RSC Advances

REVIEW

View Article Online
View Journal | View Issue

CrossMark click for updates

Cite this: *RSC Adv.*, 2015, **5**, 10588

Albumin as a promiscuous biocatalyst in organic synthesis

Domenico C. M. Albanese[a] and Nicoletta Gaggero*[b]

Received 25th September 2014
Accepted 18th December 2014

DOI: 10.1039/c4ra11206g

www.rsc.org/advances

Albumin emerged as a biocatalyst in 1980 and the continuing interest in this protein is proved by numerous papers. The use of albumin was initially confined to the field of asymmetric oxidations and reductions, but more recently it has found a broader application to chemical reactions such as additions, condensations and eliminations. This review reports the main applications of albumin in organic synthesis that have appeared in the literature in the past decade.

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

1. Introduction

Albumin, the most abundant blood protein in mammals, is a globular, water-soluble, un-glycosylated serum protein of molecular weight 65 000 Dalton, composed of three homologous domains (labeled I, II and III) each containing two similar sub-domains (A and B).

It binds a wide range of hydrophobic endogenous and exogenous compounds in specific sites, thus affecting their free concentration, distribution, metabolism and toxicity in living beings.

The binding property is extremely interesting in the clinical, pharmaceutical and biochemical fields as well as in organic chemistry. In fact, serum albumin, in particular the much studied bovine serum albumin (BSA), not only recognizes and binds a number of organic compounds, but is also able to discriminate between the enantiomers of a chiral molecule. For this reason albumin has been used since the eighties as a resolving agent on an analytical scale in the immobilized form.[1–5]

In 1978 Sugimoto developed the first enantioselective reduction of prochiral ketones in aqueous buffer promoted by BSA,[6] followed a year later by enantioselective sulfoxidation.[7] Afterwards, at least in some cases, the amount of protein could be reduced to catalytic levels, thus greatly increasing the simplicity of work-up and the efficiency of the protocol without affecting the stereoselectivity.

Later on it was highlighted that albumin is able to accelerate some organic reactions, thus working like a catalyst, although it does not have a true catalytic site as enzymes. Since the 1980s, it has found continuous success in biotransformation. The lack of a specific catalytic site makes BSA and human serum albumin (HSA) extremely versatile catalysts with a broad chemical

[a]*Dipartimento di Chimica, Università degli Studi di Milano, via Golgi 19, 20133-Milano, Italia*

[b]*Dipartimento di Scienze Farmaceutiche, Sezione di Chimica Generale e Organica "A. Marchesini", Università degli Studi di Milano, via Venezian 21, 20133-Milano, Italia. E-mail: nicoletta.gaggero@unimi.it; Fax: +39-0250314476*

Domenico Albanese received his Ph.D. degree in 1993 with Prof. Dario Landini working on phase transfer catalysis. After short stays at Imperial College London and the Technical University of Denmark, he gained a permanent position at the Università degli Studi di Milano, where he was appointed associate professor in 2008. His research interests include novel developments of phase-transfer catalysis, green chemistry and the development of new environmentally friendly antifouling agents.

Nicoletta Gaggero received her Ph.D. degree in 1992 working on stereoselective reactions with natural proteins, enzymes and models of enzymes. After working at the Laboratoire de Chimie de Coordination du CNRS of Toulouse, she obtained a permanent position at the Università degli Studi di Milano. Her research interests cover the field of biocatalysis and asymmetric synthesis.

This journal is © The Royal Society of Chemistry 2015

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

reactivity that ranges from reduction and oxidation reactions to condensations and cycloadditions.

The mode of action of both albumins has been ascribed to the basic nature of their hydrophobic pockets, in particular the IIA binding site containing a lysine, Lys-222 in BSA and the homologous Lys-199 in HSA.[8,9]

Although BSA and HSA have 76% homology in the amino acid sequence,[10,11] similar tertiary structures and binding sites, BSA finds a wider range of applications due to its lower cost and larger availability.

This mini-review covers the literature relating to the use of BSA and HSA in organic synthesis published in the past decade. It is organized in two main sections: the first deals with reactions catalyzed by transition-metal moieties complexed with albumin to give artificial metalloenzymes, whereas the second describes applications of albumin in water and organic solvents.

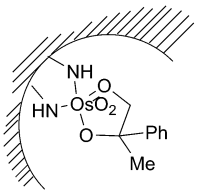
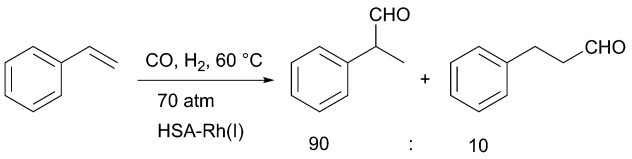


Fig. 1 OsO_4–BSA–α-methylstyrene complex.



Scheme 1 Hydroformylation of styrene promoted by a HSA–Rh complex.

2. Albumin as a metalloenzyme mimic

Metalloenzymes are among the most efficient and versatile biocatalysts able to perform complex transformations such as the selective oxidation of unactivated hydrocarbons promoted by cytochrome P-450.[12]

Many efforts have been devoted during the past decades to investigate the role of the protein scaffold in controlling the coordination number, geometry and stability of individual metal ions and metal cofactors.[13] Moreover, the protein protects the catalytic site from side reactions which could lead to self-destruction. The protein binding site establishes the orientation and the distance of the substrate from the catalytic center, ensuring the optimal stereochemical outcome of the reaction. These findings enabled chemists to rationally design metalloenzymes, which share the properties of enzymes and those of organometal catalysts.[14–17]

Anchoring a transition metal complex to an appropriate host protein is one of the most straightforward and simple methods to construct hybrid catalysts. The host protein should be tolerant to denaturing agents (for example, oxidants and heat), commercially available at a reasonable cost, easy to handle and accessible by an efficient expression system. BSA satisfies the first three requirements. Unfortunately, recombinant BSA is not available so far, whereas a good expression system has been reported for HSA, namely in the yeast *Pichia pastoris*.[18,19] In the case of HSA, therefore, the possibility of a directed evolution of stereoselectivity of hybrid catalysts might be achieved.[20]

Finally, the protein scaffold of albumin has a binding pocket large enough to bind both substrate and metal catalyst at the same time.

In 1983 the first enantioselective *cis*-dihydroxylation of a series of alkenes with ee up to 68% promoted by a 1 : 1 OsO_4/BSA complex in carbonate buffer was developed.[21] Spectrophotometric investigations supported the hypothesis that OsO_4 was coordinated by the protein *via* primary amino groups (Fig. 1).

More recently, Ward and Schirmer, inspired by this pivotal report, selected streptavidin (SAV) as the host protein for *cis*-hydroxylation.[22] SAV/OsO_4 proved a better catalyst in terms of enantioselectivity and turnover with respect to BSA/OsO_4. A genetic optimization of the performance of the complex was carried out by the authors.

Moreover, BSA was used as a supporter for binding Schiff-base metal complexes with oxidative radical scavenging activity in order to generate novel water-soluble metalloprotein conjugates.[23,24]

The non-covalent binding of porphyrins, phthalocyanines and corroles to albumins was also investigated.[25,26]

2.1 Hydroformylation

$Rh(CO)_2(acac)$/(HSA) complexes were employed in the hydroformylation reaction of several alkenes in a water/pentane biphasic system, at 40–60 °C and 50–80 atm (CO/H_2 = 1, Scheme 1).[27,28] The optimal metal-to-protein molar ratio was ≥5 : 1, the excess of Rh(I) protecting albumin from denaturation caused by heat. Also, the pH of the aqueous phase proved to be important; the best results were achieved at pH 7.[29]

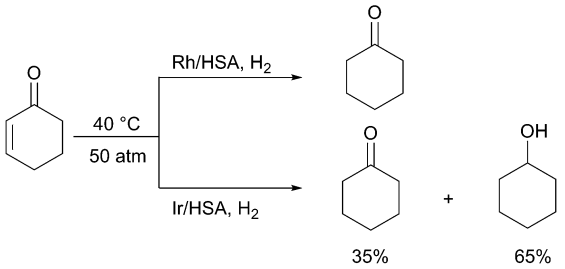
Even at a very high substrate/catalyst molar ratio (500 000 : 1), styrene was quantitatively converted to aldehydes. However, the conversion of styrene dropped after three cycles at a 780 000 : 1 substrate/catalyst molar ratio, whereas the activity remained the same after six cycles when a 10 400 : 1 ratio was used.

