Engineering stereocontrol into an aldolase-catalysed reaction†

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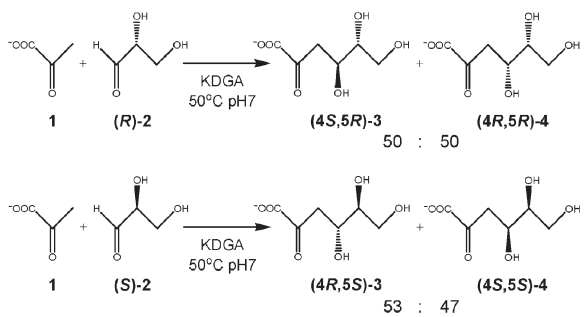
A novel thermostable aldolase has been developed for synthetic application, and substrate engineering has been used to induce stereocontrol into aldol reactions of this naturally-promiscuous enzyme.

Biocatalysis is established as a powerful tool for the asymmetric synthesis of chiral intermediates for pharmaceutical and agrochemical purposes.[1] One area of great utility is the use of aldolases in stereoselective carbon–carbon bond forming reactions,[2] particularly for asymmetric synthesis of complex carbohydrates.[3] However, many aldolases display a narrow specificity profile, often favoring phosphorylated substrates, which can limit the range of chiral aldol products that may be prepared. In order to address this problem, attempts have been made to improve/alter the biocatalytic properties of known aldolases using site-directed mutagenesis and directed evolution.[4] This approach has achieved only limited success however, and so the identification of novel aldolases with a broad specificity profile, particularly towards non-phosphorylated substrates, is of great interest.

2-Keto-3-deoxygluconate aldolase (KDGA) from the hyperthermophile *Sulfolobus solfataricus* has great potential as a biocatalyst for asymmetric aldol reactions. It is a type I aldolase with broad specificity for non-phosphorylated substrates, is extremely thermostable, and can be efficiently expressed in *Escherichia coli*.[5] However, the initial promise of this enzyme for synthesis was tempered by the unexpected discovery that it exhibits no diastereocontrol for the aldol condensation of its natural substrates pyruvate **1** and D-glyceraldehyde (*R*)-**2**, which gives a 50 : 50 mixture of *anti*-(4*S*,5*R*)-3-deoxy-2-hexulosonate **3** and *syn*-(4*R*,5*R*)-3-deoxy-2-hexulosonate **4** (Scheme 1, Fig. 1A).[6] Whilst non-stereoselective aldolase-catalysed reactions using non-natural aldehyde substrates have been reported previously, this aldolase represents a rare example of an enzyme that exhibits no stereocontrol during reaction of its *natural* substrates.[7,8] Indeed, this observation led to the discovery of a novel 'promiscuous metabolic pathway' in *Sulfolobus solfataricus*, which metabolises both glucose *and* galactose using the same series of non-specific enzymes, in a pathway that may be indicative of the primitive evolutionary state of this organism.[6]



Scheme 1

The complete lack of stereocontrol demonstrated by KDGA for D-glyceraldehyde (*R*)-**2** is problematic from a synthetic perspective however, because it affords a mixture of diastereoisomeric aldol products that must then be separated. Furthermore, we also demonstrated that KDGA-catalysed condensation of pyruvate with L-glyceraldehyde (*S*)-**2** also proceeds with no stereocontrol, yielding a similar 53 : 47 diastereoisomeric mixture of *anti*-(4*R*,5*S*)-**3** and *syn*-(4*S*,5*S*)-**4** in good yield (Scheme 1, Fig. 1B).‡ The formation of stable pyranose and furanose ring forms of *anti*-**3** and *syn*-**4** normally results in these aldol reactions occurring irreversibly under kinetic control.[6,7,9] This was confirmed for KDGA-catalysed condensation of pyruvate **1** with (*S*)-**2** and (*R*)-**2**, by

