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Stereoselective oxidations and reductions catalyzed by nonredox proteins

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Abstract—Use of common proteins with no distinctive redox functionalities as chiral templates for asymmetric oxidations and reductions represents an intriguing, but barely explored, phenomenon in biocatalysis. This review focuses on synthetically important and challenging reactions stereoselectively catalyzed or mediated by ubiquitous nonredox proteins, such as bovine serum albumin and chymotrypsin.

Contents

1. Introduction

There is an increasing demand for separated enantiomers of chiral organic compounds fuelled mainly by the ever-growing number of marketed drugs that are chiral and by the recognition that in such instances one enantiomer is usually superior to the other in terms of its biological activity and/or toxicity.[1] While biocatalytic, in particular enzymatic asymmetric conversions have contributed appreciably toward satisfying this demand,[2] their contribution has been severely hampered by such factors as narrow substrate specificity of the majority of enzymes, as well as their high cost, commercial unavailability and insufficient stability.[3] These limitations especially hold true for nonhydrolytic enzymes, including oxidoreductases, which are arguably the most

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versatile and promising class of enzymes from a synthetic chemical viewpoint.[4]

An intriguing alternative to employing oxidoreductive enzymes was the discovery of this catalytic activity in ubiquitous proteins whose natural, physiological role is nonenzymatic. Indeed, common nonenzymic proteins such as hemoglobin, myoglobin, and cytochrome c are known to catalyze a variety of oxidation and reduction reactions.[5] In retrospect, this phenomenon does not seem remarkable due to the presence in these hemoproteins of heme prosthetic groups, long recognized for their oxidoreductive capabilities.[6] In contrast, it would be surprising if nonhemoproteins, also lacking other readily oxidizable/reducible functionalities, could catalyze these reactions. However, there is emerging evidence that not only can such nonredox proteins catalyze (or at least mediate) diverse and synthetically interesting oxidations and reductions but they even often do so stereoselectively. If understood and generalized, this behavior could broaden the synthetic utility of biocatalysts; this is the subject of this mini-review.

2. Mechanistic rationale

To function as a stereoselective catalyst, a protein must meet two independent criteria. First, it should accelerate the reaction in question, typically by stabilizing its transition state. Second, it should provide a dissymmetric milieu, whereby the reactant(s) of interest will bind in such a way that favors one stereochemical reaction pathway over the other. Since all natural proteins, consisting of only L-amino acids, inevitably constitute chiral, dissymmetric environments,[7] the chief issue in the second criterion is whether the reactants will end up there, that is, whether they will bind to the proteins.

One would expect that the most synthetically interesting organic reactants, as they are hydrophobic, should bind to proteins in aqueous solutions due to the proteins' hydrophobic interiors and surface patches.[8] This hydrophobic binding, while not guaranteeing the subsequent discrimination between the stereochemical reaction pathways, makes this stereopreference probable. Therefore, proteins are likely to be at least enantioselective mediators, if not catalysts, of chemical reactions, including oxidations and reductions.

A protein devoid of redox moieties cannot be expected to necessarily accelerate oxidations and reductions. Nevertheless, binding of a reactant alters its environment (and that of its ensuing transition state) from purely aqueous to that of the protein's binding site. The net result of this transition is either an increase or decrease in the activation barrier (the $\Delta G^{\neq}$ value, that is, the difference between the Gibbs free energy of the protein–reactant complex in the transition state and that in the ground state) with the consequent inhibition or acceleration, respectively, of the reaction. Statistically, the chances of these outcomes are presumably similar. This admittedly simplistic analysis indicates that adding even a nonredox protein to the reactants could be beneficial in terms of making the redox reaction at least stereoselective (with the extent of the benefit enhanced at high protein concentration and protein–reactants affinities) and perhaps also faster. This prediction is verified below using the available experimental data from the literature.

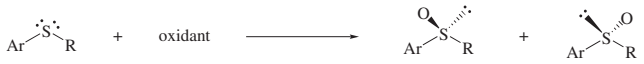
3. Stereoselective oxidations

3.1. Oxidation of sulfur-containing compounds

3.1.1. Monooxidation of sulfides (sulfoxidation). Oxidation of aryl alkyl sulfides to form optically active sulfoxides is a synthetically challenging and important reaction.[9] Such sulfides, due to their hydrophobicity, should bind to hydrophobic pockets of proteins. Assuming that the resultant protein–sulfide complexes are accessible to the oxidant molecules, enantioenriched sulfoxides could be formed.