Chemo- and regioselectivity were generally high and compete favorably with other catalytic systems such as TPPTS/Rh(I) (TPPTS = triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt). Unexpectedly, the branched aldehyde was found to be the major regioisomer.

2.2 Hydrogenation

More recently, the aqueous/toluene biphasic hydrogenation of α,β-unsaturated carbonyl compounds has been carried out in the presence of $Rh(CO)_2(acac)$/(HSA) and $[Ir(COD)Cl]_2$/HSA complexes.[30] 2-Cyclohexen-1-one was chosen as the model substrate to investigate the carbonyl/alkene reduction selectivity of the catalysts (Scheme 2).

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.



Scheme 2 Hydrogenation of 2-cyclohexen-1-one promoted by HSA–metal complexes.

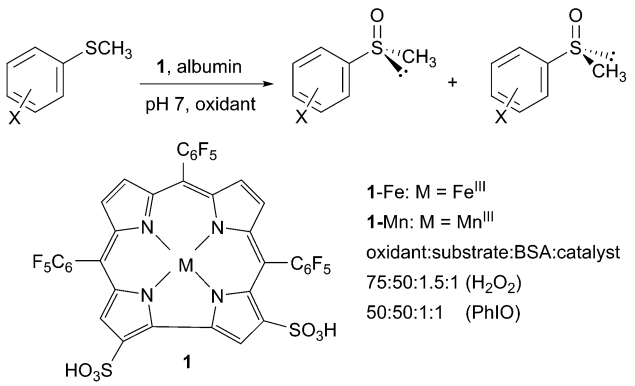
Quantitative conversions were observed with both catalytic systems working at 40 °C and 50 atm. Under these conditions Rh/HSA exclusively afforded cyclohexanone, while a mixture of cyclohexanone and cyclohexanol was obtained with Ir/HSA. By decreasing the H_2 pressure to 20 atm, the iridium catalyst showed a lower activity in comparison to rhodium and an increasing amount of cyclohexanone was obtained.[31]

α,β-Unsaturated aldehydes required a higher temperature and prolonged reaction time in order to achieve high conversions. A comparison with $Rh(CO)_2acac$/TPPTS showed that Rh/HSA was less active but more selective towards the alkene hydrogenation. Rh/HSA was not able to induce any enantioselectivity in the hydrogenation of 3-aryl-2-methyl-2-propenals to the corresponding saturated aldehydes. Both Rh/HSA and Ir/HSA were recycled without significant loss of activity.

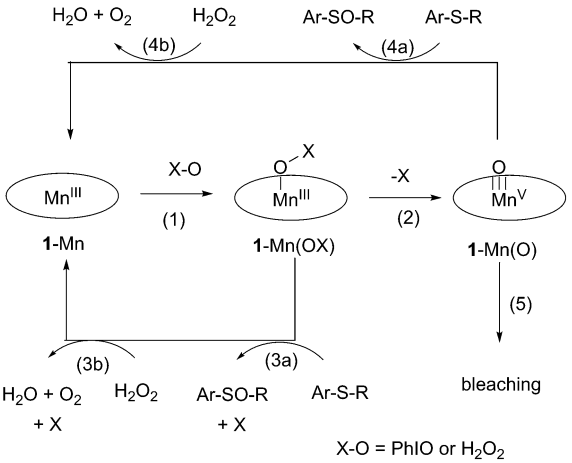
2.3 Sulfoxidation

The biomimetic sulfoxidation of a series of substituted thioanisoles and ethylphenylsulfide was carried out with hydrogen peroxide or iodosylbenzene in the presence of albumin/metal sulfonated corrole complexes **1** in up to 74% ee (Scheme 3).[32]

A competitive background oxidation could be ruled out on the basis of reaction yield and ee obtained in the presence of albumin only. Also, the direct oxidation of sulfides by H_2O_2 was negligible. The albumin source had a significant effect on the ee and the absolute configuration of the sulfoxides. With all the albumins tested the enantioselectivities and yields were



Scheme 3 Stereoselective sulfoxidations catalyzed by albumin-conjugated corrole metal complexes.



Scheme 4 Proposed catalytic cycle in the sulfoxidation reaction catalyzed by BSA-conjugated corrole metal complexes.

superior when manganese–corrole complexes and hydrogen peroxide were used if compared to the corresponding iron derivatives.[33] Manganese conjugates in the presence of H_2O_2 were also the better systems as regards catalyst stability. In fact, in the presence of less-reactive substrates, hydrogen peroxide is decomposed to oxygen and water, thus protecting the catalyst from bleaching and/or protein oxidation. This route did not occur when iodosylbenzene was used.

Studies on the mechanism highlighted the formation of two catalytic oxidant species: the species **1**–Mn(OX) prevailing when H_2O_2 is used and the species **1**–Mn(O) being the most abundant one with iodosylbenzene (Scheme 4). The activity towards the substrates was higher for **1**–Mn(OX) than for **1**–Mn(O). However, the reactivity of the latter species towards H_2O_2 was reversed.

Sulfoxides were almost exclusively produced by route 3a when X–O is H_2O_2. As route 4b is not operative when PhIO is the oxidant, sulfoxides may be obtained through both routes 3a and 4a with this oxidant. Moreover, a larger extent of catalyst bleaching may be expected with PhIO (route 5).

The different enantioselectivities observed with H_2O_2 and PhIO are evidence that the oxygen transfer occurred through different intermediates.

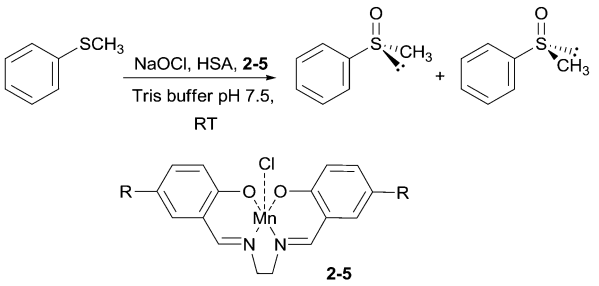
Three Mn–salen derivatives bearing substituents with pK_a ranging from −1 to 9 (R = SO_3H, CO_2H, OH), in addition to R = H, were synthesized and their complexes with HSA characterized (Scheme 5). The Mn/protein ratio of the hybrids varied from 1 for complexes **3** and **4** to 4 for the unsubstituted **2**.

Binding affinity studies demonstrated that the presence of ionizable substituents improves the complex affinity, **3** and **4** being bound more tightly.

The NaOCl oxidation of thioanisole catalyzed by these novel Mn monooxygenase mimics was studied. Although high conversions and almost complete sulfoxide selectivity were observed, no enantioselectivity was obtained.[34] Only phenylmethyl sulfone was obtained in the uncatalyzed reaction, whereas a mixture of sulfoxide and sulfone was recovered in the

 This journal is © The Royal Society of Chemistry 2015

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.



R	Complex	Conversion (%)	SO/ SO_2 (%)	Mn/protein ratio
H	**2**	75	71/4	4 ± 0.5
COOH	**3**	97	97/0	1.6 ± 0.8
SO_3H	**4**	81	80/1	1 ± 0.2
OH	**5**	91	86/5	2 ± 0.5

Scheme 5 The NaOCl oxidation of thioanisole catalyzed by Mn monooxygenase mimic Mn–salen complexes.

presence of HSA without Mn–salen. In both cases only partial conversions were observed.