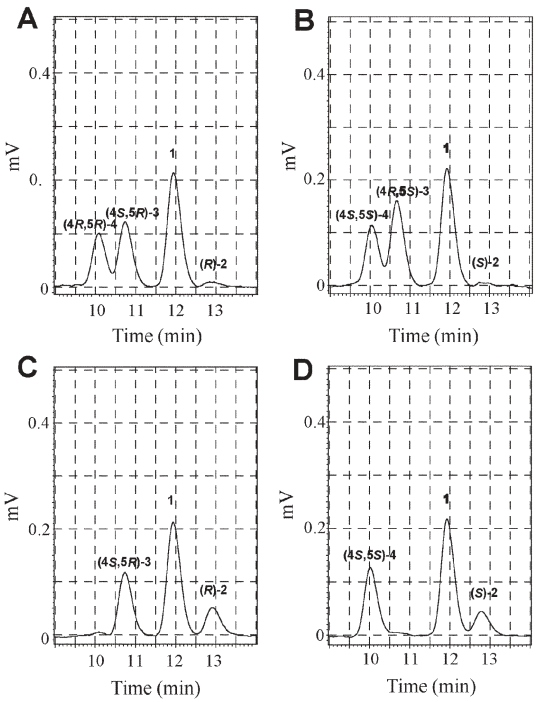


Fig. 1 HPLC traces displaying the products of the non-stereoselective aldolase-catalysed condensation of pyruvate **1** with D-glyceraldehyde (*R*)-**2** (A) and L-glyceraldehyde (*S*)-**2** (B). Hydrolysed products from the equivalent reactions with D-glyceraldehyde acetonide (*R*)-**5** (C) and L-glyceraldehyde acetonide (*S*)-**5** (D) show significant enrichment of a single diastereoisomer.

† Electronic supplementary information (ESI) available: details of the synthesis and characterisation of all compounds. See http://www.rsc.org/suppdata/cc/b4/b413255f/

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demonstrating that the approximately 50 : 50 ratio of diastereoisomeric aldol products **3** : **4** formed remained unchanged throughout the course of the biotransformation, in both cases. It was concluded therefore, that KDGA must contain a remarkably non-specific binding pocket for these aldehyde substrates, since it was capable of catalysing pyruvate addition to either the *Re*- or *Si*- face of *both* enantiomers of glyceraldehyde.

We reasoned that the use of a structurally rigid analogue of glyceraldehyde might enable us to engineer stereocontrol into these KDGA-catalysed reactions. Kinetic analysis revealed that the acetonide derivatives D-glyceraldehyde acetonide (*R*)-**5** (k_{cat}/K_m = 14 mM^{-1} min^{-1}) and L-glyceraldehyde acetonide (*S*)-**5** (k_{cat}/K_m = 7 mM^{-1} min^{-1}) were both good substrates for KDGA (Table 1). In preparative reactions using recombinant KDGA, condensation of pyruvate **1** with (*R*)-**5** or (*S*)-**5** resulted in the highly stereoselective formation of *anti*-(4*S*,5*R*)-**6** in >92% de, or *syn*-(4*S*,5*S*)-**7** in >94% de, respectively (Scheme 2). The absolute configurations of both *anti*-(4*S*,5*R*)-**6** and *syn*-(4*S*,5*S*)-**7** were established *via* acid-catalysed hydrolysis of their acetonide groups to afford *anti*-(4*S*,5*R*)-**3** (Fig. 1C) and *syn*-(4*S*,5*S*)-**4** (Fig. 1D) respectively, whose structures and de were confirmed by comparison with authentic standards using NMR and HPLC.

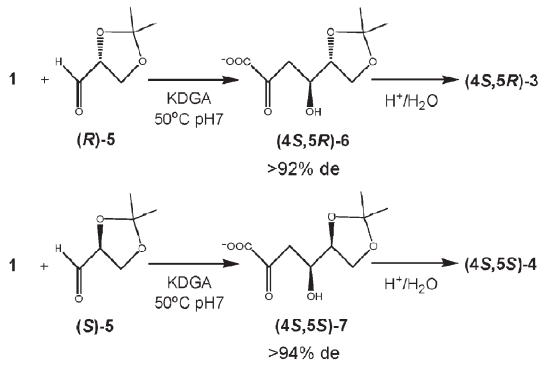
The acetonide fragments of (*R*)-**5** and (*S*)-**5** are likely to induce stereocontrol into these aldol reactions by sterically blocking access to the aldehyde binding region of KDGA that enables pyruvate to be delivered to the *Re*- face of the aldehyde, thus preventing formation of aldol diastereomers that contain a (4*R*)-hydroxyl group. Furthermore, the high de observed for (*S*)-**5** and (*R*)-**5** indicates that these aldol reactions also proceed under kinetic control, despite the fact that *anti*-(4*S*,5*R*)-**6** and *syn*-(4*S*,5*S*)-**7** exist as their open-chain form in solution.