Bovine serum albumin (BSA) is an abundant carrier protein, with no known natural catalytic function, which possesses well-defined hydrophobic binding sites. The latter bind a variety of hydrophobic compounds,[10] thus making this readily available and inexpensive protein an attractive template for asymmetric sulfoxidations.

Prochiral organic sulfides were indeed found to be oxidized into optically active sulfoxides in the presence of BSA by diverse oxidants such as $NaIO_4$, H_2O_2, or *m*-CPBA (Fig. 1).[11] While initial studies stressed that large (albeit sub-equimolar) amounts of BSA were required to attain high enantiomeric excesses of the sulfoxide products,[11] it was later demonstrated that even catalytic amounts in fact sufficed.[12,13] Also, the range of oxidizing agents was further extended (Fig. 1), with especially facile BSA-catalyzed sulfoxidation achieved with dioxiranes.[14]



Yield = 30 - 98%
Ee = 7 - 81%

Ar = Ph, *p*-tolyl
R = alkyl, Bn, Ph
oxidant = $NaIO_4$, H_2O_2, *tert*-BuOOH, *m*-CPBA, $KHSO_5$, dioxiranes

Figure 1. Asymmetric oxidation of alkyl aryl sulfides in aqueous buffer in the presence of BSA.[11–13]

A striking feature of these sulfoxidations was that the nature of the oxidant could be crucial in determining both the absolute configuration of the products and their enantiomeric purity.[11] Likewise, the pH of the reaction medium had a major impact on both stereochemical parameters.[15] In addition, the structure of the sulfides profoundly influenced the stereoselectivity of

this oxidation process, although no discernable structure–activity relationship emerged.[11–15]

Due to the multiplicity of the binding sites in BSA, a definitive determination of the binding mode of the sulfides to the protein is not possible. This situation can be remedied, however, by using a protein with a single well-defined hydrophobic binding pocket, such as bovine pancreatic α-chymotrypsin.

α-Chymotrypsin is a hydrolytic enzyme known to bind many hydrophobic substrates and ligands.[16] This non-redox protein was found not only to increase the rate of sulfoxidations, but also to yield sulfoxides of high enantiomeric purity.[17] Stereoselectivity of the chymotrypsin-catalyzed sulfoxidation process was significantly enhanced by minimizing the contribution of the spontaneous reaction. Like with BSA, the chymotryptic reaction was highly sensitive to the sulfide structure: the degree of enantioselectivity varied from as high as $E = 43$ in the case of *iso*-butylphenyl sulfide to virtually nonexistent ($E = 1.2$) for 4-*tert*-butylphenyl methyl sulfide. This keen structural discrimination by the protein can be explained by means of molecular modelling (Fig. 2). Upon binding, one of the sulfur's lone pairs of *iso*-butylphenyl sulfide was seen buried in the binding pocket of chymotrypsin, whereas the other was completely exposed to the solvent and thus to attack by the oxidant. Consequently, only one sulfoxide enantiomer was formed almost exclusively. In contrast, the orientation of the 4-*tert*-butylphenyl methyl sulfide in the binding pocket of chymotrypsin was such that both of the sulfur's lone pairs were completely accessible by the solvent; not surprisingly, nearly racemic sulfoxide was produced in this case.

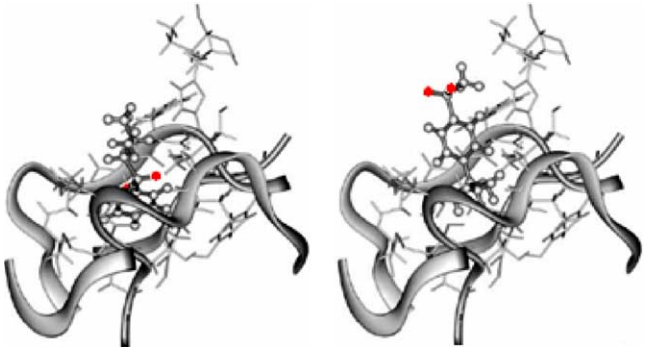


Figure 2. Molecular models of bound *iso*-butyl phenyl sulfide (left) and 4-*tert*-butylphenyl methyl sulfide (right) to the active site of α-chymotrypsin. Electron lone pairs of the sulfides are shown in red (adopted from Ref. 17).

