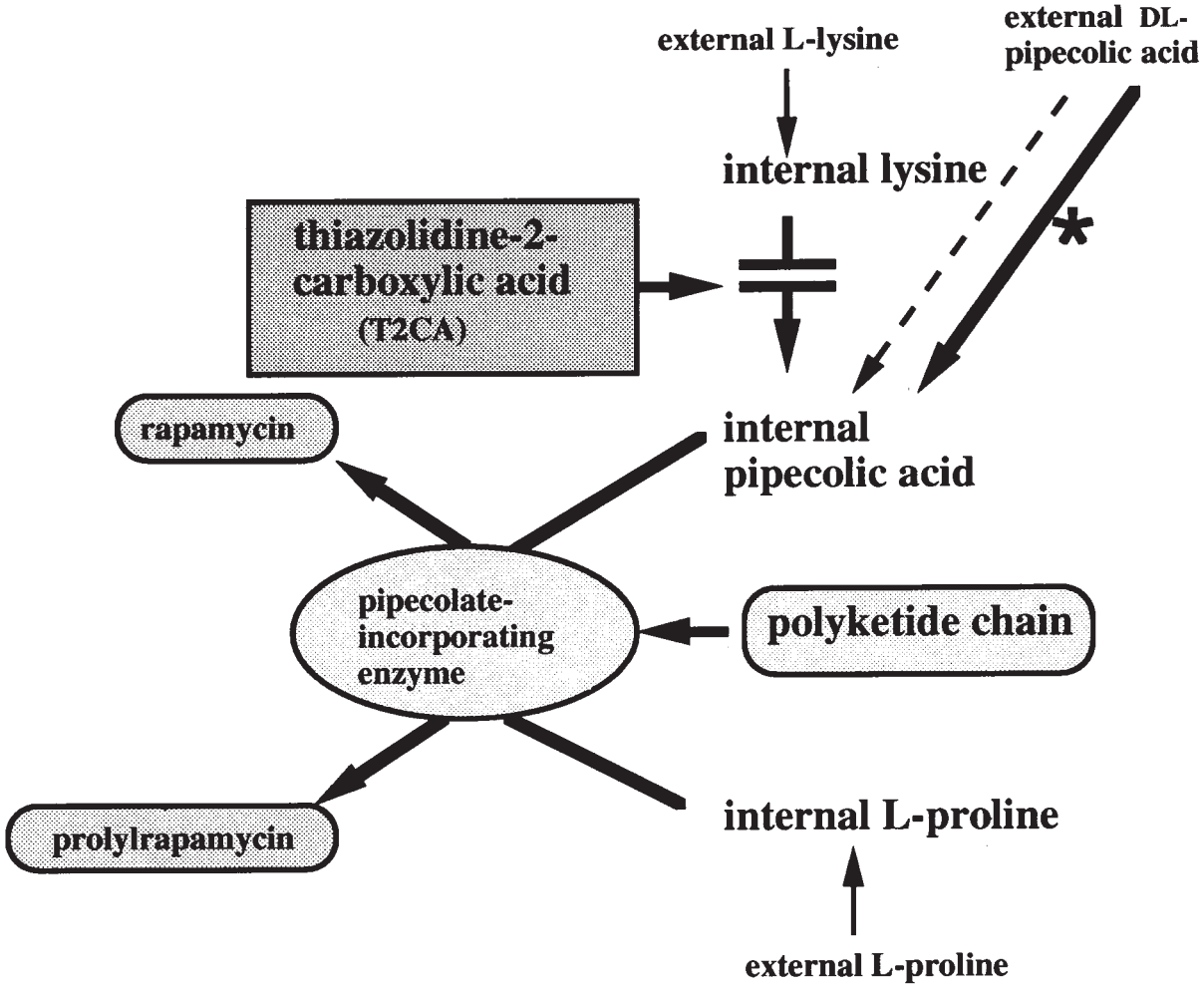


Figure 5 Hypothesis: action of thiazolidine-2-carboxylic acid in biosynthesis of rapamycin and prolylrapamycin. *Activity stimulated by l-proline and/or T2CA.

the endogenous conversion of l-lysine to pipecolate is inhibited by T2CA. Also, in the presence of added l-proline, dl-pipecolic acid stimulates rapamycin formation, suggesting that uptake of dl-pipecolate is increased by the presence of l-proline. Indeed, dl-pipecolate may enter the cell by an l-proline- or T2CA-induced permease.

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