2.4 Diels–Alder cycloaddition

The Diels–Alder reaction is one of the most useful methods for carbon–carbon bond construction and many efforts have been devoted to the search for enantioselective variants of this process. Chiral Lewis acid complexes that selectively activate a diene or dienophile while providing a stereodefined environment are among the most effective catalysts. In particular, the synthetic utility of copper(II) complexes is well known and several recent reports deal with Cu(II)-catalyzed Diels–Alder reactions in water.[35–38]

The Diels–Alder reaction of 1,4-naphthoquinone derivatives with different dienes has been carried out by using a catalytic amount of BSA without metal in an aqueous buffer solution, with an ee up to 38%.[39]

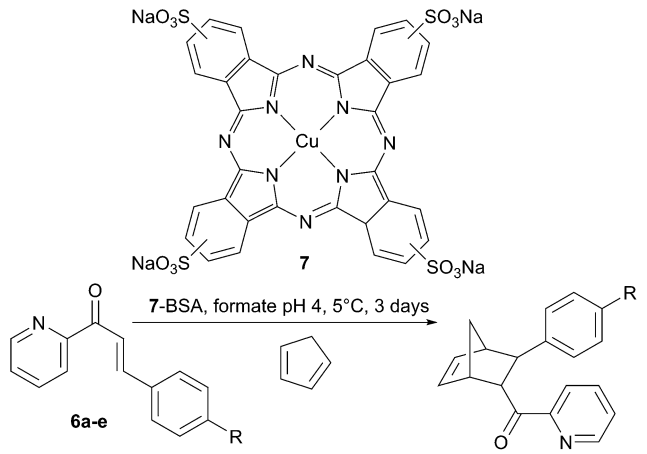
Commercially available, water-soluble phthalocyanine–copper complex **7** was chosen as the ligand for serum albumins in the cycloaddition of a series of azachalcones **6** with cyclopentadiene (Scheme 6).[40]

Remarkably, 7–BSA proved to be a highly selective catalyst for the reaction of azachalcone **6a** that furnished the corresponding adduct with a 96/4 *endo*/*exo* ratio and 93% ee of the major diastereoisomer with only a 2 mol% catalyst loading. It is noteworthy that BSA–$Cu(NO_3)_2$, BSA–$CuCl_2$, BSA–$Cu(OTf)_2$ and BSA–$Cu(BF_4)_2$ led to almost racemic adducts. On the other hand, only a 4% conversion was obtained by using BSA in the absence of any metal source. Other commercially available albumins, in particular rabbit serum albumin and chicken-egg serum albumin, furnished poor ee, whereas HSA, porcine serum albumin and sheep serum albumin afforded 85%, 68% and 75% ee, respectively. Enantioselectivities of 85–98% and conversions of 71–91% were obtained with the substituted azachalcones **6b–e**.

These results compare favorably with those obtained using a homogeneous metal catalyst[41] or in the presence of various hybrid catalysts taking advantage of proteins,[42,43] or DNA[44,45] to induce stereoselectivity.

Although in some cases good to excellent results have been obtained, the BSA approach seems to be more practical due to the low cost of the catalyst and its simple preparation.

Under optimized conditions, 7–BSA catalyzed the Diels–Alder reaction of cyclopentadiene and *trans*-1,3-diphenyl-2-propenone (**8**) in only 5% yield and 56% ee. Investigations on the role of the nitrogen atom in the pyridyl ring on the outcome of the reaction are necessary in order to elucidate the reaction mechanism.



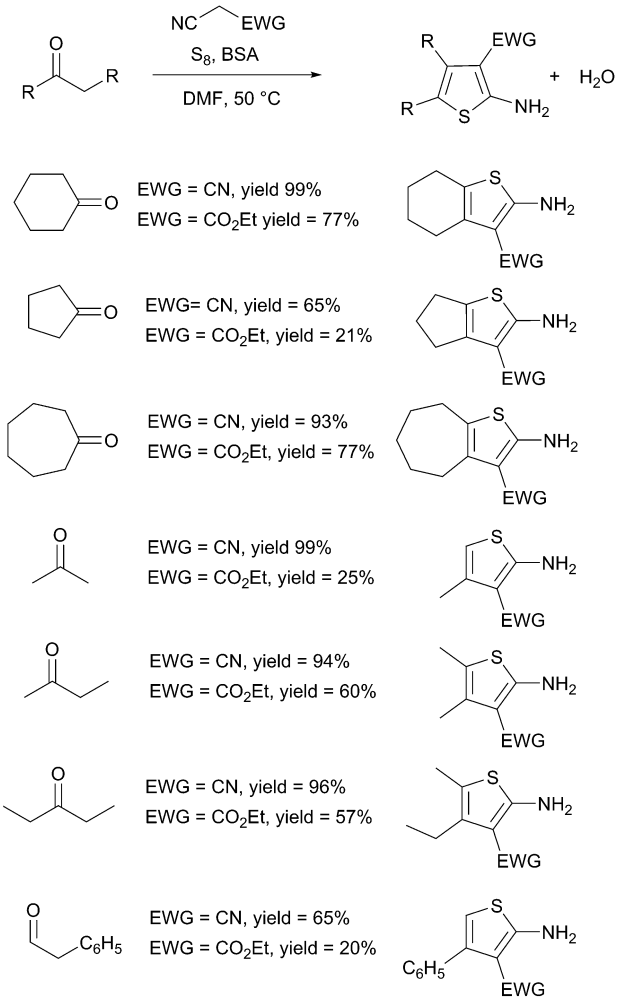
a, R = H; **b**, R = CH_3; **c**, R = OCH_3; **d**, R = NO_2; **e**, R = Cl

Scheme 6 BSA-conjugated phthalocyanine Cu(II) complex catalyzed Diels–Alder reaction.

3. Albumin in an organic solvent

Albumin, as well as true enzymes, operates in water, favoring compartmentalization of insoluble substrates in hydrophobic pockets, thus improving local concentration and reactivity, and imparting unique chemo-, regio- and stereoselectivity. In the past twenty years the so-called "nonaqueous enzymology" has emerged, expanding the versatility of biocatalysis. A lot of studies devoted to enlighten the catalytic behavior of enzymes in organic media have shown that polar solvents are the most denaturing ones.[46] Notwithstanding, albumin tolerates even water-miscible, polar solvents such as ethanol, acetone, DMF and DMSO. Under these conditions it is possible in some cases to perform more than one catalytic cycle.

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.



Scheme 7 Gewald reactions catalyzed by BSA.

3.1 Gewald condensation

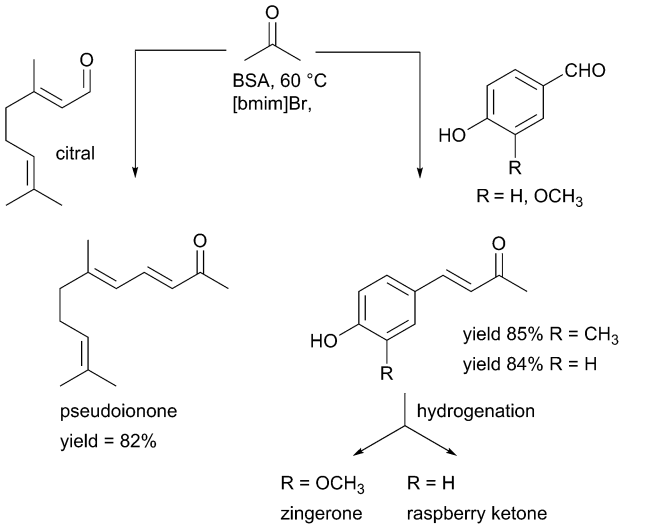
Substituted 2-aminothiophene scaffolds exhibit several pharmacological activities and constitute useful building blocks for the synthesis of natural products, dyes and agrochemicals. The Gewald condensation is the most general method for the preparation of substituted 2-aminothiophenes.