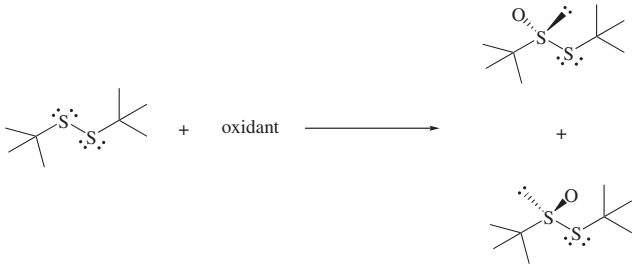
Subtilisin Carlsberg, another hydrolytic enzyme with a hydrophobic binding site albeit less defined than in chymotrypsin, also accelerated the aforementioned sulfoxidation, but with a moderate level of stereoselectivity.[17]

BSA was found to catalyze not only the sulfoxidation of nonfunctionalized substituted sulfides but also those containing ester and keto groups.[13,18] In contrast to the reactions depicted in Figure 1,[11,15] no significant effect of the pH on the enantiomeric purity of the products was observed. However, as above, the nature of the oxidant affected the absolute configuration of the resulting sulfoxide,[13] although the effect was not as pronounced as in the case of the alkyl phenyl sulfides.[11] Less efficient asymmetric sulfoxidation was observed in the presence of another globular protein and a close analogue of BSA, human serum albumin.[18]

3.1.2. Monooxidation of disulfides (thiosulfination). Antibacterial and anti-tumor properties of thiosulfinates make them interesting targets for medicinal chemistry.[19] Since disulfides resemble (although are chemically distinct from) sulfides, one would expect that biocatalytic thiosulfinations can be carried out similarly to sulfoxidations.

BSA was indeed found to catalyze the oxidation of disulfides to produce optically active thiosulfinates.[20] Unfortunately, due to inherent chemical and/or thermal instabilities of thiosulfinates, only a few disulfides yielded stable products. As the hydrophobicity of the disulfide substrate increased (1,2-dithiane < di-*iso*-propyl disulfide < di-*tert*-butyl disulfide), the rates of the protein-catalyzed and spontaneous oxidations rose and dropped, respectively. This dual trend led to the greatest stereoselectivity being observed in the case of the most hydrophobic di-*tert*-butyl disulfide, which was the best substrate for this biocatalytic oxidation affording the thiosulfinate product with a high ee. Interestingly, akin to BSA-mediated sulfoxidations, the nature of the oxidant had a dramatic effect on the absolute stereochemistry of the produced thiosulfinate (Table 1).

Table 1. Effect of the oxidant on the absolute configuration and efficiency of the thiosulfination of di-*tert*-butyl disulfide in aqueous buffer in the presence of BSA[20]



Oxidant	Initial rate, *p*mol/h mg of BSA		
	(*S*)-Isomer	(*R*)-Isomer	(*E*) (*S*/*R*)
H_2O_2	960 ± 60	56	17 ± 1
tert-BuOOH	2.1 ± 0.5	41 ± 3	0.051 ± 0.012

The synthetic utility of the BSA-catalyzed thiosulfination of di-*tert*-butyl disulfide was also demonstrated: after about 3 days, *S*-*tert*-butyl *tert*-butane thiosulfinate was obtained with 66% yield and 88% ee, using H_2O_2 as an oxidant.

3.1.3. Kinetic resolution of sulfoxides. Racemic sulfoxides are readily accessible via chemical oxidation of the corresponding sulfides. Since sulfoxides can bind to BSA, albeit less tightly than sulfides,[21] one would expect that further partial oxidation to sulfones (Fig. 3) can be conducted, thus potentially affording the resolution of racemic sulfoxides.[15,22] Unfortunately, the enantiomeric excess of the sulfoxides thus obtained was found to be quite low (<33%).[15,22]

