Cycling the potential negative of 190 mV causes the (2 × 2)-Bi lattice to disappear and a new ordered rectangular Bi structure to form (Figure 1c). The left side of Figure 1d illustrates the relative positions of the Bi adatoms and the Au(111) substrate. The average spacing between two neighboring Bi adatoms is 0.34 ± 0.02 nm. On the upper left corner of Figure 1d, $\mathbf{P}_1$ and $\mathbf{P}_2$ are vectors forming the primitive unit cell of this lattice. The measured angle between $\mathbf{P}_1$ and $\mathbf{P}_2$ is 94 ± 4°. On the lower left corner of Figure 1d we introduce a nonprimitive unit cell deduced from the spacings and angles in the primitive unit cell as well as the orientation of the overlayer relative to the underlying Au. The nonprimitive cell, given by vectors $\mathbf{U}_1$ and $\mathbf{U}_2$, shows a 0.49 ± 0.03 nm spacing in the direction of $\mathbf{U}_1$ which is $\sqrt{3}$ the Au distance. The rotation of $\mathbf{U}_1$ relative to the Au is 30 ± 3°. In consequence, vector $\mathbf{U}_1$ is commensurate with the [2,1] direction of Au(111). In the $\mathbf{U}_2$ direction, however, a 0.46 ± 0.04 nm spacing is found which is not commensurate with the Au(111) lattice. The Bi structure is then best described as uniaxially commensurate with Au(111). This Bi full monolayer is consistent with the proposed full monolayer structure of Bi on Ag(111) found in a recent X-ray scattering study;[19] however, the Ag(111)/Bi upd system does not exhibit the (2 × 2) open structure.

We observed these structures during both deposition and stripping in the corresponding potential regions and also in solutions containing H_2O_2. We also found the same images and voltammetry in 0.1 M H_2SO_4 and 0.1 M HNO_3 electrolytes, a result which stands in contrast to previous studies of Cu[20,21] and Ag[22] upd on Au(111). This lack of sensitivity toward electrolyte most likely arises from the influence of the considerable partial charge remaining on the Bi adatom in the upd potential region.[9-11] A similar result was found for upd of Hg on Au(111).[23]

The (2 × 2)-Bi structure is observed at the same potentials at which maximum activity for H_2O_2 reduction is found, while both the bare Au(111) and the rectangular lattice are found at potentials where electroreduction activity is substantially less. We thus consider the structural differences between the two inactive lattices and the (2 × 2)-Bi structure. The bare Au surface of course contains no Bi. The rectangular lattice exhibits Bi adatoms, but the packing density of Bi here is substantially greater than that of the 2 × 2 adlattice (64% vs 25% coverage). The (2 × 2)-Bi structure exhibits both open Au and Bi sites, while the rectangular lattice contains almost no open gold sites. Hence, both Au and Bi are required to effect the catalytic reduction of H_2O_2.

The above observation leads to two possible origins of the electrocatalytic activity of the (2 × 2)-Bi lattice. First, H_2O_2 is thought to bind end-on to bare Au[24] giving an Au-O-O intermediate. The end-on binding of H_2O_2 to Au could be enhanced by the presence of Bi, possibly through steric or electronic interactions. Second, the (2 × 2)-Bi lattice could enable the formation of an intermediate wherein H_2O_2 adsorbs on one Au and one Bi atom with a heterobimetallic bridge (Au-O-O-Bi). The peroxide bond length (0.148 nm) is too short to bridge Bi adatoms alone in either the (2 × 2) or rectangular structures. On the surface with the full Bi monolayer, there is not enough open Au fo peroxide to form a bimetallic bridge. A heterobimetallic bound peroxide would polarize the O-O bond and enhance its cleavage, leading to formation of H_2O.

We have shown that two different Bi adlattice structures form during upd of Bi onto Au(111) in acid electrolytes. The (2 × 2)-Bi adlattice exhibits enhanced reactivity toward H_2O_2 reduction relative to the rectangular adlattice because the first structure exhibits both open Bi and Au sites. A heterobimetallic bridge model for H_2O_2 on this surface could explain the enhanced reactivity.