The first biocatalytic protocol to carry out this condensation was promoted by BSA.[47] The reaction has been carried out at 50 °C in DMF with a low catalyst loading (20 mg $mmol^{-1}$ of ketone/aldehyde). Moreover, the biocatalyst has been recycled five times without a decrease in the yield (Scheme 7).

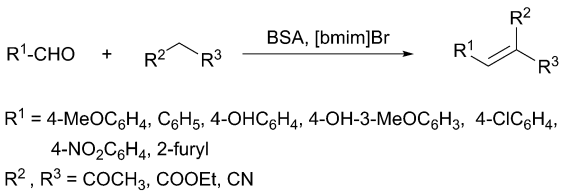
The authors proposed that a lysine residue located in an apolar pocket could be responsible for the catalytic activity of the protein.

3.2 Aldol and Knoevenagel condensation

An investigation on the biocatalyzed formation of the olefinic bond by aldol and Knoevenagel condensations in ionic liquids highlighted that BSA is a good catalyst for both reactions.[48] A wide range of substituted aromatic aldehydes was tested in the aldol condensation with acetone in 1-butyl-3-methyl imidazolium bromide ([bmim]Br) as solvent affording good to excellent yields. Of particular interest is the facile access to enones bearing a free phenol moiety that otherwise require longer synthetic paths involving additional protection–deprotection steps.



Scheme 8 BSA-[bmim]Br-catalyzed synthesis of key intermediates of biologically active compounds.



Scheme 9 BSA mediated Knoevenagel condensation in [bmim]Br.

The method was successfully applied to citral for the synthesis of *E*-pseudoionone, a key starting material for the preparation of vitamin A and carotenoids, and for the synthesis of the intermediates of raspberry ketone and zingerone (Scheme 8).

Also, the BSA-catalyzed Knoevenagel reaction towards different active methylene groups in [bmim]Br as solvent provided olefins in good to excellent yields and with (*E*)- selectivity when ethylacetoacetate or ethylcyanoacetate were used (Scheme 9).

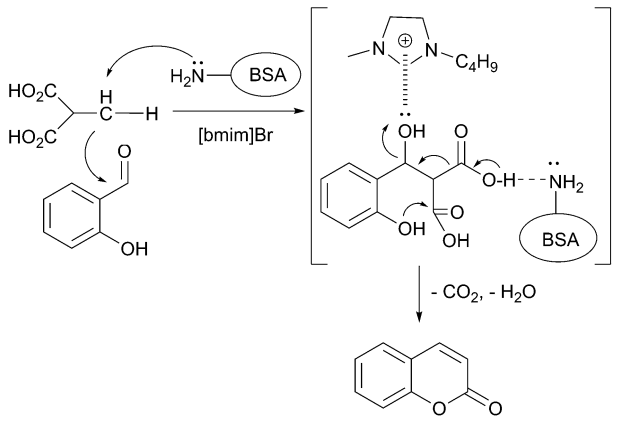
The reaction of aldehydes with malonic acid is followed by decarboxylation to give the corresponding cinnamic acids according to the Knoevenagel–Doebner condensation.

In the case of *o*-hydroxy substituted benzaldehydes a final cyclization step furnished coumarins in good yields. The postulated mechanism of this multi-step reaction involves the concerted action of the ionic liquid as well as a basic amino group of an amino acid of BSA (Scheme 10).

Catalyst recyclability up to four times and scaling up to 1 gram of substrate have been demonstrated.

 This journal is © The Royal Society of Chemistry 2015

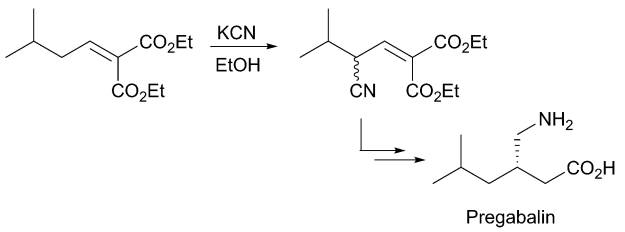
Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.



Scheme 10 Proposed mechanism for the synthesis of coumarins.

R = *i*-Bu, *i*-Pr, *n*-C_7H_{15}, *p*-F-C_6H_4, *p*-MeO-C_6H_4, 2-furyl, 2-thienyl, 3-Py

Scheme 11 BSA mediated Knoevenagel condensation in DMSO.



Scheme 12 Diethyl 2-(2-methylpropylidene)malonate route to pregabalin.

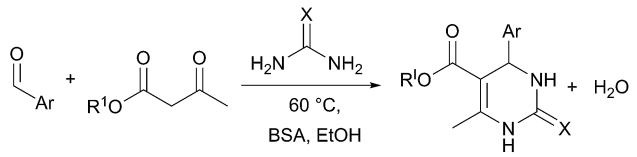
The Knoevenagel condensation between diethylmalonate and aliphatic and aromatic aldehydes in DMSO at RT has been catalyzed by BSA covalently immobilized on an epoxy-functionalized polymer (Scheme 11). The reaction gave high yields and the catalyst could easily be recycled up to five times by filtration of the reaction mixture.[49]

Usually a large excess of diethylmalonate is used to avoid aldehyde self-condensation; however, under these reaction conditions only 1.2 equivalents are enough to ensure high yields of the desired product.

Diethyl 2-(2-methylpropylidene)malonate, obtained from the condensation of iso-valeraldehyde, is of particular interest since it can be used in the manufacture of pregabalin, a drug for the treatment of several central nervous diseases (Scheme 12).[50]

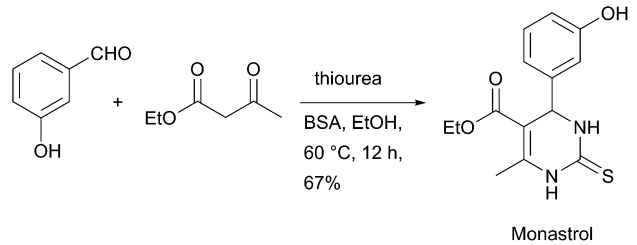
3.3 Biginelli reaction

Several benzaldehydes were treated with urea and acetoacetates in the presence of BSA for the preparation of 3,4-dihydropyrimidin-2-(1*H*)-ones with yields of 70–83% according to the Biginelli condensation (Scheme 13).[51]



Ar = 4-$MeOC_6H_4$, C_6H_5, 4-OHC_6H_4, 3-OHC_6H_4,4-OH-3-$MeOC_6H_3$, 4-ClC_6H_4, 3-BrC_6H_4, 4-$NO_2C_6H_4$, 4-MeC_6H_4, 4-*N*,*N*-$(CH_3)_2C_6H_4$
R^1 = Et, Me, *t*-Bu
X = O, S

Scheme 13 BSA-mediated Biginelli condensation in EtOH.



Scheme 14 BSA catalyzed gram-scale synthesis of Monastrol.

The reaction can be performed also with thiourea to give the corresponding 3,4-dihydropyrimidine-2-(1*H*)-thiones which are of interest for their biological activity (*e.g.* Monastrol, Scheme 14). Also in this case it has been suggested that the amino group of an amino acid side chain in BSA participates in the catalytic cycle. This hypothesis was supported by the drastic decrease of yield observed by using acetylated BSA. The recyclability (up to three cycles) of BSA was demonstrated; moreover, the reaction has been scaled-up to 1 g of aldehyde in the case of Monastrol.