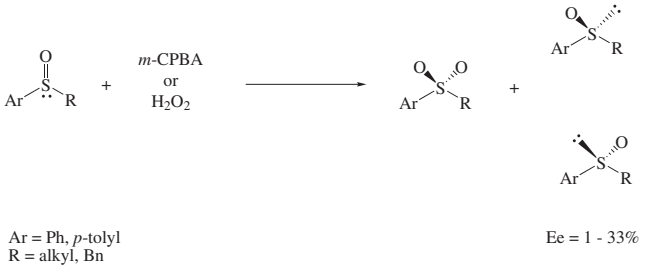


Figure 3. Kinetic resolution of racemic sulfoxides in aqueous buffer in the presence of BSA.[15,22]

Interestingly, the overoxidation of sulfides using a large excess of an oxidant (either H_2O_2 or *m*-CPBA) led to sulfoxides with ees as high as over 90%. However, this resolution was very sensitive to the sulfide structure, and in many instances sulfoxides of lower enantiomeric purities were obtained.[15]

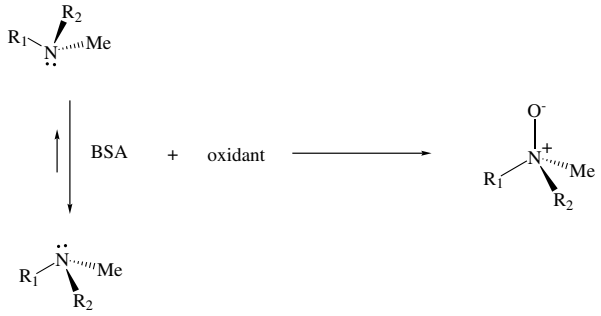
3.2. Oxidation of amines

Chiral *N*-oxides are promising auxiliaries for enantioselective transformations wherein they can act as both ligands and catalysts.[23] However, their use in asymmetric synthesis has not been extensively explored because individual enantiomers of *N*-oxides are difficult to prepare. Especially challenging is the synthesis of optically active acyclic tertiary *N*-oxides due to a facile *N*-inversion of the amines even at room temperature. It is conceivable that this inversion could be hindered by binding to a protein. Furthermore, in the case of unsymmetrical tertiary amines, the chirality of a protein imposes constraints that could lead to stereoselective oxidation of the nitrogen. Also, the unbound amine would be present as a racemate, thus creating the possibility for conducting a dynamic kinetic resolution.

Recently, it has been reported[24] that tertiary amines can be oxidized into the corresponding *N*-oxides in the presence of BSA, although the oxidation is sluggish and takes several days (Table 2). More stereoselective oxidations were observed with amines bearing a long alkyl chain in combination with a benzyl group. However, as with BSA-catalyzed sulfoxidations, no general structure–reactivity trends transpired.

Unfortunately, no attempt was made to estimate the extent of the spontaneous reactions, which are likely to contribute to the overall process and thus diminish the enantiomeric purity of the *N*-oxides. Despite moderate stereoselectivities being obtained, this was the first demonstration that BSA could act as a chiral mediator in the oxidation of heteroatoms other than sulfur.

Table 2. Effect of the oxidant and the structure of the amine on stereoselective oxidation of tertiary amines in aqueous buffer in the presence of BSA (only one enantiomer of *N*-oxide is shown, its absolute configuration was not determined)[24]

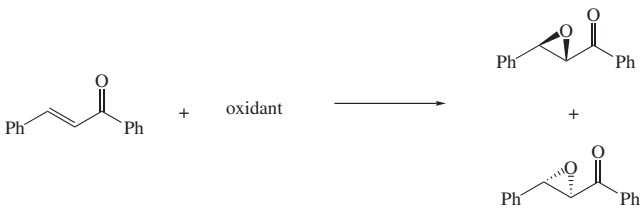


R_1	R_2	Oxidant	Yield (%)	Ee (%)
Ph	Et	$NaIO_4$	25	18
Ph	Et	$NaIO_4$	35	20
Bn	Pentyl	$NaIO_4$	87	64
Bn	Pentyl	H_2O_2	100	67
Bn	Pentyl	*m*-CPBA	100	4

3.3. Epoxidation of electron-deficient alkenes (Weitz–Scheffer oxidation)

Optically active epoxides are valuable synthons in organic chemistry,[25] thus making asymmetric oxidation of electron-deficient alkenes important.[26] It is recognized that binding of a hydrophobic α,β-unsaturated ketone to the hydrophobic interior of BSA could lead to the formation of an optically active epoxide (Table 3).[27] The absolute configuration of the epoxides formed was controlled by the nature of the oxidant, as in the aforementioned BSA-catalyzed sulfoxidations and thiosulfinations. The low enantiomeric induction observed was attributed to high conformational mobility of the α,β-enone. Therefore, the oxidation of more rigid substrates was expected to proceed with greater stereoselectivity.

