The Fate of Valine-Oxygen during Penicillin Biosynthesis

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[4-$^{2}H_6$, $^{18}O_2$]valine has been fed to intact cells of *Cephalosporium acremonium*; isolation of the biosynthesised α-aminoadipoylcysteinyl valine (ACV) tripeptide has demonstrated that an intracellular exchange of *one* and *both* valine oxygens occurs.

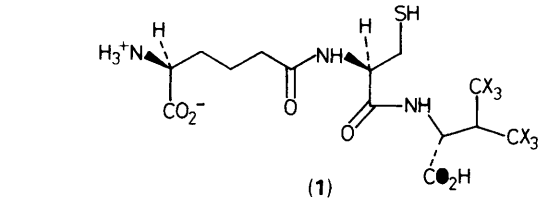
Penicillin biosynthesis from L-valine, L-cysteine, and L-α-aminoadipic acid is known[1] to proceed *via* the tripeptide L-α-aminoadipoyl-L-cysteinyl-D-valine, LLD-ACV (**1a**). During the course of formation of the tripeptide (**1a**) the stereochemistry of the valinyl residue is inverted. Exogenous feeding experiments of L-[$^{18}O_2$]valine to *Penicillium chrysogenum* performed by Thomas[2] suggested the elimination of *one* of the *two* carboxy-oxygen atoms during the overall conversion of L-valine to penicillin V (**2a**). From this it was concluded that 'the elimination of a valine-oxygen atom could take place either in the course of biosynthesis of this tripeptide [ACV (**1**)] or its subsequent oxidative cyclisation to the penam nucleus.' Subsequently we showed[3] that *no* oxygen atoms were exchanged during the conversion of the tripeptide (**1a**) to isopenicillin N (**2b**) [the biosynthetic precursor of (**2a**)]. We now report that valine is transformed by intact cells of the β-lactam negative mutant, N-2, of *Cephalosporium acremonium*[4] A.T.C.C. 14553 into ACV (**1**) with both partial and complete loss of both oxygen atoms.

A possible problem of the previous experiment[2] is that if *both* oxygen atoms of the exogenously labelled valine were exchanged prior, during, or subsequent to assembly of tripeptide (**1**), then such exchange would be masked by the endogenous production of ACV (**1**). In order to avoid such problems [4-$^{2}H_6$, $^{18}O_2$]valine (**3b**) was synthesised (*ca.* 70% from labelled 2-bromopropane; Scheme 1) so that the fate of the exogeneous precursor could be followed discretely. In addition the use of a β-lactam negative Takeda N-2 mutant of *C. acremonium*,[4] an organism which excretes ACV (**1**) and has no active penicillin-forming enzyme available, allowed direct examination of the incorporation of valine into the tripeptide (**1**), rather than in the downstream penicillin (**2**).[2]

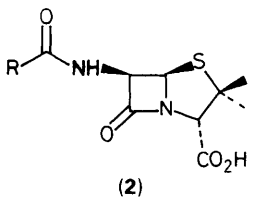
Thus racemic† [4-$^{2}H_6$, $^{18}O_2$]valine (**3b**) was fed at a level of 12.5 mg ml^{-1} to a culture of Takeda N-2 mutant of *C. acremonium*[4] as soon as the stationary phase was established. Fermentation was continued for 3 days at 250 rev min^{-1}. The culture was sonicated, centrifuged, and the supernatant filtered to provide a crude lysate from which ACV disulphide (**4**) and recovered valine (**3**) were isolated by reverse phase h.p.l.c. [octadecylsilane, (i) MeOH: 25 mM NH_4HCO_3 (1 : 4); (ii) 25 mM NH_4HCO_3 as eluants]. The ACV disulphide (**4**) was then reduced [dithiothreitol (5 equiv.); water, 5 min] and converted to its *N*,*S*-diethoxycarbonyldimethyl ester derivative (**5**).[3] The recovered valine (**3**) and ACV derivative (**5**) were then examined by mass spectrometry (Table 1, entries 1,2,3). This data indicates substantial loss of both *one* and *two* oxygen atoms from valine (**3**) to ACV (**1**).

Control experiments were also performed. Thus substitution of valine (**3b**) by [$^{18}O_2$]ACV disulphide (**4c**) demonstrated that there was no extensive extracellular exchange of

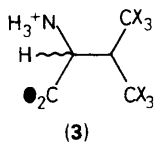
† Only L-valine is thought to be converted to ACV (**1**) in broken cell experiments (*ex. C. acremonium* C-91[5a] *or* C-10[5b]) but it is known that both L- and D-forms contribute to the intracellular pool of L-valine when added to intact cells of *P. chrysogenum*[6] (although incorporation of D-valine into the mycelium is much slower).



