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# ASYMMETRIC WEITZ - SCHEFFER EPOXIDATION PROMOTED BY BOVINE SERUM ALBUMIN. PART III[1]. HIGHLY STEREOSELECTIVE SYNTHESIS OF OPTICALLY ACTIVE EPOXYNAPHTHOQUINONES.

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Abstract - The epoxidation of 2-substituted naphthoquinones with t-BuOOH in an aqueous buffer solution containing a small amount (up to 5 % molar equiv) of bovine serum albumin (BSA) gives the corresponding epoxides with enantiomeric excess (e.e.) up to 100 %. The enantioselectivity is very sensitive to the addition of water miscible or immiscible cosolvents and to the length of the alkyl chain in position 2. The mechanism by which the cosolvents influence the e.e. was studied. Correlations between the circular dichroism spectra of the BSA-quinone complexes and the stereochemistry of the epoxidation products were found.

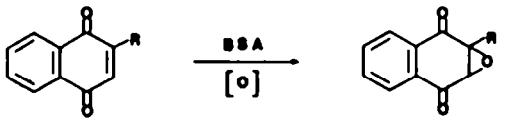
Introduction. Asymmetric chemical transformations in the presence of proteins as chiral agents are a phenomenon of general interest. According to Brewster[2] "the use of proteins as catalysts is as ancient as life itself, moreover it is at any rate, a habit that terrestrial biota would find difficult to give up". We and others have used the globular protein bovine serum albumin (BSA) as a tool for catalytic asymmetric synthesis.[3] The reactions examined were sulfoxidation,[4] borohydride reduction of ketones[5] or ketoesters,[6] cis hydroxylation[7] and epoxide formation through Darzens condensation[8] and Weitz - Scheffer oxidation in alkaline medium.[1] In particular, we have recently studied the asymmetric epoxidation of substituted naphthoquinones[1] by t-butylhydroperoxide in water containing BSA. A range of substrates and the effects of variation of pH of the aqueous buffer solution, peroxidant and metal ions were examined.

In spite of considerable work, there are still several uncertainties about the mechanism responsible for this and other asymmetric syntheses. In this paper we have examined the factors which control the enantioselectivity of the Weitz-Scheffer asymmetric epoxidation promoted by BSA. We have also studied the diastereomeric complexation of 2-alkyl naphthoquinones with BSA by CD spectroscopy and found a correlation for predicting the stereochemical outcome of the reaction.

Result and Discussion. Our previous results under heterogeneous conditions[1] indicated that t-BuOOH was the most stereoselective of the oxidants tested in the Weitz - Scheffer asymmetric epoxidation of substituted 1,4-naphthoquinones in the presence of BSA. The highest e.e. was obtained with 2-cyclohexyl-1,4-naphthoquinone (79 % e.e.). The stereochemistry of the process was very sensitive to minor structural differences in the substrates. Both the optical purity and the absolute configuration of the major product depended on the

nature of the substituent at position 2. Predicting the stereochemical outcome was therefore difficult. Steric effects were important: 2-tert-butyl-1,4-naphthoquinone was not oxidized even after a long reaction time. Low pH increased the enantioselectivity in most cases.

We have now investigated in more detail the role of BSA in this epoxidation. We first repeated the reactions under homogeneous conditions in aqueous buffer solution at pH 9, with t-BuOOH as oxidant and 5 % molar equiv (with respect to the substrate) of BSA under stirring. Naphthoquinones (1) and (3) gave the corresponding epoxides (9) and (11), with e.e. equal to or greater than those formed under heterogeneous conditions (Table I). The asymmetric induction therefore must occur in the aqueous solution and not at the interface. However, in order to perform preparative scale reactions, we preferred to work with larger amounts of substrate under heterogeneous conditions.




| | | | |
|---|---|---|---|
| (1,9) | R = $CH_3$ | (6,13) | R = $C_6H_5$ |
| (2,10) | R = $C_2H_5$ | (8,14) | R = Cyclo-$C_6H_{11}$ |
| (3,11) | R = i-$C_4H_9$ | (7,15) | R = n-$C_6H_{13}$ |
| (4,12) | R = n-$C_4H_9$ | (8,16) | R = n-$C_8H_{17}$ |