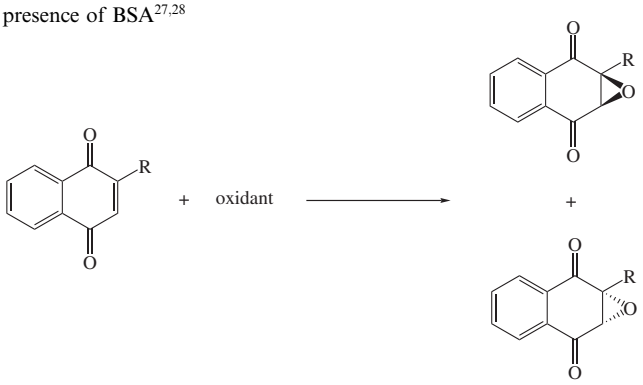
Table 3. Epoxidation of chalcone in aqueous buffer in the presence of BSA[27]



Oxidant	Ee (%) (abs. config.)
H_2O_2	12 (2*S*,3*R*)
tert-BuOOH	13 (2*R*,3*S*)

Indeed, a highly stereoselective epoxidation of conformationally fixed naphthobenzoquinones in the presence of BSA was achieved (Table 4).[27,28] Optically active epoxides were also produced in the presence of human

Table 4. Epoxidation of naphthoquinones in aqueous buffer in the presence of BSA[27,28]



R	Oxidant	Yield (%)	Ee (%) (abs. config.)
iso-Pr	H_2O_2	60	15 (2*S*,3*R*)
iso-Pr	*tert*-BuOOH	55	21 (2*R*,3*S*)
iso-Bu	H_2O_2	70	8 (2*S*,3*R*)
iso-Bu	*tert*-BuOOH	62	77 (2*R*,3*S*)
n-Octyl	*tert*-BuOOH	66	100 (2*R*,3*S*)
Bn	H_2O_2	44	15 (2*R*,3*S*)
Bn	*tert*-BuOOH	22	12 (2*R*,3*S*)

serum albumin,[28a] although the efficiency of the process was inferior to that in the presence of BSA.

Again, the absolute configuration of the resulting epoxides was sometimes controlled by the oxidant. Generally, higher stereoselectivities were obtained with *tert*-BuOOH than with H_2O_2. Increasing the alkyl chain length of the 2-substituent led to a rise in both yield and enantiomeric excess of the epoxynaphthoquinones, presumably due to a better binding of these hydrophobic compounds to the hydrophobic interior of BSA.[28b]

In accordance with our mechanistic rationale, BSA was shown to provide a chiral environment affording a stereoselective epoxidation. Furthermore, this particular case demonstrated that BSA was not always a catalyst but sometimes merely a chiral mediator, that is, the rate of the reaction for several substrates in the absence of the protein was comparable to (or even exceeded) that in the presence of BSA.[28b] This phenomenon was attributed to the hindered accessibility of the naphthoquinone–BSA complex to the oxidant, as well as to a more viscous reaction microenvironment.

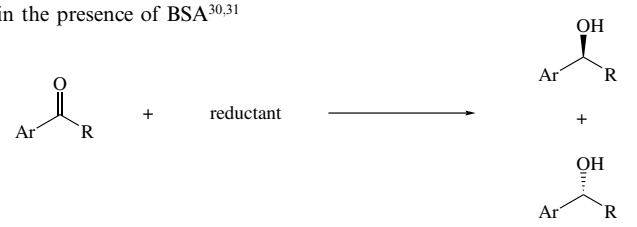
4. Stereoselective reductions

4.1. Reduction of ketones

4.1.1. Reduction of alkyl aryl ketones. Asymmetric reduction of ketones in the presence of chiral, for example, protein auxiliaries could potentially be useful for the synthesis of optically active alcohols that are indispensable building blocks in modern synthetic chemistry.[29] In particular, if a hydrophobic ketone binds to a protein, stereoselective addition of a reducing agent could yield enantiomerically enriched secondary alcohols.