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Initial-State and Transition-State Effects on Diels-Alder Reactions in Water and Mixed Aqueous Solvents

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The fact that rate constants for Diels-Alder (DA) reactions in water are dramatically larger than those in organic solvents[1-11] contradicts with common notion that DA reactions are rather insensitive to solvent effects.[12,13] For example, second-order rate constants for DA reactions[10] of cyclopentadiene (CPD) with methyl vinyl ketone (MVK) and ethyl vinyl ketone (EVK) are enhanced (relative to *n*-hexane) by a factor of ca. 400. The corresponding reaction with naphthoquinones is ca. 6800 times faster.[10] These intriguing solvent effects[14-19] evoked discussions about the molecular origin of this phenomenon. Explanations were sought in terms of high internal solvent pressure,[3,14,15] micellar catalysis,[2a,b] catalysis by hydrogen bonding,[20,21] solvent polarity or solvophobicity effects,[4,6,11] and hydrophobic association.[1f,4,6,11] Some of these explanations are fallacious. First, the internal solvent pressure of water is extremely low[13] and cannot be held

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Table I. Initial-State and Transition-State Solvent Effects on the DA reactions of CPD with MVK (I) and EVK (II) in Terms of Gibbs Energies at 298 K (kJ mol^{-1}, standard state 1 mol dm^{-3})

(I) CPD + MVK

solvent	$\Delta G_t^{\ominus}$(CPD)	$\Delta G_t^{\ominus}$(MVK)	$\Delta G_t^{\ominus}$(IS)	$\delta\Delta^{\ddagger}G^{\ominus\,a}$	$\Delta G_t^{\ominus}$(AC)
water	7.0	2.1	9.1	−10.02	0.9
ethanol	0.0	0.0	0.0	−0.13	−0.1
1-propanol	0.0	0.0	0.0	0.0	0.0
n-hexane	−1.6	*b*		4.89	
CH_3CN	−0.8	−3.8	−4.6	5.07	−0.5
CH_2Cl_2	−5.8	−5.8	−11.6	4.29	−7.3
$CHCl_3$	−5.2	−5.0	−10.2	3.13	−7.0

(II) CPD + EVK

	$\Delta G_t^{\ominus}$(CPD)	$\Delta G_t^{\ominus}$(EVK)	$\Delta G_t^{\ominus}$(IS)	$\delta\Delta^{\ddagger}G^{\ominus\,a}$	$\Delta G_t^{\ominus}$(AC)
water	7.0	4.4	11.4	−10.19	1.3
1-propanol	0.0	0.0	0.0	0.0	0.0

[a] $\Delta^{\ddagger}G^{\ominus}$ is based on second-order rate constants in $dm^3\ mol^{-1}\ s^{-1}$. [b] Could not be determined.

responsible for the observed acceleration of DA reactions in water. Secondly, in most cases the structure and concentration of dienes and dienophiles used in kinetic experiments exclude the formation of micellar-like aggregates. Finally, kinetic measurements of DA reactions in trifluoroethanol indicate that hydrogen bonding can only account for part of the rate effect in water. Therefore, we contend that the rate enhancements have to be attributed primarily to the peculiar solvent properties of water.[10] For a better understanding, it is of great interest to separate the rate increase of DA reactions in water into initial-state and transition-state effects.[13,22] To this end, we have determined thermodynamic parameters for the transfer of MVK, EVK, and CPD from 1-propanol (as a reference solvent) to water, to aqueous mixtures of 1-propanol, and to several organic solvents.[23] We present evidence that the impressive acceleration of DA reactions in water is primarily an initial-state effect.

Second-order rate constants for the DA reactions of CPD with MVK or EVK were determined as described previously.[10] The Gibbs energies of activation relative to 1-propanol ($\delta\Delta^{\ddagger}G^{\ominus} = \Delta^{\ddagger}G^{\ominus}$(solvent) − $\Delta^{\ddagger}G^{\ominus}$(1-PrOH) are listed in Table I. Gibbs energies for transfer of the reactants (CPD, MVK, and EVK) from 1-propanol to water, ethanol, acetonitrile, *n*-hexane, chloroform, and dichloromethane are also given in Table I, along with transfer parameters for the respective initial states (diene + dienophile). Solvent effects on the transition state (AC) were calculated from

$$\Delta G_t^{\ominus}(\text{AC}) = \Delta G_t^{\ominus}(\text{diene}) + \Delta G_t^{\ominus}(\text{dienophile}) - \delta\Delta^{\ddagger}G^{\ominus} \quad (1)$$

The relative standard Gibbs energies for the reactants CPD and MVK, the total initial state (CPD + MVK), the activated complex, and the (endo) reaction product in 1-propanol–water mixtures are shown as a function of the mole fraction of water in Figure 1. Similar data for the reaction of CPD with EVK are plotted in Figure 2. The accuracy of the applied method did not allow a significant distinction between the transfer parameters for the endo and exo products.