(1)



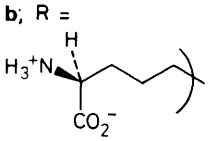
(2)



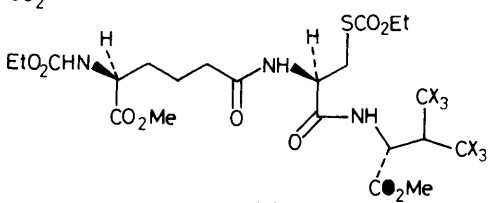
(3)

a; R = $PhOCH_2$

b; R =

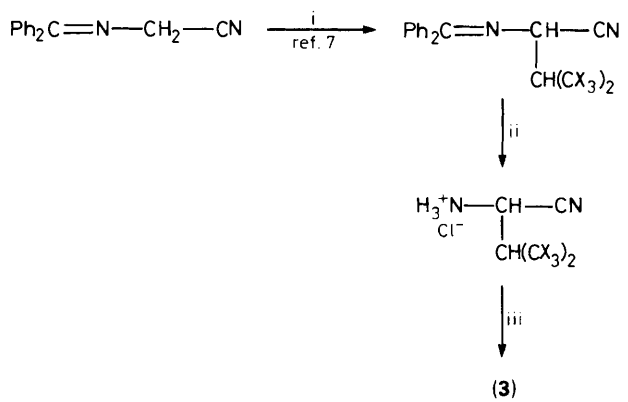


(4) = Disulphide form of (1)



(5)

For (1), (3), (4), (5) **a**; X = ^1H, ● = ^{16}O
b; X = ^2H, ● = ^{16}O, ^{18}O
c; X = ^1H, ● = ^{16}O, ^{18}O



Scheme 1. *Reagents*: i, $(CX_3)_2CHBr$, $PhCH_2\overset{+}{N}Et_3Cl^-$, 50% aq. NaOH, 0 °C → room temp. over 3 h, then room temp., 12 h; ii, 1 M HCl, 25 °C, 12 h; iii, $H_2{}^{18}O$, HCl(g) (satd.), reflux, 12 h.

valinyl oxygens from the tripeptide (Table 2, entries 1, 2) and a culture fluid only experiment (which had been separated from mycelia grown to the stationary phase) with [$^{18}O_2$] valine gave *no labelled* ACV (**1**), demonstrating that the conversion of valine (**3**) to ACV (**1**) was intracellular.

We have demonstrated that an *intracellular* exchange of *one* or *both* valinyl oxygen atoms occurs prior to ACV (**1**) excretion from intact cells of the N-2 mutant of *C. acremonium*. Whether such exchange could result from the operation of processes not related to penicillin biosynthesis, during tripeptide assembly, at the tripeptide (**1**) level, or a combination of such events, is uncertain.

Table 1. NH_3 desorption chemical ionisation results: labelled valine (**3b**) experiment.

Entry	Valine (**3b**)	*m/z*	123	124	125	126	127	128	129
1	[1-●$_2$,4-$^2H_6$]	(%)obs.	5	9	7	53	13	100	10
	Synthetic	●	–	$^{16}O_2$	—	$^{18}O^{16}O$	—	$^{18}O_2$	—
	Tripeptide derivative[a]	*m/z*	541	542	543	544	545	546	547
2	(**5b**) [from (**3b**)]	(%)obs.	5	77	32	100	35	19	5
		●	—	$^{16}O_2$	—	$^{18}O^{16}O$	—	$^{18}O_2$	—
	Recovered valine (**3b**)	*m/z*	123	124	125	126	127	128	129
3	[1-●$_2$,4-$^2H_6$]	(%)obs.	1	14	5	61	11	100	6

[a] Spectrometry also revealed endogeneous ACV (**1a**) production (*ca.* 33% of exogenous derived production), (**5a**) *m/z* obs. 535(1%), 536(100), 537(28), 538(12), 539(4); cald. for $C_{22}H_{38}N_3O_{10}S$ [*M*H]$^+$ 535(0), 536(100), 537(28), 538(10).

Table 2. NH_3 desorption chemical ionisation results: extracellular exchange control experiment.

Entry	*m/z*	536	537	538	539	540	541	542	543
	(%)								
1 Derivative (**5c**) from tripeptide disulphide (**4c**) before incubation	obs.	1	1	29	12	100	30	4	2
	●	$^{16}O_2$	—	$^{18}O^{16}O$	—	$^{18}O_2$	—	—	—
	m/z	536	537	538	539	540	541	542	543
	(%)								
2 Derivative (**5c**) from recovered tripeptide (**4c**) after incubation	obs.	45[a]	14[a]	38[a]	13	100	29	12	3
	●	$^{16}O_2$	—	$^{18}O^{16}O$	—	$^{18}O_2$	—	—	—

[a] These values are effected by endogenous ACV (**1a**) production.

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