Reduction of aromatic ketones by $NaBH_4$, $NaBH_3CN$, or 1-propyl-1,4-dihydronicotinamide (NAH) in the presence of BSA was indeed found[30,31] to afford optically active alcohols (Table 5). In some cases, the nature of the reductant determined the absolute configuration of the resulting alcohol[31] (as in the aforementioned oxidations of sulfur-containing compounds). The enantioselectivity of the reduction was also sensitive to the ketones structure. Importantly, fluorine-containing ketones were stereoselectively reduced in the presence of BSA, thus forming pharmaceutically interesting optically active fluorinated alcohols.[32]

Table 5. Asymmetric reduction of aromatic ketones in aqueous buffer in the presence of BSA[30,31]



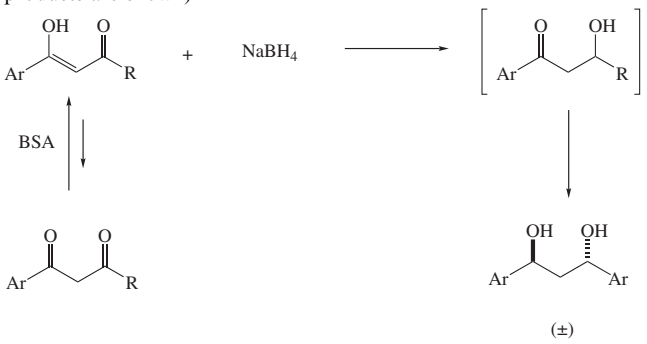
Ar	R	Reductant	Ee (%) (abs. config.)
Ph	CF_3	NAH	47 (*R*)
Ph	CF_3	$NaBH_4$	36 (*S*)
Ph	Me	$NaBH_4$	78 (*R*)
Ph	*n*-Bu	$NaBH_4$	14 (*S*)
1-Naphthyl	Me	$NaBH_4$	67 (*S*)

Further studies indicated a strong pH dependence of the enantiomeric excesses of the alcohols formed, with the largest values being obtained at pH above 9.[33] Unfortunately, substantial amounts of BSA were required to achieve reasonable enantiomeric purity, although the protein could be recycled without substantial loss of stereoselectivity.

Interestingly, the concentration of BSA was found to affect the absolute configuration of the alcohols produced.[33] This puzzling observation was explained by speculating that the reduction could occur in both the interior and exterior surface binding sites of the protein.[33] At low BSA concentrations (<0.5 mM), the reaction predominantly took place on the surface by the chiral $BSA{\cdot}BH_4^-$ ion pair (or covalently bound $BSA{\cdot}BH_3$). At higher protein concentrations (above 1 mM), the main reaction site shifted from the surface to the more hydrophobic interior of BSA, whereby the opposite enantiomer of the alcohol was produced. Unfortunately, the synthetic utility of these BSA-mediated reductions is undermined by the unimpressive enantiomeric excesses of the resulting alcohols, most likely due to a facile spontaneous reduction yielding racemates.

4.1.2. Diastereoselective reduction of 1,3-diketones. A highly asymmetric reduction of 1,3-diketones to produce *anti*-diols was observed[34] in the presence of equimolar amounts of BSA (Table 6). It is noteworthy that the reduction of 1,3-diketones proceeded with some level of diastereoselectivity even in the absence of protein.[34]

Table 6. Diastereomeric reduction of 1,3-diketones in aqueous buffer—acetonitrile mixture in the presence of BSA (only major products are shown)[34]



Ar	R	α:β	*anti*:*syn*
Ph	Me	2:98	93:7
Ph	*tert*-Bu	45:55	56:44
2-Naphthyl	Me	10:90	80:20
Ph	Ph	—	96:4
p-Tolyl	$(CH_2)_3OH$	5:95	95:5

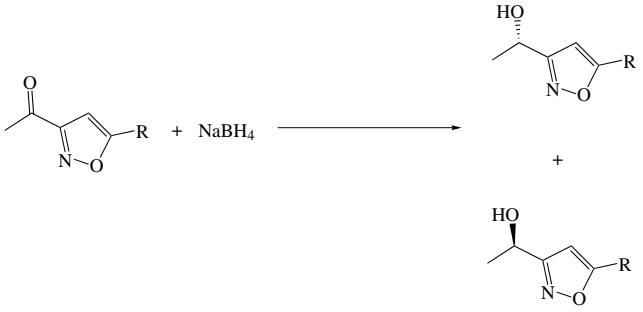
However, in the presence of BSA such diastereoselectivity was significantly amplified; interestingly, in many cases it was even inversed. Spectroscopic and chemical studies suggested that the binding of the diketone to the hydrophobic pocket of the protein shifts the keto–enol equilibrium toward the enol-form, thereby leading to a preferential reduction of the β-keto-group. The resultant β-hydroxy ketone is reduced further, producing the *anti*-diol as the major product. Thus, in this instance BSA served as a chiral template that provided both regio- and stereoselectivity. Regrettably, due to the large amounts of the protein required for this transformation, its synthetic utility is doubtful.