Although the stereoselective version of the Biginelli reaction is of great interest, the synthesis of enantioenriched 3,4-dihydropyrimidin-2-(1*H*)-ones has mainly been based on chemical or enzymatic resolution and chiral auxiliary-promoted diastereoselective approaches.[52] However, catalytic stereoselective protocols have also been developed recently. They involve both chiral ligands in the presence of 10% $Yb(OTf)_3$,[53] and BINOL-derived phosphoric acids.[54]

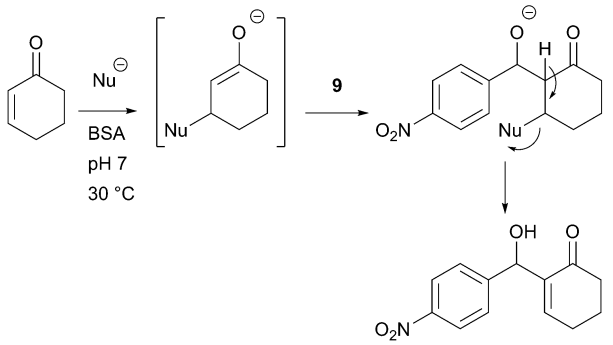
4. Albumin in water

It is well-known that albumin exist as F (pH 3.5), N (pH 7) and B (pH 9) reversible isomeric forms which influence the binding properties and the catalytic behavior.[55] Albumin is generally employed under neutral to basic conditions (pH 7–11), thus ensuring the involvement of a free amino group of lysine in the general base catalysis mechanism. However, it is also employed under acidic conditions, for example in the Diels–Alder reaction.[40]

4.1 Morita–Baylis–Hillman

BSA proved a suitable catalyst in the Morita–Baylis–Hillman (MBH) reaction of 2-cyclohexen-1-one and *p*-nitrobenzaldehyde

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.



Scheme 15 Morita–Baylis–Hillman reaction promoted by BSA.

(**9**) in pH 7.0 phosphate buffer at 30 °C (Scheme 15).[56,57] It is well-known that the MBH reaction can be catalyzed by numerous nucleophilic species such as amines or alcohols. The catalytic activity of BSA could be ascribed to several types of nucleophilic moieties in the amino acid side chains of the protein.

In this reaction BSA competes favorably with respect to other biocatalysts tested, both as regards yield (up to 35%) and ee (up to 19%). Although the results are not satisfactory, this study demonstrates the possibility of further development through genetic optimization by directed evolution.

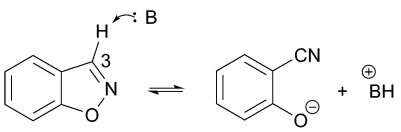
4.2 Kemp elimination

The Kemp elimination is a classic example of a concerted E2 elimination initiated by proton abstraction from the electron-poor carbon atom 3 *via* a charge-delocalized transition state (TS). It is sensitive to the base strength and the solvent nature, the polar aprotic ones affording better results than water (Scheme 16).

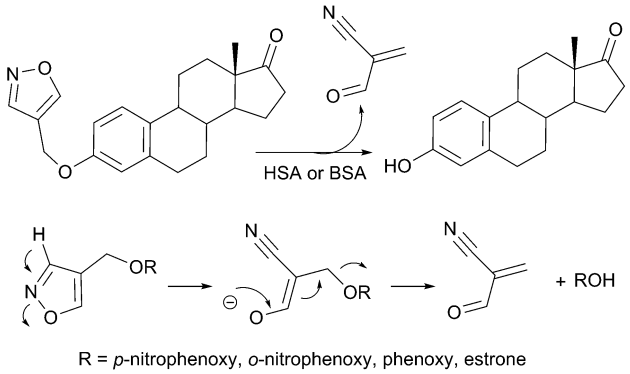
The reaction has been used as a probe of catalytic efficiency of antibodies generated against a cationic hapten mimicking the TS geometry.[58] They proved good catalysts even though their efficiency was poor with respect to enzymes.

Serum albumins have also been shown to be able to promote the reaction[59,60] with an efficiency similar to that observed with antibodies.[61]

A lot of studies have been pursued to elucidate the catalytic mechanism of both systems. Houk and Hilvert came to the conclusion that the efficiency of these biological catalysts derives from having a catalytic base located in a hydrophobic active site, a consequence of hapten design in antibodies, but the evolutionary result in the case of albumins.[62] Tawfik also showed that a completely different protein, the aldolase antibody 38C2, is able to catalyze the Kemp elimination. In common with other catalysts, 38C2 possesses a hydrophobic



Scheme 16 Kemp elimination mechanism.



R = *p*-nitrophenoxy, *o*-nitrophenoxy, phenoxy, estrone

Scheme 17 Activation of estrone prodrug by a tandem Kemp elimination/β-elimination reaction catalyzed by albumin.

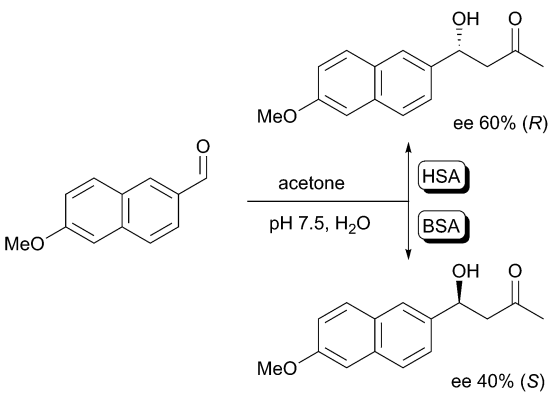
active site with a conserved lysine residue. The presence of an active site with features that are inherently catalytic was addressed by the author as the origin of promiscuity in biological catalysts.[63]

The isoxazole ring is used as a protecting group for phenols and can also find application in modulating drug pharmacokinetics in serum. For example, the deprotection of the phenol moiety of estrone has been carried out by a tandem Kemp elimination-β-elimination reaction under basic conditions (Scheme 17).[64]

This tandem reaction is efficiently catalyzed by BSA and HSA even at neutral pH. However, kinetic studies highlighted that the rate of the albumin-catalyzed reaction increased with pH. This suggests that a general base is involved in the mechanism that could be triggered by Lys-199 in HSA and Lys-222 in BSA, both placed in subdomain IIA. Inhibition kinetics with pyridoxalphosphate (PyrP) and sodium octanoate strengthen this hypothesis. The residual activity observed in BSA after incubation with PyrP was explained by the different substrate accessibility in the binding pocket IIA in the two albumins.

4.3 Aldol reaction

The acetone aldol addition to substituted aromatic aldehydes promoted by albumin in neutral aqueous solution was studied (Scheme 18).[65]



Scheme 18 Aldolase activity of albumin.

This journal is © The Royal Society of Chemistry 2015

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

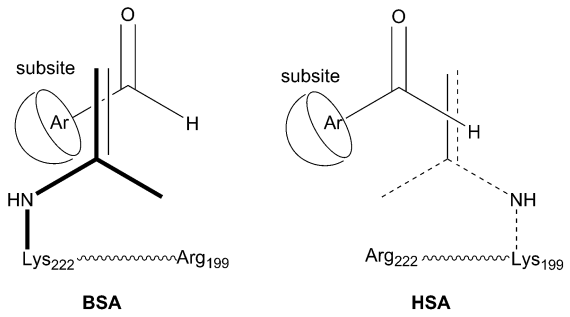


Fig. 2 Proposed mechanism for the enamine-mediated addition of acetone to 6-methoxy-2-naphthaldehyde promoted by albumin.

The BSA-catalyzed reaction follows Michaelis–Menten kinetics according to a true enzymatic process. Moreover, it is inhibited by warfarin, a well-known BSA ligand of the subdomain IIA. Lys-199 in HSA and Lys-222 in BSA could be involved in the catalytic cycle through covalent binding with acetone to give a *N*-methylethenamine intermediate. The different positions of the lysine groups in the polypeptide chain of the two proteins likely establish the opposite absolute configurations observed in the reaction products (Fig. 2).