We have noted that, in contrast to our results for the sulfoxidation reaction,[3b] BSA generally is not a chemical catalyst in the epoxidation. As shown in Table II, the reaction rate increases only for very poorly water-soluble substrates (6,8), whose solubility is enhanced by the protein. A retarding effect is observed in the other cases (1,3,5) and is probably due to hindrance of the approach of the oxidant by the protein-substrate complex and to the increased medium viscosity. Good asymmetric induction requires that a certain threshold concentration of BSA be present. The results in Table III show that for 2-phenyl-1,4-naphthoquinone (5) there is no substantial change of stereoselectivity with 0.5 % mol of BSA and the usual 5%, whereas smaller amounts of BSA (0.025%) lower the enantioselectivity. It should also be mentioned that the aqueous solution of the protein recovered from the epoxidation can be recycled, again giving good stereoselectivity ( 50 % e.e. in the case of the cyclo-hexyl derivative (14)). The process did not cause any denaturation of BSA. The epoxynaphthoquinones are optically stable in the reaction medium; the 2-phenyl derivative (13) (50 % e.e.) was recovered quantitatively after a 5 day reaction time without any appreciable racemization.

A very important goal in asymmetric synthesis is optimization of the enantiomeric excess. We have used two approaches to obtain this, namely the selection of an appropriate substrate and the addition of organic cosolvents to the aqueous medium.

Effects of the Organic Cosolvent. Water-miscible and water-immiscible organic cosolvents increase significantly the enantioselectivities of pig liver esterase hydrolysis of diesters.[8] Very recently we showed that high e.e. can be obtained by the same means in Weitz - Scheffer condensation in the presence of BSA.[9] The best asymmetric induction (90 % e.e.) was obtained with 2-isobutyl-

1,4-naphthoquinone (3) and t-BuOOH in a buffer solution containing 0.05 molar equivalents of isooctane. Larger amounts (5 % v/v) of isooctane or other cosolvents, such as EtOH or DMSO, only slightly influenced the enantioselectivity of the process (66-75 % e.e. compared to 77 % e.e. without organic cosolvents). By contrast, in $CCl_4$ (5 % v/v) the racemic epoxide (11) was obtained. The latter result can be easily accounted for, since $CCl_4$ takes the substrate and the oxidant out of the aqueous phase, so that the reaction takes place in the organic solvent. Indeed the chemical yield in epoxide (11) was similar in a blank experiment carried out under the same conditions but without BSA. Lower amounts of $CCl_4$ (0.04 % v/v, i.e., $CCl_4$ completely dissolved in the aqueous buffer ) had negligible effects on e.e..

The role of organic cosolvents and in particular that of isooctane in this reaction is difficult to understand and there is no current general rule formulating the effects of organic cosolvents in enzymatic reactions.[8] Information on this subject was obtained by measuring the binding of isobutyl naphthoquinone (3) and its epoxide (11) to BSA and by studying the influence of cosolvents on the binding. Binding studies carried out with free and immobilized BSA gave practically identical results (Figure 1). The double-reciprocal plot[10] of the binding of isobutyl naphthoquinone to BSA gave an association constant of $10^4\ M^{-1}$ and that of isobutyl epoxynaphthoquinone of $5.5\times10^3\ M^{-1}$. In both cases the intercept with the ordinate indicated that 5 moles of ligand/mole of BSA were bound and that a single class of binding sites was responsible for the interaction. Due to the low solubility of both ligands in buffer it was not possible to see whether or not there were binding sites with lower affinity in BSA. Scatchard[11] plots gave results identical to those obtained with double-reciprocal plots.

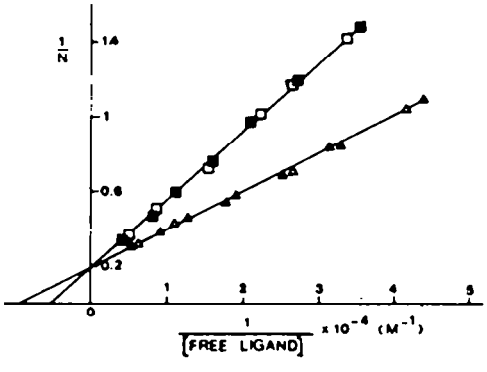



Figure 1. Double reciprocal plots of the binding of isobutyl naphthoquinone ( △ , ▲ ) and isobutyl epoxynaphthoquinone ( □ , ■ ) to free (empty symbols) and immobilized (filled symbols) BSA. N indicates the moles of ligand bound per mole of protein.