The remarkable stabilization of both reactants and activated complex in chlorinated alkanes indicates that dispersion interactions with the solvent are extremely important. However, in chlorinated alkanes initial-state and transition-state effects are similar, and consequently $\delta\Delta^{\ddagger}G^{\ominus}$ is small. It is clear that the rate enhancement in water arises primarily from the large destabilization of diene and dienophile. Interestingly, the standard chemical potential of the activated complex is hardly affected.

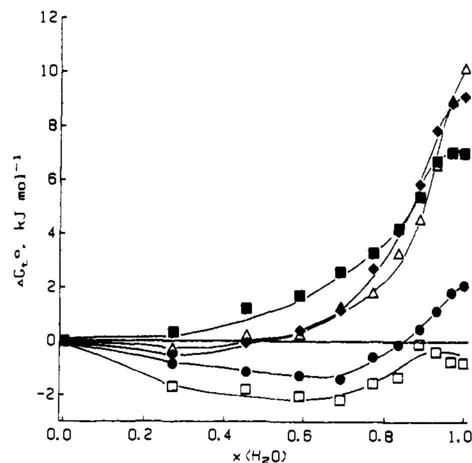

Figure 1. Relative standard chemical potentials of CPD (■), MVK (●), initial state (MVK + CPD) (◆), activated complex (□), and endo product (△) (kJ mol^{-1}, standard state is 1 mol dm^{-3}) for the reaction of CPD with MVK in aqueous mixtures of 1-propanol as a function of the mole fraction of water at 298 K.

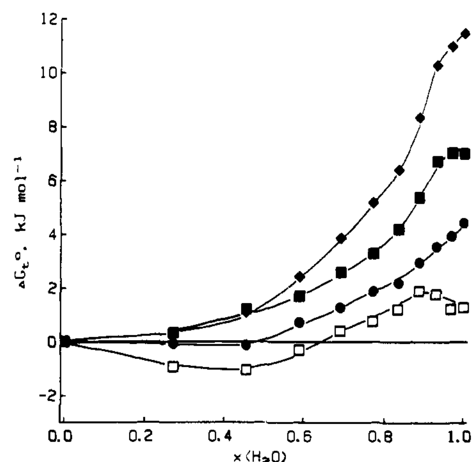

Figure 2. Relative standard chemical potentials of CPD (■), EVK (●), initial state (EVK + CPD) (◆), and activated complex (□) (kJ mol^{-1}, standard state 1 mol dm^{-3}) for the reaction of CPD with EVK in aqueous mixtures of 1-propanol as a function of the mole fraction of water at 298 K.

This chameleon-like behavior in 1-propanol–water mixtures is *not* observed for the reaction products. Apparently, the activated complex has a remarkable capacity to adapt itself to the reaction medium.[24]

The concentration dependence of the activity coefficients of CPD, MVK, and EVK (vapor pressure measurements) in water and in water-rich 1-PrOH–H_2O (<10 mol % of 1-PrOH) did not reveal any significant aggregation of the diene and dienophile at concentrations used for the kinetic measurements. This rules out "hydrophobic packing"[1f] as a major reason for the rate acceleration in water. Instead it appears that the activation process is particularly favorable in water because of the substantial reduction of the hydrophobic molecular surface exposed to water ("enforced hydrophobic interaction"[10]). The data in Figures 1 and 2 suggest that the activated complex is able to interact favorably with water molecules, possibly due to enhanced polarization of the carbonyl group induced by hydrogen bonding. Recent computer simulations by Jorgensen et al.[25] support this view.

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In summary, we have found that DA reactions involving neutral apolar reactants and a remarkably solvent-adaptable activated complex exhibit anomalous solvent effects in highly aqueous solvents. The "hydrophobic acceleration" is due to marked destabilization of the reactants as well as to hydrogen-bonding stabilization of the polarizable activated complex.