It is worth noting that BSA is also able to accelerate the retro-aldol reaction of 4-hydroxy-4-(6-methoxy-2-naphthyl)-2-butanone (methodol). Also in this case a lysine residue localized within a hydrophobic binding site of the protein seems to take part in the catalytic step. The loss of activity observed when acetylated BSA is used proved this hypothesis.[66]

A 103 amino acid sequence corresponding to HSA's residues 191–294 of the IIA binding site has been identified and expressed in *E. coli* in fusion with the maltose binding protein (MBP). This polypeptide exhibited a catalytic activity in aldol addition, comparable to that of the original albumin, whereas MBP alone proved inactive. This is the first example of an albumin fragment that retains the catalytic abilities of the whole albumin. Moreover, this result enforces the evidence that the aldolase activity is not due to impurities in commercial albumin preparation.[65] A similar acceleration in the retro-aldol reaction can be achieved using a computationally designed retroaldolase (RA-61) which bears a lysine residue in a hydrophobic binding pocket or a simple cationic micellar system in the presence of catalytic amount of butylamine.

4.4 Henry reaction

An efficient protocol for the synthesis of aromatic and heteroaromatic β-nitroalcohols in aqueous media promoted by BSA was described (Scheme 19).[67] Complete conversion of aldehyde was achieved with a 10 : 1 nitromethane : aldehyde molar ratio in a reaction medium containing nearly 90% water (nitromethane being the rest). As expected, better yields were obtained with aldehydes bearing electron-withdrawing substituents. In no case were enantio-enriched β-nitroalcohols recovered.

In order to have some insights into the role of BSA, the reaction was carried out in the presence of denatured BSA or L-lysine. Quantitative conversions were obtained in both processes, suggesting that the nitroaldol reaction proceeds *via* nonspecific catalysis. Catalyst recycling up to five times and scale-up to one gram of aldehyde were also performed.

The nitroaldol reaction has also been carried out in a MTBE/aqueous buffer system at pH 5.5 in the presence of hydroxynitrile lyase from *Hevea brasiliensis*. Good-to-excellent ees of β-nitroalcohols have been generated, although in low-to-moderate yields.[68] The same enzyme has also been used in the resolution of racemic β-nitroalcohols for the production of (*R*)-enantiomers with ee up to 95% and 49% conversion.[69]

4.5 Thio-Michael addition

An investigation on a lipase-catalyzed Michael-type carbon–carbon bond formation reported some examples promoted by BSA immobilized on Accurel MP1000 in cyclohexane as solvent.[70]

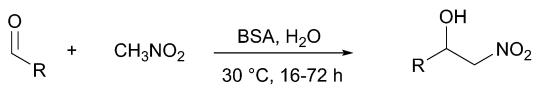
More recently, we decided to explore the ability of BSA to promote the thio-Michael addition of aromatic and aliphatic thiols to chalcone **8** (Scheme 20).[71]

Optically active adducts were obtained in high yield and ee up to 86%. In order to evaluate the influence of the structure of the acceptor on the outcome of the Michael reaction, the addition of thiophenol to various α,β-unsaturated carbonyl compounds was explored.

4.6 Sulfide oxidation

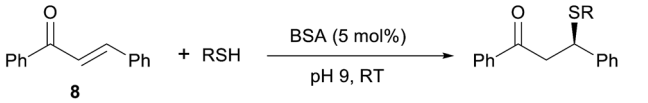
Oxidations and reductions were the first and are the most studied transformations promoted by albumin. The pivotal reports by Sugimoto[6,7] have been followed by the oxidation of sulfur-containing compounds,[72–74] the epoxidation of electron-deficient olefins,[75] and the reduction of δ-ketoacids to the corresponding enantio-enriched lactones,[76] to cite only a few. The literature of these reactions was covered by an exhaustive review that appeared in 2004.[77]

Later on, racemic β-hydroxysulfides were studied by a biomimetic approach based on a one-pot *in situ* thiolysis of epoxides followed by BSA/*t*BuOOH oxidation.[78] Moderate-to-



R = 4-NO_2-C_6H_4; 3-NO_2-C_6H_4; 4-CN-C_6H_4; 2-Pyridyl; 3-Pyridyl; 4-Pyridyl; 4-Br-C_6H_4; 4-C_6H_5-C_6H_4; 2-Nf; 1-Nf

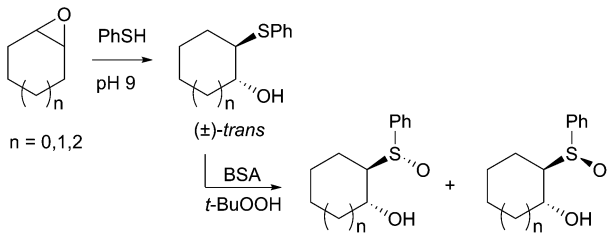
Scheme 19 BSA-mediated Henry reaction.



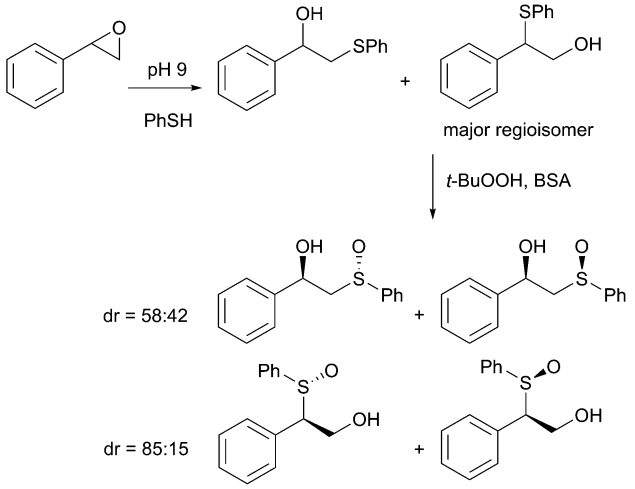
R = C_6H_5, 4-CH_3-C_6H_4, 2,6-$(CH_3)_2$-C_6H_3, 4-OCH_3-C_6H_4, 2-OCH_3-C_6H_4, 4-NO_2-C_6H_4, C_6H_5-CH_2, n-C_4H_9, n-C_8H_{17}, C_6H_5-CO

Scheme 20 BSA mediated thio-Michael addition to chalcone.

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.



Scheme 21 One-pot biomimetic approach to β-hydroxy sulfoxides.



Scheme 22 One-pot sulfoxidation of styrene oxide by BSA.

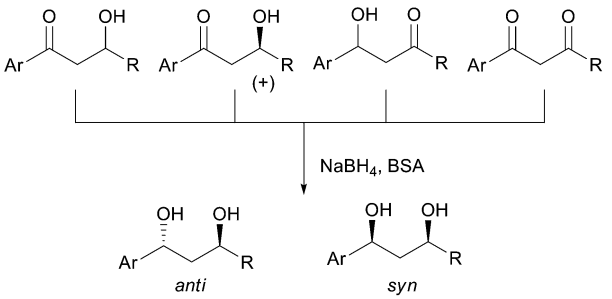
good diastereoselectivity was observed with less than 15% ee (Scheme 21).

In addition, the same approach has been investigated by using styrene oxide as an example of an α-substituted 1,2-epoxide (Scheme 22). Nucleophilic attack of thiophenol occurred preferentially at the benzylic position and the subsequent oxidation proceeded with good diastereoselectivity for the major regioisomers, but again with no appreciable enantioselectivity.

4.7 Ketone reduction

1,3-Diols are naturally occurring compounds and valuable synthetic intermediates. *Anti*-1,3-diols can be obtained by sodium borohydride reduction of the corresponding diketones or β-hydroxyketones in the presence of stoichiometric amounts of BSA with diastereoisomeric excess up to 96% (Scheme 23).[79] Control experiments carried out without BSA gave the corresponding *anti* and *syn* diols in ratios close to 1 : 1.