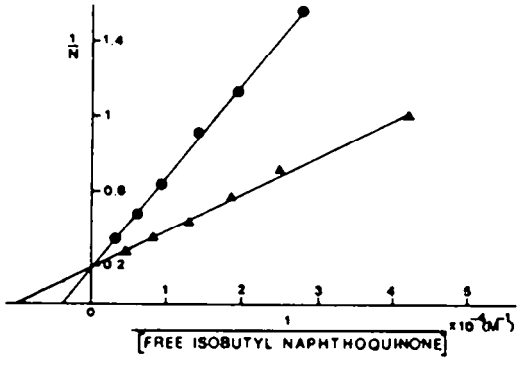



Figure 2. Double reciprocal plots of the binding of isobutyl naphthoquinone to immobilized BSA in the absence ( ▲ ) and in the presence ( ● ) of $1.5\times10^{-4}$ M isobutyl epoxynaphthoquinone.

Isobutyl epoxynaphthoquinone competitively inhibited the binding of isobutyl naphthoquinone to BSA (Figure 2), i.e., the association constant of isobutyl naphthoquinone was decreased with no effect on the number of molecules bound at saturation. The association constant of isobutyl epoxynaphthoquinone obtained from this experiment was $3.9 \times 10^3$ $M^{-1}$, a value close to that ($5.5 \times 10^3$ $M^{-1}$) obtained by direct measurement (Figure 1). This experiment, therefore, demonstrated that the two ligands bind to the same sites on BSA.

The influence of EtOH, DMSO, $CCl_4$ and isooctane on the binding of the two ligands to BSA was investigated with the immobilized protein. EtOH and DMSO (up to 9 % v/v) and $CCl_4$ (up to 0.04 % v/v) did not displace the ligands bound to BSA, in agreement with their negligible influence on the e.e.. Isooctane did not influence the binding of isobutyl naphthoquinone but concentration-dependently displaced the bound isobutyl epoxynaphthoquinone from the protein (Table IV). Therefore, isooctane was able to inhibit specifically the binding of the epoxy-derivative to BSA. We can speculate that the solvent partially occupies the binding sites of the protein in such a way that they are only accessible to the substrate and not to the bulkier, non-planar epoxy derivative.

The binding of methyl naphthoquinone (1) and of its epoxide (9) to BSA was also studied. In this case, the interaction appeared to be aspecific, with a large number of low-affinity binding sites. These data are consistent with the low enantioselectivity obtained with this substrate.

In conclusion, the process of isobutyl naphthoquinone (8) oxidation by t-butyl hydroperoxide can be schematized as follows:

$$BSA + S \longleftrightarrow BSA - S \xrightarrow{\text{oxidation}} BSA - P^* \longleftrightarrow BSA + P^* \quad (1)$$

$$S \xrightarrow{\text{oxidation}} P \quad (2)$$

in which P* is the optically active isobutyl epoxynaphthoquinone obtained from the oxidation of S bound to the chiral binding sites of BSA and P is the racemate obtained from the oxidation of unbound S. The rate of formation of P* will be proportional to the concentration of the complex BSA-S. As the reaction progresses, the concentration of the complex decreases, not only because of the decrease in S, but also because of the accumulation of P* (and P), which will compete with S for the same binding sites on BSA. The product will not, however, affect the rate of formation of the racemate P. At low concentrations, isooctane, which specifically inhibits the binding of isobutyl epoxynaphthoquinone (P* and P) to BSA, favors the formation of the BSA-S complex and therefore the asymmetric reduction. At higher concentrations (5 % v/v), two phases are formed and the reaction partially occurs in the organic phase, thus decreasing the e.e..

Effect of the Substrate. The long-chain fatty acids are among the organic ligands that bind most tightly to BSA.[12] Furthermore, the affinity for the first 2-3 sites on BSA generally increases with chain length. For these reasons we examined the epoxidation of 2-n-octyl-1,4-naphthoquinone (8) with BSA under the usual conditions. The results were beyond our expectation since the epoxide (16) was obtained enantiomerically pure and in good chemical yield (Table V). The total stereoselection for the octyl derivative (8) is also due to the negligible velocity of the competitive epoxidation of the substrate not bound to BSA, which would give racemic oxirane (16). Indeed, under the same conditions but without BSA, naphthoquinone (8) gave epoxide (16) in a lower chemical yield than structurally related compounds did (1,3,5,6), (Table II). The absolute

configuration attributed to (16) on the basis of the Cotton effect centered at 360 nm,[13] is (+)-(2S,3R).