Parallel kinetic resolution has recently emerged as a powerful strategy for stereoselective synthesis,[7] where a number of ingenious strategies have been developed that simultaneously transform both enantiomers of a racemic substrate into different chiral products.[10] The observation that KDGA transformed (*R*)-**5** into *anti*-(4*S*,5*R*)-**6**, and (*S*)-**5** into *syn*-(4*S*,5*S*)-**7**, provided us with the opportunity to use KDGA for the parallel kinetic resolution of (*rac*)-**5**. Incubation of (*rac*)-**5** with pyruvate and KDGA at 50 °C resulted in the formation of a mixture of *anti*-**6** and *syn*-**7** products, which were immediately hydrolysed to afford a 59 : 41 mixture of *anti*-**3** and *syn*-**4**. These products were separated *via* ion-exchange chromatography to afford *anti*-(4*S*,5*R*)-**3** in ⩾90% ee and *syn*-(4*S*,5*S*)-**4** in ⩾90% ee respectively (Scheme 3), as determined by comparison of their specific rotations with known literature values. Therefore, KDGA had stereospecifically converted the (*R*)-enantiomer of (*rac*)-**5** into *anti*-(4*S*,5*R*)-**6**, whilst the (*S*)-enantiomer had been simultaneously transformed into *syn*-(4*S*,5*S*)-**7**, in a rare example of a parallel kinetic resolution that employs a carbon–carbon bond forming reaction for stereocontrol.

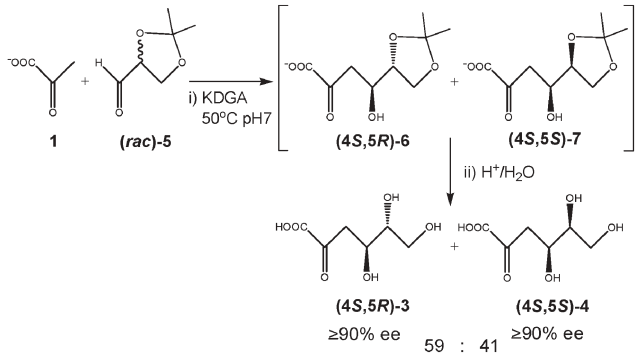
Table 1 Kinetic parameters of *Sulfolobus solfataricus* KDGA[a]

Substrate	K_m/ mM	k_{cat}/ min^{-1}	k_{cat}/K_m/ mM^{-1} min^{-1}
D-Glyceraldehyde (*R*)-**2**	3.9	594	152
L-Glyceraldehyde (*S*)-**2**	7.1	594	83
D-Glyceraldehyde acetonide (*R*)-**5**	22.7	324	14
L-Glyceraldehyde acetonide (*S*)-**5**	5.5	42	7

[a] Determined at 70 °C, pH6 using the thiobarbituric acid assay.[5]



Scheme 2



Scheme 3

In summary, this report describes one of the first applications of a thermostable aldolase for synthetic chemistry. Stereoselectivity has been induced into aldol reactions of this naturally promiscuous enzyme by employing (*R*)-**5** and (*S*)-**5** as substrates for KDGA, thus enabling a parallel kinetic resolution strategy to be developed for (*rac*)-**5**. This work represents a powerful example of substrate engineering as a mechanism for modifying/improving the enantioselectivity of an enzyme.[11] However, it is distinct from other reported examples since the enzyme's natural promiscuity has been corrected to allow stereoselective synthesis of one of its own natural substrates (4*S*,5*R*)-**3**. X-ray crystallography of KDGA with substrates docked into the active site is now being used to rationalise the induction of stereocontrol in these reactions.

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Notes and references

‡ Appropriate controls have been performed to ensure that there was no racemisation of glyceraldehyde under the conditions employed for these reactions.

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