The presence of an aromatic carbonyl group is essential for the diastereoselectivity of the process since the enolic form of the diketone is bonded to the protein IIA binding site. Studies on the mechanism of the reaction highlighted that it follows a two-step pathway. The chemoselective reduction of the aliphatic carbonyl group exposed to the solvent in the substrate–BSA complex is followed by the stereoselective reduction of the



Ar, R = C_6H_5, CH_3; 4-MeC_6H_4, CH_3; 4-naphthyl, CH_3; ferrocenyl, CH_3, 4-$NO_2C_6H_4$,CH_3; 4-IC_6H_4, CH_3; 2-furyl, CH_3; C_6H_5, $C(CH_3)_3$; 4-MeC_6H_4, $(CH_2)_3OH$; C_6H_5, C_6H_5

Scheme 23 $NaBH_4$–albumin reduction of β-hydroxyketones and 1,3-diketones.

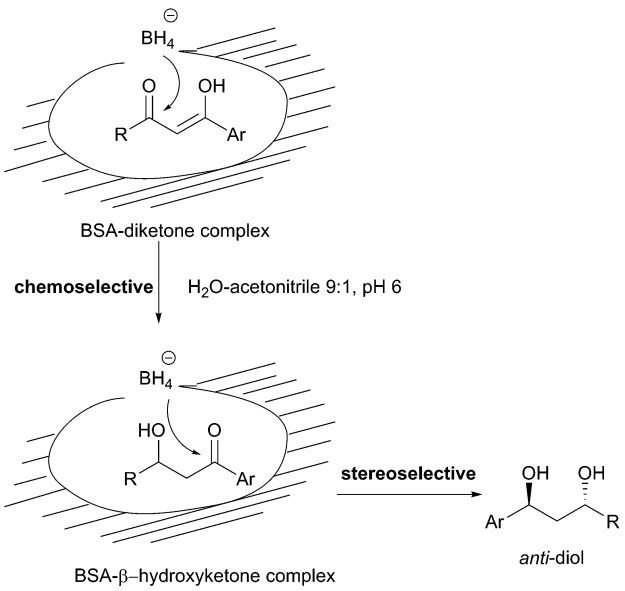


Fig. 3 Proposed mechanism of $NaBH_4$–BSA reduction.

aromatic carbonyl buried inside the hydrophobic pocket (Fig. 3).

The $NaBH_4$–BSA reduction is not stereospecific as enantiomers of 1-aryl-3-hydroxy-1-butanones are reduced with identical stereoselectivities. Racemic diols were also achieved by the albumin-mediated reduction of the corresponding diketones.

A study on the binding and catalytic potential of a 101-amino-acid peptide deriving from the A194-E294 sequence of the IIA HSA pocket (GST-HSA100) has been carried out.[80] The fragment has been cloned as a soluble glutathione S-transferase (GST) fusion protein and expressed in *E. coli.* It retained the ability to bind typical ligands of HSA, such as warfarin, and to accelerate the $NaBH_4$ reduction of 1-*p*-tolyl-1,3-butanedione to the corresponding *anti* diol with a diastereoselectivity comparable to that obtained with the native HSA. Moreover, it catalyzed the aldol addition of 6-methoxy-2-naphthaldehyde to acetone. GST-HSA100 is a promising scaffold for the construction of libraries of catalysts and binders.

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

5. Conclusions

The versatility of albumin in promoting many organic reactions lets us define it as a promiscuous biocatalyst.[81] The most important albumin mode of action derives from the ability of Lys-199 in HSA and Lys-222 in BSA, located in a hydrophobic binding site, to act as general bases or nucleophiles in a number of reaction pathways. The specific binding with substrates often favors a stereoselective outcome of the reaction. Transition metal complex binding imparts albumin with the ability to behave as a metalloenzyme. The replacement of native amino acids with different ones may modify its catalytic activity and substrate specificity, and thus widen its application. Moreover, albumin can be employed in highly denaturing polar organic solvents and in the immobilized form. These features explain why the use of albumin continues unabated and likely promises many novel applications in organic synthesis.

Notes and references

1 S. Allenmark and S. Andersson, *Chirality*, 1989, **1**, 154–160.
2 S. Allenmark, *J. Liq. Chromatogr.*, 1986, **9**, 425–442.
3 S. Allenmark and B. Bomgren, *J. Chromatogr. A*, 1983, **264**, 63–68.
4 S. Allenmark, B. Bomgren and H. Borén, *J. Chromatogr. A*, 1984, **316**, 617–624.
5 S. S. Singh and J. Metha, *J. Chromatogr. B*, 2006, **834**, 108–116.
6 T. Sugimoto, Y. Matsumura, S. Tanimoto and M. Okano, *J. Chem. Soc., Chem. Commun.*, 1978, 926–927.
7 T. Sugimoto, T. Kokubo, J. Miyazaki, S. Tanimoto and M. Okano, *J. Chem. Soc., Chem. Commun.*, 1979, 1052–1053.
8 F. Hollfelder, A. J. Kirby, D. S. Tawfik, K. Kikuchi and D. Hilvert, *J. Am. Chem. Soc.*, 2000, **122**, 1022–1029.
9 X. M. He and D. C. Carter, *Nature*, 1992, **358**, 209–215.
10 R. D. Appel, A. Bairoch and D. F. Hochstrasser, *Trends Biochem. Sci.*, 1994, **19**, 258–260.
11 K. Hirayama, S. Akashi, M. Furuya and K. Fukuhara, *Biochem. Biophys. Res. Commun.*, 1990, **173**, 639–646.
12 P. R. Ortiz de Montellano, *Cytochrome P450: structure, mechanism, and biochemistry*, Kluwer Academic/Plenum, New York, 2005.
13 N. J. M. Sanghamitra and T. Ueno, *Chem. Commun.*, 2013, **49**, 4114–4126.
14 J. C. Lewis, *ACS Catal.*, 2013, **3**, 2954–2975.
15 M. T. Reetz, *Chemical Record*, 2012, **12**, 391–406.
16 T. R. Ward, *Acc. Chem. Res.*, 2011, **44**, 47–57.
17 J. Bos, F. Fusetti, A. J. M. Driessen and G. Roelfes, *Angew. Chem., Int. Ed.*, 2012, **51**, 7472–7475.
18 K. A. Barr, S. A. Hopkins and K. Sreekrishna, *Pharm. Eng.*, 1992, **12**, 48–51.
19 W. Ohtani, Y. Nawa, K. Takeshima, H. Kamuro, K. Kobayashi and T. Ohmura, *Anal. Biochem.*, 1998, **256**, 56–62.
20 R. Woodyer, W. Chen and H. Zhao, *J. Chem. Educ.*, 2004, **81**, 126–133.
21 T. Kokubo, T. Sugimoto, T. Uchida, S. Tanimoto and M. Okano, *J. Chem. Soc., Chem. Commun.*, 1983, 769–770.
22 V. Kçhler, J. Mao, T. Heinisch, A. Pordea, A. Sardo, Y. M. Wilson, L. Knçrr, M. Creus, J.-C. Prost, T. Schirmer and T. R. Ward, *Angew. Chem., Int. Ed.*, 2011, **50**, 10863–10866.
23 G. Li, H. F. Zhang, R. M. Wang, Y. F. He and Y. B. Xiong, *Chin. Sci. Bull.*, 2013, **58**, 2956–2963.
24 X. C. Yin, X. X. Li and R. M. Wang, *Pure Appl. Chem.*, 2012, **84**, 2641–2652.
25 P. A. Zunszain, J. Ghuman, T. Komatsu, E. Tsuchida and S. Curry, *BMC Struct. Biol.*, 2003, **3**, 6.
26 A. Mahammed, H. B. Gray, J. J. Weaver, K. Sorasaenee and Z. Gross, *Bioconjugate Chem.*, 2004, **15**, 738–746.
27 C. Bertucci, C. Botteghi, D. Giunta, M. Marchetti and S. Paganelli, *Adv. Synth. Catal.*, 2002, **344**, 556–562.
28 M. Marchetti, G. Mangano, S. Paganelli and C. Botteghi, *Tetrahedron Lett.*, 2000, 3717–3720.
29 Under acidic and basic conditions no conversion was observed.
30 M. Marchetti, F. Minello, S. Paganelli and O. Piccolo, *Appl. Catal., A*, 2010, **373**, 76–80.
31 Selectivity at 76% conversion: 89% (cyclohexanone), 11% (cyclohexanol).
32 A. Mahammed and Z. Gross, *J. Am. Chem. Soc.*, 2005, **127**, 2883–2884.
33 HSA, BSA, porcine serum albumin, rabbit serum albumin, sheep serum albumin were used.
34 P. Rousselot-Pailley, C. Bochot, C. Marchi-Delapierre, A. Jorge-Robin, L. Martin, J. C. Fontecilla-Camps, C. Cavazza and S. Ménage, *ChemBioChem*, 2009, **10**, 545–552.
35 S. Otto and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1999, **121**, 6798–6806.
36 S. Otto, G. Boccaletti and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1998, **120**, 4238–4239.
37 G. Roelfes and B. L. Feringa, *Angew. Chem., Int. Ed.*, 2005, **44**, 3230–3232.
38 K. R. Rao, T. N. Srinivasan and N. Bhanumathi, *Tetrahedron Lett.*, 1990, **31**, 5959–5960.
39 S. Colonna, A. Manfredi and R. Annunziata, *Tetrahedron Lett.*, 1988, **29**, 3347–3350.
40 M. T. Reetz and N. Jiao, *Angew. Chem., Int. Ed.*, 2006, **45**, 2416–2419.
41 S. Barroso, G. Blay and J. R. Pedro, *Org. Lett.*, 2007, **9**, 1983–1986.
42 D. Coquière, J. Bos, J. Beld and G. Roelfes, *Angew. Chem., Int. Ed.*, 2009, **48**, 5159–5162.
43 P. J. Deuss, G. Popa, A. M. Z. Slawin, W. Laan and P. C. J. Kamer, *ChemCatChem*, 2013, **5**, 1184–1191.
44 M. Wilking and U. Hennecke, *Org. Biomol. Chem.*, 2013, **11**, 6940–6945.
45 L. Gjonaj and G. Roelfes, *ChemCatChem*, 2013, **5**, 1718–1721.
46 G. Carrea and S. Riva, *Angew. Chem., Int. Ed.*, 2000, **39**, 2226–2254.
47 D.-D. Zhao, L. Li, F. Xu, Q. Wu and X.-F. Lin, *J. Mol. Catal. B: Enzym.*, 2013, **95**, 29–35.
48 N. Sharma, U. K. Sharma, R. Kumar, N. Katoch, R. Kumar and A. K. Sinha, *Adv. Synth. Catal.*, 2011, **353**, 871–878.