The shortening of the alkyl chain at C-2 has dramatic effects on the degree of the asymmetric induction (Table V), since the e.e. for the corresponding epoxides (10,12) from 2-ethyl (2) and 2-n-butyl derivative (4) are only 5 % and 14 %. An intermediate behavior was found for 2-n-hexyl-1,4-naphthoquinone, which gives oxirane (15) with 30 % e.e.. It is interesting that epoxides (12), (15) and (16) all have the (+)-(2S,3R) absolute configuration. The induction of asymmetry depends on the length of the carbon chain also in the borohydride reduction of δ-ketoacids, a reaction which was reasonably stereoselective (49% e.e.) only for 5-oxohexadecanoic acid.[5] The reaction of the 2-methyl derivative (1) does not fit into the trend of 2-substituted n-alkyl-1,4-naphthoquinones (20 % e.e. and (-)-(2R,3S) absolute configuration for epoxide 9).

Spectral Properties of the BSA-Quinones Complexes. The complexes formed between BSA and various 2-alkyl-1,4-naphthoquinones were studied by CD spectroscopy, since the optical activity that originates in the electronic transitions of the quinone chromophore reflects its interactions with protein chromophores in the neighborhood of the binding sites.

We investigated the complexes formed between BSA and most of the 2-alkyl-1,4-naphthoquinones at various quinone-BSA molar ratios. The electronic bands of the 1,4-naphthoquinone chromophores which are useful here because they fall outside the protein absorptions are a moderately intense band near 335 nm (ε 2000-4000 $M^{-1}$ $cm^{-1}$) and a broader and weaker band between 400 and 500 nm.[14-16] From the intensity of the absorption spectra above 300 nm at various quinone-BSA ratios it is possible to estimate that the number of quinone molecules bound to BSA under buffer saturation conditions varies from 2.5 to 5, except for the 2-n-octyl derivative (8), a single molecule of which apparently binds to the protein even when excess substrate is present. This result may provide a clue for understanding the origin of the stereospecificity observed in the epoxidation of (8).

CD spectra of representative quinone-BSA complexes are shown in Figure 3. In general, the increase in the number of quinone molecules bound to BSA is accompanied by a roughly parallel increase in intensity of the various CD bands, indicating that when several binding sites are present they must have similar binding constants, as we have found for (3) by equilibrium dialysis experiment (see Figure 1).

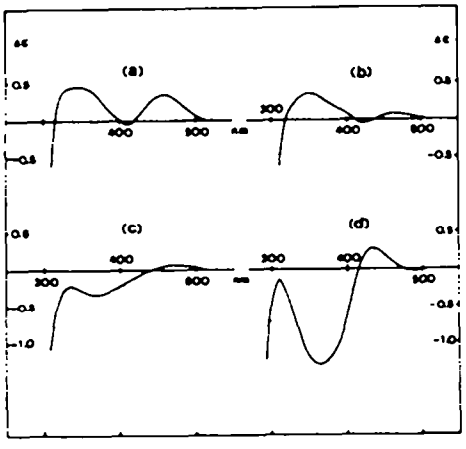



Figure 3. Circular dichroism spectra of representative 1:1 BSA-quinone complexes in aqueous buffer solution at pH 9: (a) R=isobutyl; (b) R=cyclohexyl; (c) R=hexyl; (d) R=n-octyl

Although it is obviously difficult to undertake a detailed analysis at this time, the CD spectra exhibit some regularities that are worth mentioning. In particular, there is an apparent relationship between the CD pattern of the quinone-BSA complexes and the stereochemistry (Table V) of the epoxide formed in the subsequent epoxidation reaction. The series of complexes formed by the 2-R 1,4-naphthoquinones with R=n-butyl (4), n-hexyl (7) and n-octyl (8) have CD spectra with a weaker positive band between 400 and 500 nm and a prominent negative band between 300 and 400 nm. All these n-alkyl chain substrates preferentially or exclusively yield the epoxide with (+)-(2S, 3R) configuration. By contrast, the BSA complexes formed by quinones with branched alkyl chains, R=isobutyl (3) and cyclohexyl (6), have spectra with positive CD activity within the dominant electronic bands and preferentially produce epoxides with (-)-(2R, 3S) configuration. The CD spectrum of BSA complexes of the quinone with R=ethyl (2) is somewhat intermediate between those of the two main classes but the epoxidation reaction still preferentially produces the (+)-(2S, 3R) isomer, as for the other n-alkyl-1,4-naphthoquinines, albeit with modest e.e.. Finally, the CD spectra of BSA complexes of the quinone with R=methyl (1) are entirely different from all the others. In particular, these systems cannot be included in the class of BSA complexes of n-alkyl chain naphthoquinones and, in fact, the epoxide produced (9) has the opposite configuration.