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Redox-Dependent Molecular Recognition in Proteins: Site-Directed Mutagenesis Suggests That Cytochrome *c* Oxidation State Governs Binding and Recognition to Cytochrome *c* Peroxidase

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Understanding the way in which redox proteins interact is central to understanding their function.[1] Molecular recognition is crucial to insuring the physiological specificity of redox proteins. In addition, studies of redox protein interactions may lead to some understanding of the coupling of conformation and redox state. Such coupling plays a central role in biology energy transduction. To these ends, we and others have pursued detailed studies of recognition, binding, and reactions between redox proteins (for recent reviews, see refs 1-5). The cytochrome *c* (cyt *c*):cytochrome *c* peroxidase (Ccp) system is particularly attractive, since both proteins are readily isolated in pure form, high-resolution structures are known for both proteins,[6,7] and detailed static and dynamic models of the interaction have been proposed.[8-10] Finally, both proteins have been cloned,[11,12] opening the possibility for site-directed mutagenesis as a tool to probe specific interactions.

A variety of approaches have been used previously to probe the interaction of cyt *c* and Ccp including UV spectroscopy, magnetic resonance, and fluorescence energy transfer.[13-17] Such methods have demonstrated, for example, the dependence of binding on ionic strength. Recent thermodynamic and kinetic data have shown that Ccp binding by yeast cyt *c* is far less dependent on ionic strength than for the homologous horse cyt *c*.[17c] Thus, qualtitative and quantitative comparisons of binding and recognition of these proteins require comparison over a range of ionic strengths. In the present work, an approach based on affinity chromatography[17b,19] is used to map the binding of cyt *c* to several site-specific Ccp mutants, over a range of solution conditions. In essence, binding yeast iso-1 cyt *c* to thiosepharose (via cyt *c* Cys 107)[19b,c] creates an affinity column to which Ccp (and single-site Ccp mutants) are strongly bound. Ccp is then eluted with a salt gradient. Those derivatives that bind more strongly elute at a higher salt concentration.

In this way, the relative binding of several site-specific mutants of Ccp has been assessed. The mutations chosen for study (D37K, D217K, D79K) include the acidic residues which are thought to provide the central electrostatic interactions in the models of cyt *c*:Ccp binding.[17-19] An example of the (relative) binding patterns for Ccp and these mutants is shown in Figure 1. The implication is that D37 is central to the binding of cyt *c* to Ccp, while D217 and D79 are less strongly involved. Surprisingly, the substitution D79K actually improves binding to Fe(III) cyt *c*. These observations accord well with independent binding measurements made by fluorescence energy transfer at a single ionic strength.[17] Note that the binding constants are defined by the vertical line(s) at a given salt concentration. Thus (for a given oxidation state), the apparent differences in binding at an ionic strength of 10 mM, a condition commonly used for binding studies, are quite different from those at, e.g., 50 mM. Absorbance measurements suggest that the concentration of cyt *c* on the column is $\approx 10^{-4}$ M. Thus, when the fraction of Ccp eluted is equal to 0.5, then $K_{binding} \approx 10^4$ M^{-1}.[24]

The primary focus of this study is the comparison of Fe(III) cyt *c* and Fe(II) cyt *c*. Note that the reactant, Fe(II) cyt *c*, remains bound at higher ionic strengths than does the product, Fe(III) cyt *c*. Such differences arise from known redox-state and species-dependent differences in (competitive) anion binding.[22,23] While such differences are known, they had not been measured up to physiological [Cl^-] concentrations, where these differences can become particularly dramatic. Such differences can be quantitated, using the approach to quantitative affinity chromatography outlined by Chaiken.[24] At 0.2 M KCl (pH 6.2), Ccp elutes from the 1-mL Fe(III) cyt *c* affinity column in a 5-mL elution volume, whereas 200 mL is the volume required to elute Ccp from an identical Fe(II) cyt *c* column. This difference in elution volume corresponds to a 50-fold difference in binding affinity between Fe(II) cyt *c*/Ccp and Fe(III) cyt *c*/CCp, under these conditions.[24] These differences were confirmed under the present experimental conditions, by a gel permeation chromatography experiment which shows that while Fe(II) cyt *c* remains bound to Ccp throughout elution with 0.2 M NaCl (pH 6), Fe(III) cyt *c* is not bound, under the same conditions, and so migrates

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