Open Access Article. Published on 18 December 2014. Downloaded on 2/8/2026 12:03:55 AM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

49 P. Ramesh, B. Shalini and N. Fadnavis, *RSC Adv.*, 2014, **4**, 7368–7373.
50 C. A. Martinez, S. Hu, Y. Dumond, J. Tao, P. Kelleher and L. Tully, *Org. Process Res. Dev.*, 2008, **12**, 392–398.
51 U. K. Sharma, N. Sharma, R. Kumar and A. K. Sinha, *Amino Acids*, 2013, **44**, 1031–1037.
52 L.-Z. Gong, X.-H. Chen and X.-Y. Xu, *Chem.–Eur. J.*, 2007, **13**, 8920–8926.
53 Y. Huang, F. Yang and C. Zhu, *J. Am. Chem. Soc.*, 2005, **127**, 16386–16387.
54 X.-H. Chen, X.-Y. Xu, H. Liu, L.-F. Cu and L.-Z. Gong, *J. Am. Chem. Soc.*, 2006, **128**, 14802–14803.
55 P. J. Sadler and A. Tucker, *Eur. J. Biochem.*, 1993, **212**, 811–817.
56 Under these reaction conditions no detectable background reaction has been observed even after 5 days.
57 M. T. Reetz, R. Mondière and J. D. Carballeira, *Tetrahedron Lett.*, 2007, **48**, 1679–1681.
58 S. N. Thorn, R. G. Daniels, M.-T. M. Auditor and D. Hilvert, *Nature*, 1995, **373**, 228–230.
59 F. Hollfelder, A. J. Kirby and D. S. Tawfik, *Nature*, 1996, **383**, 60–63.
60 K. Kikuchi, S. Thorn and D. Hilvert, *J. Am. Chem. Soc.*, 1996, **118**, 8184–8185.
61 A. J. Kirby, F. Hollfelder and D. S. Tawfik, *Appl. Biochem. Biotechnol.*, 2000, **83**, 173–181.
62 Y. Hu, K. N. Houk, K. Kikuchi, K. Hotta and D. Hilvert, *J. Am. Chem. Soc.*, 2004, **126**, 8197–8205.
63 L. C. James and D. S. Tawfik, *Protein Sci.*, 2001, **10**, 2600–2607.
64 G. Boucher, S. Robin, V. Fargeas, T. Dininger, M. Mathé-Allainmat, J. Lebreton and C. Tellier, *ChemBioChem*, 2005, **6**, 807–810.
65 F. Benedetti, F. Berti and S. Bidoggia, *Org. Biomol. Chem.*, 2011, **9**, 4417–4420.
66 J. Schmidt, C. Ehasz, M. Epperson, K. Klas, J. Wyatt, M. Hennig and M. Forconi, *Org. Biomol. Chem.*, 2013, **11**, 8419–8425.
67 E. Busto, V. Gotor-Fernandéz and V. Gotor, *Org. Process Res. Dev.*, 2011, **15**, 236–340.
68 M. Gruber-Khadjawi, T. Purkarthofer, W. Skranc and H. Griengl, *Adv. Synth. Catal.*, 2007, **349**, 1445–1450.
69 R. Yuryev, S. Briechle, M. Gruber-Khadjawi, H. Griengl and A. Liese, *ChemCatChem*, 2010, **2**, 981–986.
70 G. A. Strohmeier, T. Sović, G. Steinkellner, F. S. Hartner, A. Andryushkova, T. Purkarthofer, A. Glieder, K. Gruber and H. Griengl, *Tetrahedron*, 2009, **65**, 5663–5668.
71 N. Gaggero, D. C. M. Albanese, G. Celentano, S. Banfi and A. Aresi, *Tetrahedron: Asymmetry*, 2011, **22**, 1231–1233.
72 S. Colonna and N. Gaggero, *Tetrahedron Lett.*, 1989, **30**, 6233–6236.
73 S. Colonna, N. Gaggero and M. Leone, *Tetrahedron*, 1991, **47**, 8385–8398.
74 S. V. Dzyuba and A. M. Klibanov, *Biotechnol. Lett.*, 2003, **25**, 1961–1965.
75 S. Colonna, N. Gaggero, A. Manfredi, M. Spadoni, L. Casella, G. Carrea and P. Pasta, *Tetrahedron*, 1988, **44**, 5169–5178.
76 M. Utaka, H. Watabu and A. Takeda, *J. Org. Chem.*, 1986, **51**, 5423–5425.
77 S. V. Dzyuba and A. M. Klibanov, *Tetrahedron: Asymmetry*, 2004, **15**, 2771–2777.
78 S. Colonna, V. Pironti, F. Zambianchi, G. Ottolina, N. Gaggero and G. Celentano, *Eur. J. Org. Chem.*, 2007, 363–367.
79 F. Berti, S. Bincoletto, I. Donati, G. Fontanive, M. Fregonese and F. Benedetti, *Org. Biomol. Chem.*, 2011, **9**, 1987–1999.
80 I. Luisi, S. Pavan, G. Fontanive, A. Tossi, F. Benedetti, A. Savoni, E. Maurizio, R. Sgarra, D. Sblattero and F. Berti, *PLoS One*, 2013, **8**(9), e74775.
81 U. T. Bornscheuer and R. J. Kazlauskas, *Angew. Chem., Int. Ed.*, 2004, **43**, 6032–6040.

This journal is © The Royal Society of Chemistry 2015