The increase in e.e. for the (+)-(2S, 3R) epoxide as the n-alkyl chain is lengthened (Table V) suggests that these substrates progressively tend to occupy, or become more immobilized in, the binding site for the n-octyl derivative. The CD spectrum of the BSA-8 complex is, in fact, more intense than the others. Such a binding site may be inaccessible to the naphthoquinones with branched side chains, but they probably share another site of relatively high immobilization. Support for this interpretation comes from the observation that addition of the oxidant does not displace the substrates from the binding sites, i.e., the reaction occurs at the binding sites.

The relationship between CD spectra of the BSA-substrate complexes and stereochemistry of the product provides a simplified view of the steric course of the reaction. Since the enantioselectivity must originate in a differentiation of the two faces of the quinone residue, the CD features of the BSA-quinone complexes may characterize the face of the substrate at which the attack of the oxidant preferentially occurs: the *si* face at C-2 for naphthoquinones with normal alkyl chains and the *re* face at C-2 for those with branched alkyl chains. The stereoselection in these epoxidations would thus be determined by the relative accessibility of the two faces of the naphthoquinone residue to the oxidant, different binding sites having different relative accessibility.

Conclusions. Vitamin K and, more generally, substituted 1,4-naphthoquinones and their epoxides are of considerable importance in metabolic processes. The asymmetric Weitz-Scheffer epoxidation in aqueous buffer solution promoted by BSA competes favorably with the reaction performed under phase transfer conditions[13a] and represents a significant improvement in terms of enantioselectivity. The enantioselectivity is favoured by high BSA concentration, long chain alkyl groups at C-2 of the substrate and reduced competitive inhibition by P* (equation 1) with isooctane. The oxidation of free substrate according to equation 2 is retarded by high solution viscosity, bulky substituent groups at C-2 and poorly soluble substrates. The binding studies indicate that a single class of binding sites is responsible for the interaction of isobutyl naphthoquinone and its

epoxide with BSA and explains the role played by the protein as a chiral auxiliary in the presence of isooctane as a cosolvent. Study of the spectral properties of the BSA-quinone complexes provides a satisfactory rationale for the stereoselection of these epoxidations.

## Experimental Section

**Materials and General Methods.** BSA was the fraction V Fluka commercial product; t-BuOOH (70 % in water) and the aqueous buffer solutions are commercially available. Sepharose CL-4B was obtained from Pharmacia and the dialysis tubing (0.6 cm diameter, cellulose membrane) from Sigma. $^1$H NMR spectra were recorded in $CDCl_3$ on a Varian 390 instrument. Enantiomeric excesses were determined by $^1$H NMR with the aid of Eu(hfc)$_3$ or Eu(dcm)$_3$ as chiral shift reagents, using a Varian XL 200 instrument. Electronic spectra were recorded on a Perkin Elmer Lambda 5 spectrophotometer and CD spectra on a Jasco J-600 C dichrograph. Melting points are uncorrected. Elemental analyses were performed with a Perkin-Elmer 240 instrument.

**Starting Quinones.** 2-Methyl-1,4-naphthoquinone (1) was a commercial product. Compounds 2-7 were prepared has described in the literature.[13a] 2-n-Octyl-1,4-naphthoquinone (8), in 50 % yield, was prepared by the same method[13a] from nonanoic acid ; mp 50-52°C; $^1$H NMR ( $CDCl_3$ ) δ 0.85 ( 3H,t ), 1-1.75 ( 12H,m ), 2.3-2.7 ( 2H,m ), 6.7 ( 1H,s ), 7.5-7.75 ( 2H,m ), 7.8-8.1 ( 2H,m ). Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.95; H, 8.22. Found: C, 79.89; H, 8.25.

