Stereospecific Lysis of a Range of β-Hydroxy-α-amino Acids catalysed by a Novel Aldolase from *Streptomyces amakusaensis*

Richard B. Herbert,*[a] Barrie Wilkinson,[a] George J. Ellames[b] and Ellen K. Kunec[b]

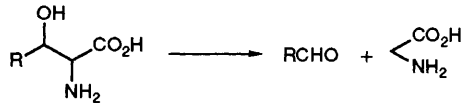
[a] *School of Chemistry, The University, Leeds LS2 9JT, UK*
[b] *Department of Chemical Development, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Research Centre, Alnwick, Northumberland NE66 2JH, UK*

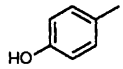
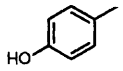
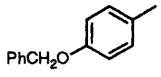
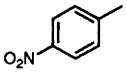
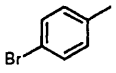
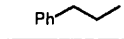
Cell-free preparations of *Streptomyces amakusaensis* contain a novel aldolase which catalyses the conversion of β-hydroxy-α-amino acids (as **2**) into the corresponding aldehyde plus glycine; the aldolase is stable, shows broad substrate tolerance, is highly selective for *threo* stereochemistry and, where examined, is stereospecific for the (2*S*,3*R*) configuration.

In the course of a biosynthetic study on tuberin **1**[1] in *Streptomyces amakusaensis* we uncovered in a preliminary way an aldolase active on *threo*-(4-hydroxyphenyl)serine **2**. Experiments with simple cell-free preparations of *S. amakusaensis* showed that a reverse aldol reaction occurred with the racemic *threo*-isomer **2** as a substrate, but not with the erythro-isomer, to give 4-hydroxybenzaldehyde **4** plus presumably glycine **5**.[2] Such an aldolase is rare, not previously, we believe, having been reported from microbiological sources. Aldolase activities have been identified in mammalian sources, however. One was shown to be present in human brains which acted on *threo*-(3,4-dihydroxyphenyl)serine **6**[3] and one from mammalian kidneys and livers which acted on *threo*-phenylserine **7** (E.C. 4.1.2.26) and was also active with the *erythro*-isomer.[4] However, this latter enzyme, at least, is most probably simply serine hydroxymethyltransferase.[5] The stereoselectivity of the *Streptomyces* enzyme is quite different and we report here on this and its substrate tolerance, indicating applications in organic chemistry.

Aldolase activity was simply obtained from *S. amakusaensis* which had been cultured until tuberin production had begun;[1] cells were isolated by centrifugation and were disrupted by

Table 1 Reaction of β-hydroxy-α-amino acids to give the corresponding aldehyde plus glycine catalysed by the *Streptomyces* aldolase

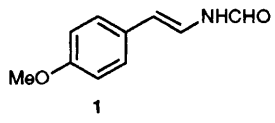


Entry	Substrate, R[a]		Aldehyde formation (%)[b]
1		*threo*[7]	37
		erythro[7]	<1
2		2*S*,3*R*[8]	72
		2*R*,3*S*[8]	<1
3	Ph	*threo*[7]	23
4	Ph	2*S*,3*R*[8]	47
		2*R*,3*S*[8]	<1
5		*threo*[7]	41
6		*threo*[9]	39
		erythro[9]	11
7		*threo*[7]	20
8		*threo*[10]	12

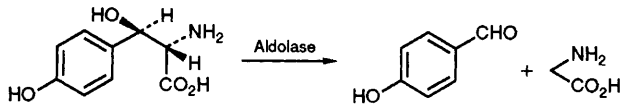
[a] Synthesis: following literature methods: refs. 7–10. Detection was by reverse phase HPLC, using a Spherisorb ODS 2 column with MeCN–H_2O (1:1) as eluent, against standard aldehyde samples. [b] Incubation was of a *S. amakusaensis* cell-free preparation (from 200 cm^3 cluture) plus substrate (0.13 mol dm^{-3}) in 0.05 mol dm^{-3} trizma buffer, 0.1 mmol dm^{-3} dithiothreitol, $FeSO_4$ (0.01 mmol dm^{-3}), ascorbic acid (0.5 μmol dm^{-3}) and 0.17 mmol dm^{-3} phenylmethylsulfonyl fluoride (protease inhibitor), total volume 50 cm^3, pH 7.0, 25 °C, 4 h. Yields are not optimized; this will be done with pure enzyme. A measure of the efficiency of the aldolase is that partially purified enzyme (0.1 mg cm^{-3} protein) under the above conditions converted **2** into **4** to the extent of 31% in 60 min. In all cases **2** was used as a parallel reference substrate; each substrate was additionally incubated with boiled enzyme (in no case was aldehyde formed); cell-free preparation minus substrate was also incubated to serve as a blank.

sonication in buffer solution to give a cell-free preparation which was used for experiments with different substrates. The results are shown in Table 1.

The previously observed[2] specificity for *threo* as against *erythro* amino acid was confirmed for **2** (entry 1). Further, and notable, stereospecificity was apparent with (2*S*,3*R*)-(4-hydroxyphenyl)serine **3** when it was compared as a substrate with its enantiomer: only the former underwent cleavage (entry 2). Note: the racemate reacted as required to half the extent of the enantiomerically pure substrate. The same stereospecificity was shown with (2*S*, 3*R*)-phenylserine as **3** and its enantiomer (entry 4). This is notably different from serine hydroxymethyltransferase (E.C. 2.1.2.1) which shows selectivity for the L-configuration at the α-centre but poor *threo–erythro* discrimination.[5,6]

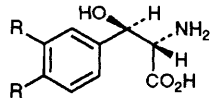


1



2 Racemate
3 2*S*,3*R*

4 **5**



6; R = OH
7; R = H

A number of different aromatic compounds were tested as substrates for the *Streptomyces* aldolase and remarkably broad substrate tolerance was observed; the reaction proceeds whether or not the hydroxy group in the substrate is benzylic (compare entries 8 and 3), proceeds with considerable steric bulk in the substrate (entry 5), and proceeds whether any substituent on the aromatic ring is electron releasing (entry 1) or withdrawing (entry 6). With the last entry some reaction was found with *erythro*-(4-nitrophenyl)serine. The results of an experiment with [1,2-^{14}C]-*threo*-(4-hydroxyphenyl)serine confirmed that glycine **5** was indeed the other product of the aldolase reaction. We conclude by noting that we have identified a new, and stable, aldolase from a microbial source which shows broad substrate tolerance. Immediate applications lie in resolving racemic samples of *threo*-β-hydroxy-α-amino acids to give the uncommon (2*R*,3*S*) isomer and also in establishing the configuration of β-hydroxy-α-amino acids. These compounds are of noted importance (see, *e.g.*, ref. 6.).

Further work is concerned with purification of the *Streptomyces* aldolase and its characterization; also applications in organic synthesis. The search for similar enzymes in other microbial cultures is justified by our findings.

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