4.1.3. Reduction of functionalized ketones. The versatility of biocatalytic ketone reductions can be significantly enhanced if various substituents are present. This would also allow easy subsequent transformation of optically active alcohols into other useful synthons.

Isoxazoles are versatile building blocks that provide a convenient entry into functional diversity.[35] In the presence of BSA, the keto-group of substituted keto-isoxazoles was asymmetrically reduced by $NaBH_4$ to produce optically active hydroxy-isoxazoles (Table 7).[36] The reduction of more hydrophobic substrates proceeded with higher asymmetric induction, consistent with their tighter binding to the protein.

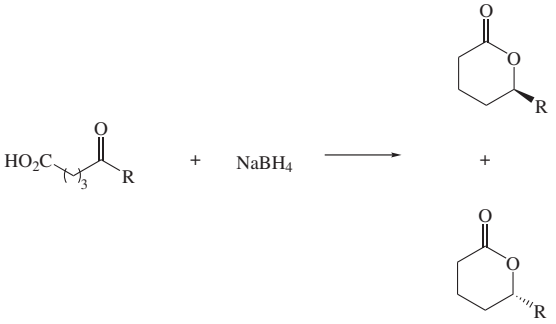
Lactone motif is widely represented in a variety of natural products, and thus constitutes an important target.[37] BSA was shown[38] to catalyze the reduction of δ-keto acids to produce optically active δ-lactones (Table 8). For more hydrophobic δ-keto acids, more enantiomerically pure lactones were obtained, although the levels of stereoinduction were moderate. Interestingly, temperature dramatically influenced the stereoefficiency of this transformation. Notably, decreasing the ratio of δ-keto acid to BSA also lowered the enantiomeric purity of the lactone, which is in sharp contrast

Table 7. Asymmetric reduction of substituted keto-isoxazoles in aqueous buffer in the presence of BSA (the absolute configuration was not determined)[36]



R	Yield (%)	Ee (%)
CH_2Cl	80	15
Pr	10	71

Table 8. Stereoselective reduction of δ-keto acids in aqueous buffer in the presence of BSA leading to formation of chiral lactones[38]



R	*T* (°C)	Yield (%)	Ee (%) (abs. config.)
Me	0	22	0
n-Bu	0	74	0
n-Octyl	0	54	26 (*R*)
n-Undecyl	0	61	44 (*R*)
n-Undecyl	25	50	16 (*R*)

to reductions of aromatic ketones in the presence of BSA.[30,31,33]

5. Conclusions

Nonredox proteins can act as versatile chiral templates for a number of synthetically useful oxidations and reductions. Thus far, BSA has been the most widely used protein in such redox transformations, although others, such as α-chymotrypsin, also hold promise as chiral mediators. Unfortunately, the efficiencies of the processes described to date are inferior to the best chemical methods. However, they can be often enhanced if the undesired, inevitably nonstereospecific spontaneous reactions can be suppressed. However, the contributions of such reactions to overall processes have rarely been addressed in the literature.

The absolute configurations of the products are often governed by the nature of the oxidants or reductants, thus underscoring the importance of not only protein–reactant but also protein–oxidant/reductant interactions. This intriguing, but poorly understood,

phenomenon has also received little attention and awaits further exploration.

Molecular modelling have been shown to be a valuable tool in explaining the stereochemical pathways of chymotrypsin-catalyzed sulfoxidations.[17] Similarly, it should be profitably applicable to other redox processes and lead to their better understanding and improvement. Finally, the biocatalytic potential of nonredox proteins such as BSA presumably could be enhanced further by means of directed evolution,[39] akin to a recently reported activity enhancement of peroxidase catalytic antibodies.[40]

Acknowledgements

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