**Epoxidation of 2-Substituted-1,4-Naphthoquinones. General Procedure.** TO A magnetically stirred solution of 0.05 mmol of BSA (3.3 g) in 12.5 mL of buffer solution, 1 mmol of quinone was added. The mixture was stirred for 15-20 min, then 2 mmol of the oxidant were added. The reaction was stirred at room temperature for the recommended times,[1] then extracted with 5x80 mL of diethyl-ether and the organic layers dried ($MgSO_4$) and concentrated under vacuum. The aqueous phase was stirred overnight with 300 mL of $CHCl_3$ and filtered over a cake of celite; the filtrate was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude combined residues were purified by silica gel column chromatography ( light petroleum-diethyl ether 95:5 v/v as eluent).

The epoxynaphthoquinones (9-15) are known in the optically active form and the physical properties of our specimens were in agreement with those reported in the literature.[13a] 2-n-Octyl-1,4-naphthoquinone-2,3-epoxide (16) was obtained in optically pure form in 66 % yield; mp 64-66°C; $[\alpha]_{436}^{21}$ + 81 (c=0.65, H, m ), 2.15-2.35 ( 1 H, m ), 3.85 ( 1 H, s ), 7.68-7.78 ( 2 H, m ), 7.88-8.06 ( 2 H, m ). Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.48; H, 7.76. Found: C, 74.90; H, 7.79.

**Weitz-Scheffer Epoxidation under Homogeneous Conditions.** To a solution of 0.05 mmol of BSA (3.3 g) in 12.5 mL of pH 9 buffer solution, 0.15 mmol of enone and 0.3 mmol of oxidizing agent (70 % aqueous t-BuOOH) were added. The resulting clear solution was stirred at room temperature for the times indicated in Table I and tne usual work-up gave the crude epoxides, which were purified by silica gel chromatography ( light petroleum-diethyl ether 95:5 v/v as eluent).

**Racemic Epoxides. General Procedure.** To a magnetically stirred mixture of 12.5 mL of pH 11 buffer solution and 1 mmol of quinone, 2 mmol of oxidant (70 % aqueous t-BuOOH) were added. After the appropriate reaction times (see Table II), the mixture was extracted with 5x80 mL of diethyl ether, the organic layer dried over $MgSO_4$ and, after removal of the solvent, the residue was purified as usual.

**Gel Filtration of Used BSA.** BSA employed in the epoxidation of isobutyl naphthoquinone was extracted with diethyl ether, rotoevaporated and diluted with 0.025 M potassium phosphate buffer, pH 7, containing 0.1 M NaCl, to a final concentration of 0.2 %. The solution, which did not contain insoluble aggregates, was gel filtered (0.5 mL) on a Bio-Gel A-0.5 m (200-400 mesh) column (1 cm x 100 cm) at a flow rate of 5 mL/h. The elution profile showed a single protein peak with an elution volume, i.e., protein hydrodynamic volume, coincident with that of native BSA. Therefore, using the BSA in the epoxidation process did not cause aggregation or unfolding of the protein.

**Immobilization of BSA onto Sepharose CL-4B.** Sepharose CL-4B was activated at pH 10.5 with 100 mg CNBr/mL of settled gel, following the method described by Axen et al.[17] The coupling of BSA (400 mg) to the activated matrix (20 mL) was carried out in 0.013 M sodium borate, pH 9, under gentle stirring at 4°C, overnight. The unreacted groups on the matrix were blocked by treating the gel with 0.1 M ethanolamine, pH 9, for 3 h. The supernatant was withdrawn and the immobilized protein washed with the buffer. The amount of immobilized BSA, which was determined by amino acid analysis of known volumes of gel after hydrolysis with 6 M HCl for 24 h at 110°C, was of 16 mg/mL of matrix. A reference Sepharose CL-4B was prepared in the same way but without the addition of BSA.

**Binding of Isobutyl Naphthoquinone and Isobutyl Epoxynaphtho-quinone to BSA.** The binding studies were carried out with BSA in dialysis tubings or with Sepharose CL-4B immobilized BSA. With the first method, BSA (16 mg), dissolved in 1 mL of 0.013 M sodium borate buffer, pH 9, was put inside the tubing and equilibrated (about 2 h) under gentle stirring, at 25°C, with ligand solutions (in 10 mL of sodium borate, pH 9) of various concentrations. The concentration of free ligand was determined by spectrophotometric measurement of the solution outside the tubing at 338 nm (isobutyl naphthoquinone) or 272 nm (isobutyl epoxynaphthoquinone). The amount of bound ligand was calculated by subtracting the free ligand from the total amount of added ligand. With the second method, 1 mL of immobilized BSA (16 mg of protein) was equilibrated (about 20 min) under gentle stirring with ligand solutions (2 mL) of various concentrations. The concentration of free ligand was determined by spectrophotometric measurement of the supernatant after centrifugation for 3 min at 1,500 g. Reference Sepharose CL-4B, treated analogously, did not bind the ligands.

**Preparation of the Solutions of BSA-Quinone Complexes for the UV-Vis and CD Spectra.** BSA (0.05 mmol) was dissolved into 10 mL of buffer solution at pH 9 with stirring. Then 1 protein equiv of the quinone was added to the solution under an inert atmosphere. Solution of the quinone was complete after 2-15 h of stirring. A 1 mL sample of the solution was withdrawn and then diluted to 5 mL with buffer before recording the spectrum. Preparation of a 2:1 solution of quinone-BSA complex was carried out by adding the appropriate amount of the quinone to the remaining solution of 1:1 complex under nitrogen. Except for 8, solution of the substrate was again complete after 2-15 h of stirring. The complexes with higher quinone to BSA molar ratios were prepared as above; any undissolved quinone in the mixture after 15 h stirring was centrifuged out prior to dilution and spectral recording. For evaluating the number of quinone molecules in the complex, difference spectra against $10^{-3}$ M BSA in the range 280-600 nm were used.

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Table I. Epoxidation in Homogeneous Conditions

| substrate | reaction time (days) | epoxide yield[a,b] ( % ) | e.e (± 2%)[b] |
|---|---|---|---|
| 1 | 3 | 20 ( 27 ) | 47 ( 36 ) |
| 3 | 2 | 35 ( 53 ) | 78 ( 75 ) |

[a] All the reactions were performed using a 3:1 quinone-BSA molar ratio ( see Experimental ). [b] The data relative to the epoxides obtained in heterogeneous conditions are given in brackets.

Table II. Epoxidation with or without BSA

| substrate | reaction time ( days ) | epoxide yield ( % ) without BSA | with BSA |
|---|---|---|---|
| 1 | 3 | 74 | 34 |
| 3 | 2 | 84 | 62 |
| 5 | 7 | 93 | 46 |
| 6 | 7 | 51 | 64 |
| 8 | 7 | 15 | 66 |

Table III. Epoxidation of 2-Phenyl-1,4-Naphthoquinone (5) with Different Amounts of BSA

| BSA (% mol) | reaction time (days) | epoxide yield (%) | e.e. (±2%) |
|---|---|---|---|
| 5 | 8 | 46 | 50 |
| 0.5 | 8 | 58 | 40 |
| 0.025 | 4 | 90 | 18 |

Table IV. Effect of Isooctane on Binding of Ligands to Immobilized BSA[a]

| isooctane concentration ( %, v/v ) | free ligand ( μM ) | |
|---|---|---|
| | isobutyl naphthoquinone | isobutyl epoxynaphthoquinone |
| 0 | 37 | 50 |
| 0.01 | 37 | 56 |
| 0.03 | 37 | 68 |
| 0.05 | 37 | 77 |
| 0.07 | 37 | 82 |

[a] Immobilized BSA (1 mL) was equilibrated with 2 mL of buffer containing isobutyl naphthoquinone (3) (0.42 μmol) or isobutyl epoxynaphthoquinone (11) (0.39 μmol). Then, isooctane was added up to the indicated concentrations and the free ligand spectrophotometrically determined after equilibration.

Table V. Influence of the Side - Chain on Enantioselectivity

| substrate | reaction time (days) | epoxide yield ( % ) | absolute configuration[a] | e.e. (±2%) |
|---|---|---|---|---|
| 1 | 3 | 34 | (-) (2R,3S) | 20 |
| 2 | 2 | 44 | (+) (2S,3R) | 5 |
| 3 | 2 | 62 | (-) (2R,3S) | 77 |
| 4 | 2 | 35 | (+) (2S,3R) | 14 |
| 6 | 7 | 64 | (-) (2R,3S) | 70 |
| 7 | 2.5 | 29 | (+) (2S,3R) | 30 |
| 8 | 7 | 66 | (+) (2S,3R) | 100 |

[a] See ref. 13; (+) and (-) refer to the sign of the Cotton effect at 360 nm.